



Universität Bremen

**ORAL CANCER VIA THE BARGAIN BIN: THE ROLE
OF SMOKELESS TOBACCO IN THE ETIOLOGY OF
ORAL CANCER**



*A dissertation submitted to the University of Bremen in partial
fulfillment of the requirements for the degree of
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by

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DEDICATION

On the morning of December 16, 2014, I was sitting on a chair across an oral cancer patient in a hospital in Peshawar, noting down her verbal responses on a data collection tool for the case-control study described in this dissertation. On that same morning, 144 children and staff members of the Army Public School, Peshawar, went to school, but unlike me, they never returned to their homes in the afternoon. Those 144 human beings had been brutally killed in the name of “religion” by people who follow the religion of “TERRORISM”. Pakistan had probably witnessed its darkest day. A day that made parents think twice about sending their children to schools anymore, and a day, which seemingly suggested that bullets were mightier than the pen.

As the reality of the barbaric events of December 16, 2014, began to sink in, the Pakistani nation realized that it cannot afford to see another “December 16”, and that it cannot let the terrorists win. This realization culminated in a single-minded commitment, among both the people and the Government of Pakistan, to eradicate terrorism from the country. Almost two years and three successful anti-terrorism military operations later, Pakistan is a much safer haven for its people, parents are not afraid to send their children to schools and perhaps, the balance is tilting in the favor of the pen again. *This dissertation is dedicated to the architects of a better Pakistan, who in their martyrdom induced a positive change that perhaps no research could or ever will.*

Dear *Khaula Bibi, Ahmed Ali, Abdullah Ghani, Abrar Hussain, Adnan Hussain, Bilal Arshad, Shahzad Ijaz, Shahzeb, Ziaullah Islam, Azaan toryalay, Fahid Hussain, Gul Sher, Hamid Ali, Azhar Naseer, Rizwan Sareer, Asfand, Hammad Malik, Wahabuddin, Wasif Ali, Yasirullah, Adil Shahzad, Ailian Khan, Ammar Iqbal, Arham Khan, Atifur Rehman, Awais Ahmed, Awais Nasir, Baqir Ali, Fahad Hussain, Fazal Raheem, Muhammad Furqan, Humayun Iqbal, Hamid Saif, Hamza Kamran, Haris Nawaz, Hasnain Sharif, Khushnood Zeb, Abdullah Zafar, Ghasaan Khan, Haris Khan, Muhammad Salman, Tayyab Fawad, Nangyal Tariq, Shaheer Khan, Omer Khan, Saifullah, Shahood Alam, Sher Nawaz, Mujahid Hussain, Ali Abbas, Sohail Aslam, Husnain Shah, Tanweer Hussain, Uzair Ahmad, Uzair Ali, Umair Arshad, Ahmed*

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ABSTRACT

Background: Oral cancer combined with the other cancers of the head and neck region constitute the sixth most common cancer in the world. Oral cancer is a major public health challenge in South Asia. Pakistan, India, Bangladesh and Sri Lanka have some of the highest incidence and prevalence rates of oral cancer in the world. Approximately 16,000 new cases of oral cancer are diagnosed each year in Pakistan and around 6,000 Pakistanis lose their lives to this malignancy every year. There is a disconnect between the research findings from the developed countries and the developing countries, with regards to the risk of oral cancer associated with smokeless tobacco use. Given the differences in the composition of the smokeless tobacco products used in different parts of the world, it is imperative that the health risks related with each of these products are assessed on an individual basis.

Methods: A case-control study was carried out in the Khyber Pakhtunkhwa province of Pakistan from September 2014 until May 2015, to quantify the risk of oral cancer associated with the use of *Naswar* (smokeless tobacco). Additionally, three systematic reviews (including meta-analyses) of observational studies and two narrative reviews were carried out to address the secondary objectives of this dissertation.

Results: We found an increased risk of oral cancer associated with the use of smokeless tobacco products in South Asia. The use of smokeless tobacco was also associated with an elevated risk of oral potentially malignant disorders. In Khyber Pakhtunkhwa, Pakistan, Ever-users of *Naswar* had a 21-fold increase in the risk of oral cancer compared to Never-users [Odds ratio (OR)]=21.2 [(95% Confidence Interval (CI), (8.4-53.8)]. An elevated risk of oral cancer associated with *Naswar* use was found among both women and men. The risk of oral cancer increased with the increasing frequency, total duration, and the intensity of *Naswar* use. 70% of the oral cancer burden of Khyber Pakhtunkhwa was attributable to *Naswar*.

Discussion: The findings of the systematic reviews and the case-control study on *Naswar* are comparable to the existing literature. The results clearly demonstrate a high risk of oral cancer and related disorders, associated with the use of smokeless tobacco products like *Naswar*, Betel quid, and Gutkha. There is a lack of oral cancer research in Pakistan and the tobacco control policies of the country largely focus on tobacco smoking while neglecting smokeless tobacco. In order to tackle the growing burden of oral cancer in South Asia in general and Pakistan in particular, policies need to be in place to curb the use of smokeless tobacco products. These products need to be regulated and brought under the tobacco tax-net. Changes to the composition of these products, to make them less harmful to health, should also be looked into as an intervention.

ABSTRAKT

Hintergrund: Weltweit bildet Mundkrebs zusammen mit anderen Krebsarten der Kopf-Hals-Region die sechsthäufigste Krebsart. Mundkrebs ist eine große Herausforderung im Bereich der öffentlichen Gesundheit in Südasien. Pakistan, Indien, Bangladesch und Sri Lanka haben einige der höchsten Inzidenz- und Prävalenzraten von Mundkrebs. Rund 16.000 neue Fälle werden jedes Jahr in Pakistan berichtet und rund 6.000 Pakistanis verlieren jährlich ihr Leben aufgrund dieser Krebserkrankung. Rauchen gilt als wichtiger Risikofaktor, allerdings gibt es Unterschiede in den Forschungsergebnissen aus den entwickelten Ländern und den Entwicklungsländern in Bezug auf die Gefahr des Mundkrebses durch die Verwendung von rauchlosem Tabak. Angesichts der unterschiedlichen inhaltlichen Zusammensetzung von rauchlosen Tabakprodukten in verschiedenen Teilen der Welt ist es zwingend erforderlich, dass die gesundheitlichen Risiken im Zusammenhang mit jeder Form dieser Produkte auf individueller Basis quantifiziert werden. **Methoden:** Es wurde eine Fall-Kontroll-Studie in der Provinz Khyber Pakhtunkhwa Pakistan zwischen September 2014 und Mai 2015 durchgeführt, um das Risiko von oralen Krebs im Zusammenhang mit Naswar (rauchlosem Tabak) zu verwenden. Ferner wurden drei systematische Reviews (inklusive Meta-Analysen) von Beobachtungsstudien sowie zwei narrative Bewertungen erarbeitet, die die sekundären Ziele dieser Dissertation umfassen. **Ergebnisse:** Im Zuge der Fall-Kontroll-Studie wurde ein stark erhöhtes Risiko für Mundkrebs in Zusammenhang mit der Verwendung von rauchlosen Tabakwaren in der Khyber Pakhtunkhwa Region, Pakistan, beobachtet. Zudem war die Verwendung von rauchlosem Tabak mit einem erhöhten Risiko von möglicherweise malignen oralen Erkrankungen assoziiert. *Naswar* Nutzer hatten ein 21-fach erhöhtes Mundkrebsrisiko im Vergleich zu Personen, die *Naswar* nie genutzt haben [Odds ratio (OR)]=21.2, [95% Konfidenzintervall, (8.4-53.8)]. Ein erhöhtes Risiko für Mundkrebs wurde bei Frauen und Männern beobachtet. Das Mundkrebsrisiko stieg mit zunehmender Häufigkeit, Dauer und Intensität der Nutzung von *Naswar*. Circa 70% aller Mundkrebsereignisse in der Khyber Pakhtunkhwa Region sind der Nutzung durch *Naswar* zuzuschreiben. **Diskussion:** Die Ergebnisse der Bewertungen und der Fall-Kontroll-Studie zu *Naswar* sind im Einklang mit der vorhandenen Literatur. Die vorliegenden Ergebnisse zeigen ein hohes Risiko für Mundkrebs und verwandte Erkrankungen, die im Zusammenhang mit der Anwendung von rauchlosen Tabakprodukten wie *Naswar*, Betel quid, Gutkha stehen. Es besteht ein Mangel an entsprechender Krebsforschung in Pakistan sowie bei Anti-Tabak-Maßnahmen des Landes, die die Risiken rauchlosen Tabaks vernachlässigen. Um die zunehmende Krankheitslast durch Mundkrebs in Südasien im Allgemeinen und insbesondere in Pakistan anzugehen, bedarf es Maßnahmen vor Ort, um die Verwendung von rauchlosen Tabakwaren einzudämmen. Diese Produkte müssen reguliert und unter die Tabaksteuer gestellt werden. Änderungen in der Zusammensetzung dieser Produkte müssen angestoßen werden, damit sie weniger schädlich für die Gesundheit sind. Diese Maßnahmen sollten ferner hinsichtlich ihres Erfolges übergeprüft werden.

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ABBREVIATIONS/ACRONYMS

DNA	Deoxyribonucleic Acid
EMRO	World Health Organization's Eastern Mediterranean Regional Office
FATA	Federally administered tribal areas of Pakistan
HPV	Human Papilloma Virus
IARC	International Agency for Research on Cancer
ICD	International Classification of Disease
LMIC	Low and middle-income countries
NCD	Non-Communicable Disease
OPMD	Oral Potentially Malignant Disorders
OR	Odds Ratio
RR	Relative Risk
SLT	Smokeless Tobacco
WHO	World Health Organization

FOREWORD

Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less.

-Marie Curie

Pakistan has been a perennial member of the Ivy League of oral cancer incidence and prevalence. Oral cancer ranks as the second most common malignancy in Pakistan among both sexes, and the most prevalent cancer among men. My interest in oral cancer piqued during the formative years of my dentistry career (2006-07). While working as a “House Officer” at the “Khyber College of Dentistry” in Peshawar, Pakistan, I would come across at least one oral cancer patient on a daily basis. It was through the skimming of the documented social/personal histories of these patients that made me realize, that most of them had one thing in common i.e. the habit of *Naswar* (a form of smokeless tobacco). A *Naswar* packet roughly costs 1/10th of a cigarette pack in Pakistan. The easy availability and cheap prices make *Naswar* an attractive product to tobacco users. *Naswar* use is considered as a cultural practice and a social norm in the Khyber Pakhtunkhwa, Pakistan.

It was not, until much later (2014), that I finally got a chance to be able to assess the association between *Naswar* use and the risk of oral cancer in Khyber Pakhtunkhwa, Pakistan, in a formal manner. I, with the help of my advisor Prof. Dr. Hajo Zeeb, and the valuable input from Prof. Dr. Wolfgang Ahrens, designed a case-control study to assess the association between *Naswar* use and oral cancer. The study was based on the template of the “Alcohol Related Cancer and Genetic susceptibility in Europe (ARCAGE)” study [1]. We devised the data collection tools and study protocols from October 2013 until March 2014. Ethical approval for the study was granted by the Research and Ethics Board of the Khyber medical University, Peshawar and data collection in Pakistan began in September 2014. Although hampered by some local events in Peshawar, we were successful in recruiting the required number of participants by the end of May 2015. The data cleaning and analysis was carried out until December 2015. We have, since then submitted a manuscript to International peer-reviewed journals based on the data collected during the study. During this time i.e. from the conception of the case-control study until the submission of the

manuscript related to the study, I also carried out a scoping review of oral cancer research in Pakistan and two systematic reviews on the risk of oral cancer, and oral potentially malignant disorders, associated with smokeless tobacco use. Additionally, a pooled analysis of case-control studies from Pakistan, a review of the smokeless tobacco control policy in Pakistan, and a guest editorial on the use of *Naswar* and the associated risk of oral cancer constitute my doctoral research work. The details of the research studies carried out in the context of this body of work, including the objectives, methods, results and a discussion of the results, are provided in the various chapters of this dissertation.

I am confident that my findings will add new knowledge to the existing knowledge base on the deleterious effects of smokeless tobacco use. These findings have the potential to influence tobacco control policies and inform cancer control strategies in Pakistan.

LIST OF ARTICLES

This dissertation is based on eight methodologically different but thematically intertwined research articles. Six of these articles have been published, one article is under review and one article is in preparation. I am the first author of all the afore-mentioned articles.

Following is the list of the articles that forms the basis of this dissertation. These are referred to in the text by their roman numerals.

- I. Khan Z, Muller S, Ahmed S, Tonnie J, Nadir F, Samkange-Zeeb F. Quantitative Review of Oral Cancer Research Output from Pakistan. *Asian Pacific Journal of Cancer Prevention*. 2015;16(11):4733-9.
- II. Khan Z, Tönnies J, Müller S. Smokeless tobacco, and oral cancer in South Asia: a systematic review with meta-analysis. *Journal of Cancer Epidemiology*. 2013 Dec;2014:394696.
- III. Khan Z, Khan S, Christianson L, Rehman S, Ekwunife O, Samkange-Zeeb F. Smokeless tobacco and oral potentially malignant disorders in South Asia: a protocol for a systematic review. *Systematic Reviews*. 2016 Aug 24;5(1):142.
- IV. Khan Z, Khan S, Christianson L, Rehman S, Ekwunife O, Samkange-Zeeb F. Smokeless tobacco and oral potentially malignant disorders in South Asia: a systematic review and meta-analysis. *Nicotine Tob Res* 2017 ntw310. doi: 10.1093/ntr/ntw310.
- V. Khan Z, Shah SMH, Rehman B, Ullah Z, Khan S, Dreger S, Pohlabein H, Zeeb H. Oral cancer via the Bargain Bin: findings of a case-control study on the risk of oral cancer associated with a smokeless tobacco product (*Naswar*). Under peer-review in "PLOS ONE".
- VI. Khan, Z. A gaping gap (smokeless tobacco control in Pakistan). *Tob Induced Dis*. (2016) 14: 36. doi:10.1186/s12971-016-0102-y
- VII. Khan Z. Naswar (smokeless tobacco product), oral cancer and tobacco control in Khyber Pakhtunkhwa, Pakistan. *Khyber Med Univ J* 2016; 8(3): 113-114."

- VIII. Khan Z, Heise T, Dreger S, Rizwan SA. Use of *Naswar* and the risk of oral cancer in Pakistan: A systematic review and meta-analysis. (In preparation).

1 INTRODUCTION

“It's a disease so horrible, it defies metaphor - people use cancer as a metaphor for the worst things in life, but there are no metaphors dreadful enough to describe cancer”

-Charlie Jane Anders

Oral cancer is an ancient disease. The earliest description of the condition can be found in the “Sushruta Samhita”, a Sanskrit surgery text from the 600 BC [2], that refers to it as “Mukharbuda”, a malignancy of the buccal mucosa [3]. Oral cancer is characterized by an aggressive course, culminating in severe local tissue destruction and distant metastasis [4]. The sequelae of oral cancer, in addition to disability and mortality, include a diminished quality of life because of the anatomical, aesthetic, and functional role of the oral cavity [5]. Oral cancer combined with the other head and neck cancers constitutes the sixth most common cancer in the world [6]. According to the latest estimates of the International Agency for Research on Cancer (IARC), worldwide, more than 300,000 new cases of oral cancer are diagnosed each year with a five-year prevalence of more than 700,000 cases. Oral cancer accounts for nearly 200,000 deaths each year. Oral cancer has surpassed lung cancer as the most prevalent cancer among men in Pakistan and is the second most common malignancy in women. Approximately 16,000 new cases of oral cancer are diagnosed each year in Pakistan and about 6,000 Pakistanis die annually of oral cancer [7].

Although oral cancer has a multifactorial etiology, tobacco use and alcohol are universally considered as the major risk factors for oral cancer [8]. Recent estimates suggest that smokeless tobacco (SLT) use led to a loss of 1.7 million “disability adjusted life years” and 62,283 deaths due to cancers of the mouth, pharynx, and esophagus [9]. SLT is a form of tobacco that is used without burning the product [10]. An estimated 250 million people use SLT in some form in South and South East Asia, implying that more than 90% of the world’s SLT burden lies in this region [11]. 13.3% of Pakistan’s population uses SLT products [12], and its use is gaining more popularity in the wake of rising cigarette prices in the country. Given the high prevalence of oral cancer and the use of SLT, there is a striking lack of etiological research on oral cancer in Pakistan, with negligible local evidence to inform tobacco and cancer control policies in the country [13]. Of particular note is the scarcity of research on

Naswar and the associated risk of oral cancer in Pakistan. *Naswar* is an SLT product that is used predominantly in Pakistan and Afghanistan, and to a lesser extent in India, Bangladesh, and Central Asia [14]. In 2007, the IARC also acknowledged the lack of epidemiological data to establish the carcinogenicity of *Naswar* in humans, in its monograph on smokeless tobacco [15].

Although evidence from South Asia (mostly India) points toward a potentially causal association between oral cancer and smokeless tobacco use [16-19], some investigations from industrialized countries, particularly Sweden, where SLT use is common, do not show an increased risk of oral cancer linked to the use of SLT products [20, 21]. This has led to recommendations from certain sections of the scientific community that smokeless tobacco can be used as means of “tobacco harm reduction” [20, 22]. There are also suggestions that some forms of SLT can be used as less hazardous and considerably cheaper alternatives to smoking and nicotine replacement therapies in smoking cessation [23]. Additionally, in some parts of Pakistan, it is also considered as a medicinal herb used to relieve pain, rather than a potentially addictive and perilous tobacco product [24].

1.1 RATIONALE

The disconnect between research findings from the Industrial and developing countries about the risk of oral cancer associated with the use of smokeless tobacco, the lack of etiological research on *Naswar* and oral cancer in Pakistan, and the “harm reduction” debate regarding smokeless tobacco, served as rationale to conduct the studies carried out in the context of this body of work.

1.2 OBJECTIVES

This body of work is guided by the following objectives.

1.2.1 General Objective

To study the role of smokeless tobacco as a determinant of oral cancer incidence and prevention in Pakistan.

1.2.2 Specific Objectives

- i. To assess the risk of oral cancer associated with *Naswar* use in Khyber Pakhtunkhwa, Pakistan.
- ii. To appraise the quantitative as well as thematic aspects of the oral cancer research output from Pakistan.
- iii. To determine the risk of oral cancer associated with SLT use in South Asia by systematically reviewing evidence.
- iv. To assess the risk of Oral Potentially Malignant Disorders associated with SLT use in South Asia.
- v. To estimate the pooled risk for oral cancer associated with *Naswar* use, from epidemiological studies carried out in Pakistan.
- vi. To identify gaps, regarding smokeless tobacco control, in the tobacco control policy of Pakistan.
- vii. To formulate policy recommendations based on the evidence generated by this body of work.

1.3 DISSERTATION STRUCTURE

The work done in the context of this dissertation is hereafter described in eight chapters and nine articles (Three published, three accepted for publication, two under peer review and one in preparation), which are provided in the appendices (I – VIII).

Chapter 2 provides an overview of oral cancer including its clinical and histopathological features, the global epidemiology of oral cancer, and a description of its risk factors.

Chapter 3 deals with smokeless tobacco and covers its nomenclature and global distribution. It sheds light on the association of different disease, particularly cancers, with the use of smokeless tobacco. The chapter also provides an insight into the biochemical basis of smokeless tobacco carcinogenesis.

Chapter 4 addresses the public health domains of Non-communicable disease prevention, and causality, and how the intersection of these two provided the conceptual framework for this body of work.

Chapter 5 includes a description of the research methods that were used for the case-control study that was carried out in Khyber Pakhtunkhwa province, Pakistan (Article V). It also briefly sheds light on the research methods used in preparing the other manuscripts included in this dissertation.

Chapter 6 elucidates the scientific findings, listed according to the objectives that guided this body of work.

Chapter 7 presents a discussion of the research findings, how these findings fit the criteria for causality, and the strengths and limitations pertaining to the methods used to address the aims of this body of work.

Chapter 8 provides specific conclusions related to the objectives of this body of work, as well as providing overall conclusions, and implications for policy.

2 THE CURIOUS CASE OF ORAL CANCER

"...the radium has once again begun to eat away at something...and my world is what it was previously, a small island of pain floating on an ocean of indifference."

-Sigmund Freud (on suffering from oral cancer) to Marie Bonaparte (16.6.1939).

2.1 ORAL CANCER DEFINITION

The many peculiarities of oral cancer begin with the very definition of the disease, as there is none, which is universally agreed upon [25]. A 2011 literature search in PubMed returned 17 different terms used for oral cancer [26]. The problem stems from the range of anatomical sites that are included in oral cavity and oropharynx, as the definition of cancer itself, is very clear in the literature [27]. This discourse on the nomenclature of oral cancer often leads to difficulties in retrieving research material on oral cancer for comparative purposes, thus potentially hampering the research process [26].

For the purpose of this dissertation, the term “oral cancer” will be used collectively for the malignancies of oral cavity and oropharynx. In accordance with the “International Classification of Disease 10th revision-(ICD-10)” [28], a malignant neoplasm of the lip, tongue, buccal mucosa or any other part of the oral cavity or oropharynx will be considered as oral cancer (Table 2.1).

2.2 CLINICAL FEATURES

Oral cancer usually begins as a silent disease with no or minimal clinical signs and symptoms [29]. It may be preceded by pre-malignant conditions, collectively known as “Oral Potentially Malignant Disorders” [30]. Although oral cancer develops in a visible anatomical site, the malignancy is often at an advanced stage, at the time of the initial diagnosis [31]. Some of the middle to late stage signs and symptoms include the ulceration of the tumor that does not heal, difficulty in speaking, trismus, dysphagia, bad breath, and mobile teeth. Pain may be felt when the tumor is infected or compresses the nearby nerves [32].

Table 2.1. Anatomical subsites for oral cancer based on the international classification of disease-10th revision.

ICD-10 code	ICD-9 equivalent	Anatomical site
C00	140.0-140.9	Lip
C01	141	Base of tongue
C02	141.1-141.9	Other & unspecified parts of tongue
C03	143.0-143.9	Gum
C04	144.0-144.9	Floor of mouth
C05	145.2-145.5	Palate
C06	145.0-145.1,145.6-145.9	Other & unspecified parts of mouth
C09	146.0-146.2	Tonsil
C10	146.3-146.9	Oropharynx
C14	149.0-149.9	Other & ill-defined sites of lip, OC* & Pharynx

*Oral cavity

2.3 HISTOPATHOLOGICAL FEATURES

More than 90% of the oral cancers are squamous cell carcinomas arising from the epithelial lining of the oral cavity [33]. The rest of the oral cancers are comprised of verrucous carcinoma, adenosquamous carcinoma, mucoepidermoid cancer, adenoid carcinoma cuniculatum and some other very rare forms [34].

2.4 DIAGNOSIS

A provisional diagnosis of oral cancer is usually through visual inspection and clinical signs and symptoms. Histological confirmation (Gold standard) of the tumor is used to establish a definitive diagnosis. More recently, Magnetic Resonance Imaging and Computed Tomography have been increasingly used to aid in the diagnosis of oral cancer [34].

2.5 GLOBAL EPIDEMIOLOGY OF ORAL CANCER¹

Globally, there is a marked geographical variation in oral cancer incidence and mortality, with two-thirds of the oral cancer burden lying in the developing countries [35]. Oral cancer is twice more common in males as compared to females. The usual onset of the disease is in the sixth and seventh decade of life, although in some parts of the world the onset may be at an early age [36]. Figure 2.1 describes the incidence of, and mortality due to, oral cancer in different geographic regions of the world. Figure 2.2 highlights the top 20 countries in the world with the highest mortality and incidence of oral cancer.

2.5.1 Oral Cancer in South Asia

Oral cancer is a huge public health problem in South Asia [37]. It accounts for one-third of all cancers in India [38]. Bangladesh and Sri Lanka rank second and fourth in the world respectively, with regards to oral cancer incidence. In Pakistan, it accounts for 10% of all new cancer cases. Recent trends show an increase in the incidence rates of oral cancer in Pakistan, and it is feared that the country may face an oral cancer epidemic by the year 2030 [39]. The five-year prevalence of oral cancer in Pakistan, in men and women, is 16,781 and 13,866, respectively.

2.6 RISK FACTORS FOR ORAL CANCER

Oral cancer has a multifactorial etiology. Analogous to its incidence, there are marked differences in risk factors for oral cancer between different geographic locations.

2.6.1 Tobacco use²

Tobacco and alcohol constitute the biggest preventable risk factors for oral cancer [40]. Smoking tobacco in any form e.g. cigarette, cigars, cigarillo, bidi, hookah, chillum or sheesha is considered as a risk for oral cancer [41]. IARC has placed tobacco in the group I of

¹ Unless cited otherwise, all figures reported in the sub-section 2.5 have been taken from most recent edition of GLOBOCAN [7]. All rates are per 100,000 population.

² Smokeless tobacco is described in chapter 3.

carcinogens for oral and pharyngeal cancers [42]. A 2004 meta-analysis of 12 observational studies reported a pooled relative risk (mRR) of 3.4 [95% Confidence Interval (CI), 2.3-4.9] for oral cancer associated with cigarette smoking, compared to non-smokers [43]. Another meta-analysis of 15 case-control studies revealed a pooled odds ratio (mOR) of 4.6 (95% CI, 3.1-6.7) for smokers compared to non-smokers [44]. Similarly, Bidi smoking, which is a common practice in some Asian countries was reported to increase the risk of oral cancer by more than three-fold compared to non-smokers in a meta-analysis of 12 studies, mOR=3.1 (95% CI, 0.7-1.8) [45]. Chang et al., in a meta-analysis of cohort studies on smoking-related mortality, reported mortality ratios ranging from 4.0 to 7.9 for oral cancer from cigar smoking [46]. All the cited reviews reported a dose-response relationship between tobacco smoking and oral cancer.

2.6.2 Alcohol Drinking

Alcohol is a major risk factor for oral cancer and along with tobacco is responsible for about 70% of all oral cancer cases in the world [47]. A 2015 meta-analysis of 16 studies concluded that the risk of oral cancer is almost five times elevated among long-term alcohol users compared to non-users, mRR=4.6 (95% CI, 3.1-6.7) [48]. Another pooled analysis reported a 13-fold higher risk of oral cancer associated with the consumption of 125 grams of alcohol per day, mRR=13.0 (95% CI, 9.8-17.1) compared to non-drinkers, but this estimate was not adjusted for smoking [49]. Li et al., reported pooled relative risks of 1.2 (95% CI, 0.9-1.6) for light (≤ 12.5 g/day), 1.7 (95% CI, 1.2-2.5) for moderate (12.6-49.9 g/day), and 3.6 (95% CI, 2.6-5.0) for heavy (≥ 50 g/day) drinkers respectively, when compared to non-drinkers [50]. Although alcohol is an independent risk factor for oral cancer, it is the synergistic effect of alcohol consumption and tobacco use, which might be a reason for the very high risk of oral and pharyngeal cancers associated with these habits. Among the 74% cases attributed to tobacco and alcohol use, in a multi-center European study on upper aero-digestive tract cancers, only one percent was attributed to alcohol, 29% to tobacco, while 44% were accredited to the joint effect of both alcohol and tobacco use [51].

The curious case of oral cancer

International Agency for Research **Lip, oral cavity, Other pharynx: both sexes, all ages**

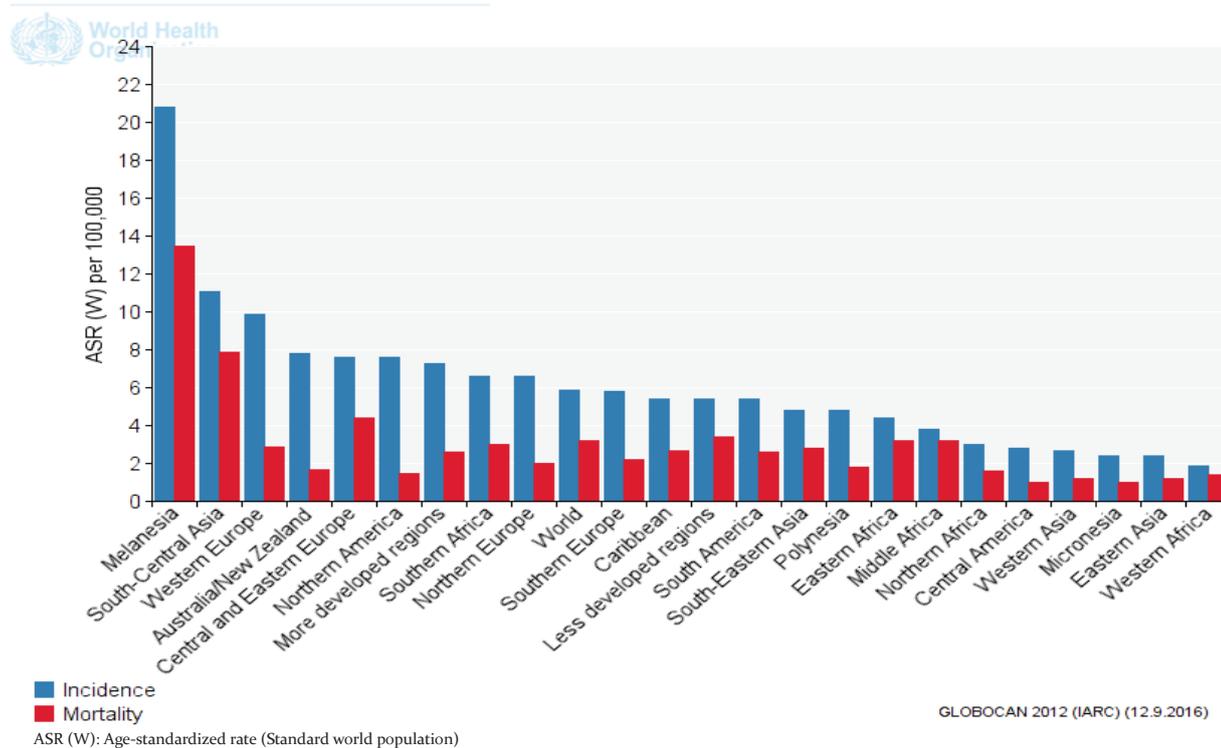


Figure 2.1. Global Incidence and mortality related to oral cancer based on the 2012 estimates by the IARC [7].

International Agency for **Lip, oral cavity, Other pharynx: both sexes, all ages**

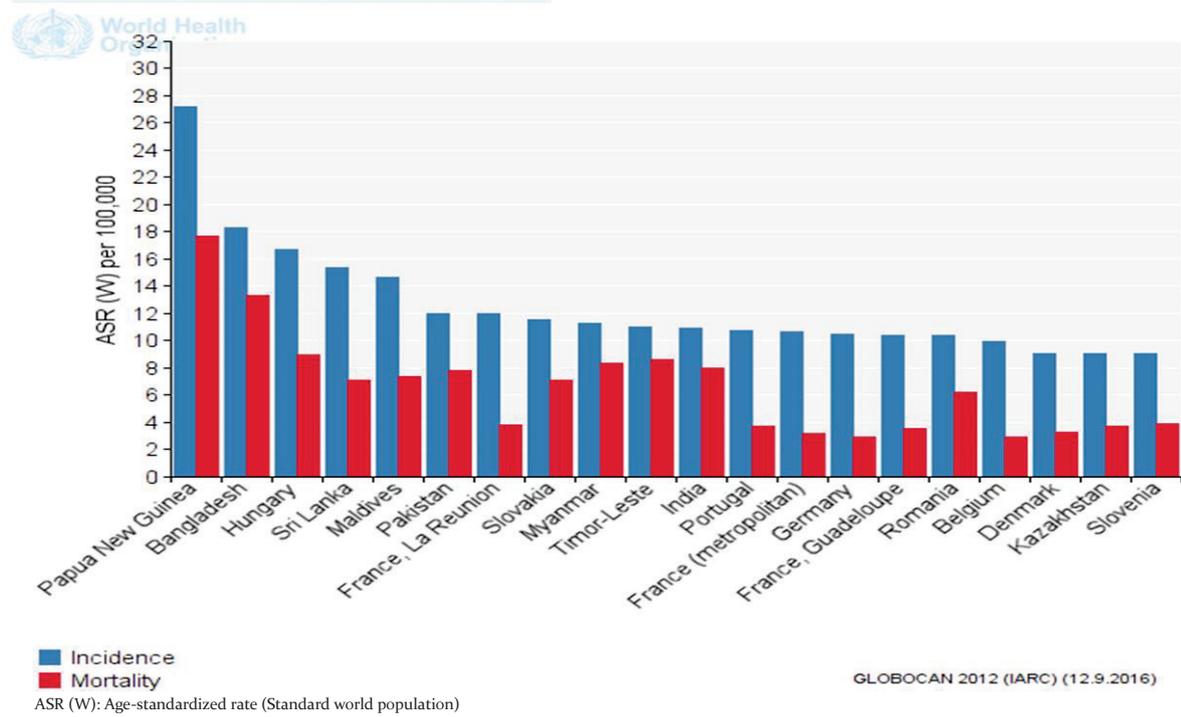


Figure 2.2. Countries with the highest incidence and mortality of oral cancer based on the 2012 estimates by the IARC [7].

2.6.3 Diet

Diet has a modifying effect on the risk of cancer. Diet rich in saturated fats and processed meat is associated with an elevated risk of cancer. Conversely, a diet consisting of fruit, fibrous food items, and dairy products has been shown to have a protective effect [52]. Notani et al reported a two-fold increase in the risk of upper aero-digestive tract cancers in subjects who did not consume vegetables daily compared to those who did [53]. A cohort study of 34,651 post-menopausal women reported an RR of 0.5 (95% CI, 0.3-0.8) for oral cancer, in the highest tertile of whole grain users compared to the lowest tertile. The same study reported an RR of 0.5 (95% CI, 0.3-0.8) for oral cancer, among users of yellow/orange vegetables in the highest tertile, compared to the users in the lowest tertile [54]. Mediterranean diet, which is high in vegetables and monounsaturated fats, is associated with a decrease in the risk of upper aero-digestive tract cancers [55]. A meta-analysis by Xu et al. reported an increased risk of oral cancer in South America with the use of meat mRR= 2.1 (95% CI, 1.4-3.2) [56]. Levi et al., reported a four-fold increase in the risk of oral cancer among the highest quartile of processed red meat consumers compared to the lowest quartile, odds ratio (OR)=4.6 (95% CI, 2.5-8.6) [57].

2.6.4 Oral Hygiene

Oral hygiene plays an important role in the development of oral cancer [58]. Results from a large multicenter case-control study from Europe reported that a poor condition of the mouth almost tripled the risk of head and neck cancer, OR=2.8 (95% CI, 1.7-4.8) [59]. A large study from Latin America reported a comparatively lower but still significant risk of oral cancer associated with poor oral hygiene compared to good oral hygiene, OR=1.8 (95% CI, 1.4-2.4) [59]. Similarly, the lack of toothbrush use, OR=2.3 (95% CI, 1.2-4.3) and daily mouthwash use, OR=3.4 (95% CI, 1.9-5.8) increased the risk of head and neck cancers in Latin America [59]. A study from Brazil reported a three-fold increase in the risk of oral and pharyngeal cancer with bleeding gums, which is a sign of periodontal disease and often results from poor oral hygiene, OR=3.1 (95% CI 1.2-7.9) [60]. Shamami et al., in a systematic review of case-control and cohort studies concluded that independent of smoking and alcohol use, periodontal disease and tooth loss, are a risk factor for oral cancer [61].

Mouthwashes, which are used as an aid to maintaining good oral hygiene, pose a unique dilemma in this regard. Recent evidence suggests an increase in the risk of oropharyngeal cancer with the use of mouthwash [62]. This may be due to the alcoholic content of some of these mouthwashes. Contrastingly, some systematic reviews have reported no significant increase in the risk of oral cancer with the regular use of mouthwash, mRR=1.1 (95% CI (0.9-1.3) [63]. This disconnect in evidence regarding the risk of oral cancer associated with mouthwashes, warrants further scientific investigation.

2.6.5 Social and demographic risk factors

Oral cancer is similar to the other health-related phenomena, in the sense that a socio-demographic gradient exists, with regards to its incidence. Conway and colleagues have reported that a lower occupational and social class was associated with the incidence of oral cancer in both high and low-income countries, mOR=1.8 (95% CI, 1.4-2.3) [64]. A literature review carried out by Liu suggests that lower levels of education (OR range, 1.85 ~ 5.3) and lower monthly income (OR range 1.7 ~ 2.41), are related to an increased risk of oral cancer [65]. A study from India reported a five-fold increase in the risk of oral cancer associated with low education, compared to higher education, OR=5.3 (95% CI, 3.7-7.6) [66]. Another Indian study reported a three-fold increase in the risk of oral cancer among subjects with an income lower than 5,000 Rupees compared to the ones in the highest salary stratum, OR=2.9 (95% CI 1.9-4.2) [67].

Oral cancer is more common among men than in women. This difference is more pronounced in Europe and the Americas and less so in South Asia [68]. There have been suggestions that differences in the hormonal environment might be one of the reasons for this disparity [69]. Ethnic differences also play a major role with regards to oral cancer. The Non-Hispanic white races in the USA have a far higher rate of oral cancer incidence as compared to the white Hispanic races [70]. Moreover, black males have a higher incidence of oral cancer as compared to white males [40]. The very high incidence of oral cancer in the Indian subcontinent as compared to other parts of the world also suggests a role of ethnicity. Csikar et al. demonstrated a higher incidence of oral cancer among women of South Asian origin as compared to other ethnic groups in England [71]. These differences exist both inter

and intra-countries, and even though most can be explained by diet and habits like smoking and alcohol etc, there are suggestions that genetic factors may be involved [68].

2.6.6 Human Papilloma Virus

The incidence of smoking is decreasing worldwide but the incidence of oral cancers seems to be increasing [72, 73], suggesting the emergence of new risk factors. Human Papilloma Virus (HPV) is a one such risk factor for oral cancer. Mehanna et al., have reported a 32% increase in the prevalence of HPV among oropharyngeal cases when comparing studies conducted prior to the year 2000 and studies carried out after 2000 till 2009 [74]. The increase in the prevalence of HPV infection among oropharyngeal cancers has been more pronounced in Europe and to a lesser extent but still high, in the United States [72]. HPV type 16 and 18 are considered as the main subtypes responsible for oral cancers, moreover, HPV 33 has also been found in cases of oropharyngeal cancers [75]. Evidence from Industrialized countries suggests a causal link between oral cancer and HPV. However, more research is needed in the context of developing countries. Tobacco remains the major risk factor in the developing countries and there are suggestions that prevalence of HPV DNA in cases who were current or former tobacco users are low compared to non-users. [76].

3 SEVERAL SHADES OF SMOKELESS (TOBACCO)

“...tobacco is the only legally available consumer product which kills people when it is used entirely as intended”.

- The Oxford Medical Companion

The term “smokeless tobacco” as the name implies, refers to all forms of tobacco use that do not involve the burning of the product [77]. Smokeless tobacco (SLT) use includes either, or a combination of, chewing, sucking, inhaling, or dipping tobacco. SLT is used as such (leaves) or mixed with other ingredients to make it more palatable, and/or to facilitate a more efficient delivery of the active ingredients to the bloodstream [78]. Biologically, SLT differs from smoking in the mechanism of delivery of the active agent i.e. nicotine. During smoking, nicotine reaches the bloodstream via the smoke that is inhaled through the lungs, while for smokeless tobacco the absorption of nicotine into the blood is usually through the oral or nasal mucosa [79]. Additionally, for some forms of SLT e.g. chewing leaves, the absorption of the active ingredients may also take place through the lining of the other parts of the gastrointestinal tract e.g. the stomach or intestine [80].

Smokeless tobacco use is prevalent all over the world. Recent estimates suggest that more than 300 million people in 70 countries use SLT [81]. The highest prevalence of SLT is in developing countries, particularly in the South and South East Asian countries of India, Pakistan, and Bangladesh [82]. According to the WHO estimates, almost 90% of the SLT burden of the world lies in South Asia [19]. The types of SLT products, however, vary between regions even within a single country. Among industrialized countries, America and Sweden have the highest prevalence of SLT use [83]. The prevalence of SLT use across the globe is provided in figure 3.1 .

3.1 A BRIEF HISTORY OF SMOKELESS TOBACCO

The first documented use of tobacco can be traced back to the Native American Indians during the 15th century [84]. Columbus and other explorers of that time got acquainted with the tobacco use habits of the “New World”, where it was consumed to curb the appetite and

thirst, as well as for medicinal and cosmetic purposes [85]. Tobacco was brought to Europe during the 16th century [86]. Before long, the cultivation of tobacco started in mainland Europe. However, the American colonies remained the chief exporters of tobacco, as it gained more popularity in Europe [87]. Tobacco was introduced in Asia during the British colonial era, where it soon found its way into the centuries-old tradition of betel quid chewing, by becoming an ingredient of the quid [88]. The European colonizers were also responsible for the spread of tobacco to Africa, where it was first introduced in Egypt [86]. Commercial production of the powdered form of tobacco, usually known as snuff, started in the sixteenth century in Spain and by the 17th century, snuff factories had opened in various parts of the British Isles [89]. Snuff quickly became a status symbol, used by the royals and the dandies. By the mid 18th century, snuff factories had also opened in Northern America and Sweden, but the North Americans still preferred chewing tobacco to the more formal European method of snuff use [86, 88].

3.2 SMOKELESS TOBACCO IN SOUTH ASIA

South Asia is the largest consumer of SLT in the world with approximately 250 million people using it in some form [90]. It is estimated that 1.2 million south Asians die every year due to the use of different forms of tobacco [91]. Tobacco was first introduced in south Asia mainly for smoking purposes. The British naval staff started chewing tobacco when smoking got banned from ships to avoid the risk of fire. This prompted the use of tobacco in betel quid. The use of tobacco in quid spread rapidly, and soon cultivation of tobacco started in the Indian subcontinent [92], other forms of SLT e.g. Gutkha, Nass, and Paan masala, quickly followed [93]. In South Asia, SLT has been embraced in various forms, with regional variations, and has become culturally and socially acceptable [94].

Several shades of smokeless (Tobacco)

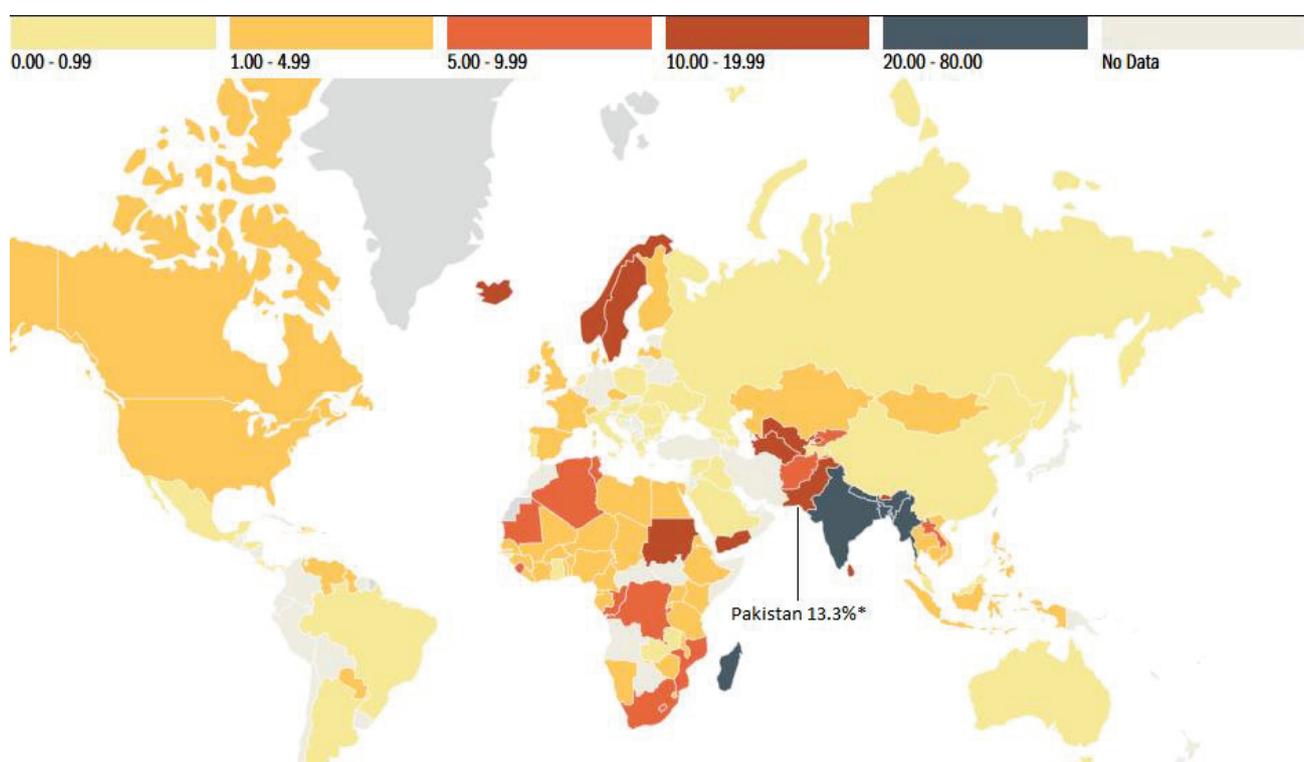


Figure 3.1. Prevalence(%) of smokeless tobacco use in different parts of the world, adapted from the World Tobacco Atlas [12].

3.3 TYPES OF SMOKELESS TOBACCO

The IARC classifies SLT products according to the method of their use i.e. chewing, sucking, other oral uses, and nasal uses. It has identified 28 types of products that are prevalent world wide [77]. The classification of the smokeless tobacco products according to their method of use is presented hereafter.

3.3.1 Chewing Tobacco products

These include Betel quid, Gutka, Iq'mik, Khaini, Khiwam, Loose leaf, Mawa, Plug, Tobacco chewing gum, Twist or roll and Zarda.

3.3.2 Sucking Tobacco products

Chimó, Dry snuff, Gutkha, Khaini, Loose-leaf, Maras, Mishri, Moist snuff, *Naswar*, Plug, Shammah, Snus, Tobacco tablets, Toombak

3.3.3 Other oral products

Creamy stuff, Gudhaku, Gul, Mishri, Red tooth powder, Tuibur

3.3.4 Nasal use products

Dry snuff and Liquid snuff

The distribution of SLT products by the regions of their use are described in Table 3.1.

3.3.5 Types of smokeless tobacco used in Pakistan

According to the 2012 Global Adult Tobacco survey, around 10 million Pakistanis indulge in the habit of SLT use [95]. The most common forms of SLT products used in Pakistan are Paan (Betel quid) with tobacco, Gutkha, and *Naswar* [96]. Using SLT products is a culturally and socially acceptable practice in the Pakistani society [94, 97]. There are marked regional variations in the type of SLT use practices in Pakistan. In the southern parts of the country, the use of paan with tobacco is more common, while in the northern part of Pakistan *Naswar* is the predominant type of SLT form [98, 99]. Following is a brief description of the different SLT products used in Pakistan. Since "*Naswar*" is the focus of this dissertation, it is described in more detail.

Table 3.1. Prevalence of different types of smokeless tobacco products by region.

Region	Type of smokeless tobacco
Americas	<ul style="list-style-type: none"> ➤ Chewing (<i>North America</i>): Loose leaf, Plug-Moist, Plug-chew, Twist or roll ➤ Dry snuff (<i>North America</i>): Iq'mik, Ariva (nicotine lozenges) ➤ Moist snuff (<i>Venezuela</i>): Chimo
Europe	<ul style="list-style-type: none"> ➤ Snus or snuff (<i>Sweden</i>) ➤ Gutkha (<i>United Kingdom</i>) ➤ Dry snuff (<i>United Kingdom</i>)
Asia	<ul style="list-style-type: none"> ➤ <i>Central Asia</i>: Gul, Nass or <i>Naswar</i>, Pan Masala or Betel quid, Zarda ➤ <i>East/Southeast Asia</i>: Gutkha, Pan Masala or Betel quid ➤ <i>South Asia, including Indian subcontinent</i>: Creamy snuff, Gul, Gutkha, Khaini, Mawa, Mishri or Masherri, Misherri, Qiwam or Kima, Red tooth powder, Snus or snuff (<i>Naswar</i>)
Middle East	<ul style="list-style-type: none"> ➤ Nass or <i>Naswar</i>, Niswar, Shammah, Zarda
Africa	<ul style="list-style-type: none"> ➤ <i>Sudan</i>: Toombak

Data source: Smokeless tobacco fact sheet - National Cancer Institute and Center for Disease control, USA [81].

3.3.5.1 Paan

Paan is usually made by vendors or prepared at home, although commercial preparation is also becoming common. To make a Paan, slaked lime and catechu (extract from the acacia tree) are pasted onto a betel leaf. Other ingredients such as tobacco, areca nut, and flavoring agents are then added, and the leaf is folded into a funnel shape to be chewed. Raw, sun dried or roasted tobacco is used in paan, depending on the preference of the customer [100]. According to a nationally representative survey from Pakistan, 7.4% of the study participants regularly chewed Paan with tobacco [96].

3.3.5.2 *Gutkha*

Gutkha usually comes in the form of dry granules that are packaged in an easy to carry plastic packet. In essence, it is the commercially manufactured version of Paan or betel quid with tobacco, but unlike Paan, it is not perishable [101]. One province in Pakistan has banned the sale and consumption of Gutkha due to its health risks. A 2012 study from Pakistan found the prevalence of Gutkha to be 6.4% [96].

3.3.5.3 *Naswar*

Naswar (or *Nass*) is a mixture of sun-dried, partially cured and powdered local tobacco (*Nicotiana Rustica*), ash, oil, flavoring agents (e.g. cardamom, menthol), coloring agents, and slaked lime [102]. It is made locally in a cement lined cavity to which water and lime are added. Thereafter, the tobacco is added, followed by the coloring and flavoring agents [81]. A heavy wooden mallet pounds the ingredients into a mixture, to which water and oil are added for binding [100]. *Naswar* can also be used in an unbounded mixture form. The product is then packed into plastic packets or ornamental boxes, ready to be used. The usage involves the shaping of *Naswar* into a round pellet with fingers, and placing it in the buccal vestibule or under the tongue [81]. It is estimated that more than 7% of Pakistan's population use *Naswar* [96]. The product is particularly more popular in the Khyber Pakhtunkhwa province of the country, where the prevalence of its use is more than 15% [96].

3.4 SMOKELESS, NOT HARMLESS

A variety of diseases have been linked with SLT use, including cancers, potentially malignant conditions, cardiovascular diseases, gastrointestinal disorders, and dental conditions [103-107]. For the purpose of this dissertation, the focus of this section will be on oral cancer.

3.4.1 Biochemical considerations

SLT has been labeled as carcinogenic by the IARC [101]. SLT contains more than 30 carcinogenic agents [108]. These include the non-volatile alkaloid-derived tobacco-specific N-nitrosamines (TSNA), N-nitrosoamino acids, volatile N-nitrosamines, metals, certain volatile aldehydes, urethane, polynuclear aromatic hydrocarbons, lactones, and radioactive

material like polonium-210 and uranium-235 and -238 [109]. Nicotine is the main psychoactive agent in smokeless tobacco and to facilitate its rapid absorption, the manufacturers keep the alkalinity (pH) of SLT products very high [110]. This, in turn, induces the formation of more TSNAs [111].

Nicotine causes dependence [112], and a higher nicotine level means stronger cravings, and a more frequent or prolonged use of the product, leading to a higher exposure to the carcinogenic agents in SLT [81]. Nicotine plays an important role in cancer initiation through the activation of signaling pathways, tumor cell growth, angiogenesis, migration, and invasion [113]. Slaked lime, another constituent of various SLT products, has been shown to have carcinogenic potential. It induces the production of reactive oxygen species (ROS) in the saliva of chewers and facilitates the hydrolysis of arecoline into arecaidine. This enables increased fibroblast proliferation and collagen synthesis, which are essential for premalignant and malignant transformation of the affected tissues [114-116].

Table 3.2 indicates that the *Naswar* used in Pakistan has one of the highest pH among the different types of SLT products used around the world. The *Nicotiana Rustica* species of tobacco, from which *Naswar* is made, has a higher nicotine content than *Nicotiana Tabacum*, which is usually used in cigarettes [81, 117, 118]. A study of 30 brands of *Naswar* from Pakistan revealed a nicotine content of 7.35-26.7 mg/g, of which more than 70% was in free form, making *Naswar* one of the highest free-form nicotine containing SLT product in the world [119]. *Naswar* also has a very high TSNA content compared to the other forms of SLT used in Pakistan. Table 3.2 refers to the comparison of *Naswar* with some other popular SLT products. *Naswar* also contains cadmium, chromium, nickel, arsenic, and beryllium, which are Group I carcinogens, and lead, nitrate, and nitrite, which are Group II carcinogens [120]. The IARC classifies Group I carcinogens as agents/mixture/exposure circumstances that have been shown to be carcinogenic in humans, and Group II carcinogens as those agents/mixture/exposure circumstances that have been proven carcinogenic in experimental animals but evidence for their carcinogenicity in humans is yet, inadequate.

Table 3.2. Biochemical comparison of *Naswar* with other SLT products

Product name	pH	Total nicotine*	Free nicotine*	TSNA
<i>Naswar</i> (Pakistan)	8.76–9.14	10.5–14.2	8.84–13.2	478–1,380
<i>Nasway</i> (Uzbekistan)	8.43	8.89	6.36	1,100
<i>Gutkha</i> (Pakistan)	8.20–8.54	0.16–2.08	0.12–1.08	83.9–1,560
<i>Gutkha</i> (India)	8.46–8.88	1.09–2.33	0.86–1.78	370–2,250
<i>Mainpuri</i> (Pakistan)	7.65	1.28	0.38	219
<i>Mawa</i> (India)	8.31	0.16	0.11	96
<i>Snus</i> (Sweden)	7.9	16.7	7.6	6.0-25.2

*Milligram per gram; TSNA=tobacco-specific nitrosamines; Source: Stanfill et al. 2011 [119].

3.4.2 No smoke, ample evidence

Tobacco smoke is recognized as injurious to health but despite the absence of smoke, SLT products can be equally harmful to the health of the user.

3.4.2.1 *Smokeless tobacco and cancer*

Smokeless tobacco is associated with multiple cancers. Boffetta et al., reported elevated risks of esophageal cancer, mRR=1.6 (95% CI, 1.1-2.3) and pancreatic cancer mRR=1.6 (95% CI, 1.1-2.2) [121], associated with SLT use. Another review of European and North American studies, reported a slight increase in the risk of stomach, mRR=1.33 (95% CI, 1.0–1.7), pancreatic, mRR=1.2, (95% CI, 0.6–2.3), and prostate, mRR=1.2 (95% CI, 1.0–1.4), cancers associated with the use of SLT [106]. A recent meta-analysis of Indian literature stated a significant association between the risk of pharyngeal, mRR=2.6 (95% CI, 2.2-3.1), laryngeal, mRR=2.8 (95% CI, 2.1-3.7), esophageal, mRR=3.1 (95% CI, 2.7-3.6) and stomach, mRR=1.2 (95% CI, 1.0-1.6), cancers and the use of SLT products [17]. There are suggestions in the literature that smokeless tobacco increases the risk of penile cancer and renal cell carcinoma [122].

3.4.2.2 Smokeless tobacco and oral cancer

The majority of SLT products are used via the oral cavity, hence, the oral cavity is at the greatest risk of malignant transformation associated with the use of these products [105]. Based on studies carried across Europe and the Americas, Bofetta et al. reported a pooled RR of 2.6 (95% CI, 1.3-5.2) for oral cancer among SLT users compared to non-users [121]. Stratification by geography, however, limited the risk to studies from the U.S, with the European studies not reflecting an increased risk of oral cancer with the use of SLT. Other reviews state a minimal increase in the risk of oral cancer with the use of SLT products used in the western world [20]. In contrast, a study carried out in Sudan reported a considerable increase in the risk of oral cancer with the use of a local form of SLT product, “Toombak”, OR=11.0 (95% CI, 4.8-25.1) [123]. The difference between the reported risks from various geographical regions may be attributed to the manufacture of relatively safer SLT products, with significantly lower known carcinogen levels e.g. Swedish Snuff [21, 124].

3.4.3 Evidence from South Asia

Four independent systematic reviews of observational studies from South Asia, with varying focus and inclusion criteria, have reported a high risk of oral cancer associated with SLT. Guha et al., found an mRR of 7.7 (95% CI, 5.3-11.1) among the users of Paan, compared to non-users [125]. Gupta et al., reported a pooled OR of 7.4 (95% CI, 5.8-9.5) and an mRR of 5.48 (95% CI, 2.5-11.7) among users of betel quid compared to non-users when combining case-control and cohort studies respectively [18]. Sinha et al., reported an mOR of 5.5 (95% CI, 5.0-6.7) for oral cancer among users of SLT compared to non-users, without specifying the subtype of SLT [17]. Khan et al., reported an mOR of 4.7 (95% CI, 3.1-7.1) among users of SLT products other than betel quid, compared to non-users [115]. The reviews were unanimous in reporting a comparatively higher risk of oral cancer among female SLT users than men, and the existence of an exposure-response relationship between SLT and oral cancer.

3.5 BIO-EPIDEMIOLOGICAL MODEL OF *NASWAR* INDUCED CARCINOGENESIS

Figure 3.2 refers to a proposed bio-epidemiological model of carcinogenesis induced by *Naswar* use. This model is adapted from the smoking-induced lung carcinogenesis model proposed by Hecht [126]. The process begins with the initiation of *Naswar* use followed by nicotine addiction, leading to the sustained use of the *Naswar*. Carcinogens present in *Naswar* are absorbed into the blood and processed by the body, resulting in the metabolic activation of TSNAs and the subsequent formation of DNA adducts. This is followed by genetic mutations, which may ultimately lead to tumor formation. At the same time, co-risk factors, some of which work through the same biological mechanism as *Naswar*, exert their influence, resulting in either hastening or retarding the process of carcinogenesis.

During the metabolic activation stage, cytochrome P450 enzymes activate the TSNAs [127]. The activated TSNAs induce primary lesions in the DNA, which usually include nucleotide methylations and pyridyloxo-butylations [128]. When DNA adducts persist unrepaired, permanent DNA mutations, such as in the RAS oncogene or the TP53 tumor suppressor gene, can occur. This may result in uncontrolled cell growth and cancer [129]. Other contributory mechanisms to tumor promotion and co-carcinogenesis include chronic local inflammation and irritation, oxidative stress, and Reactive Oxygen Species [130].

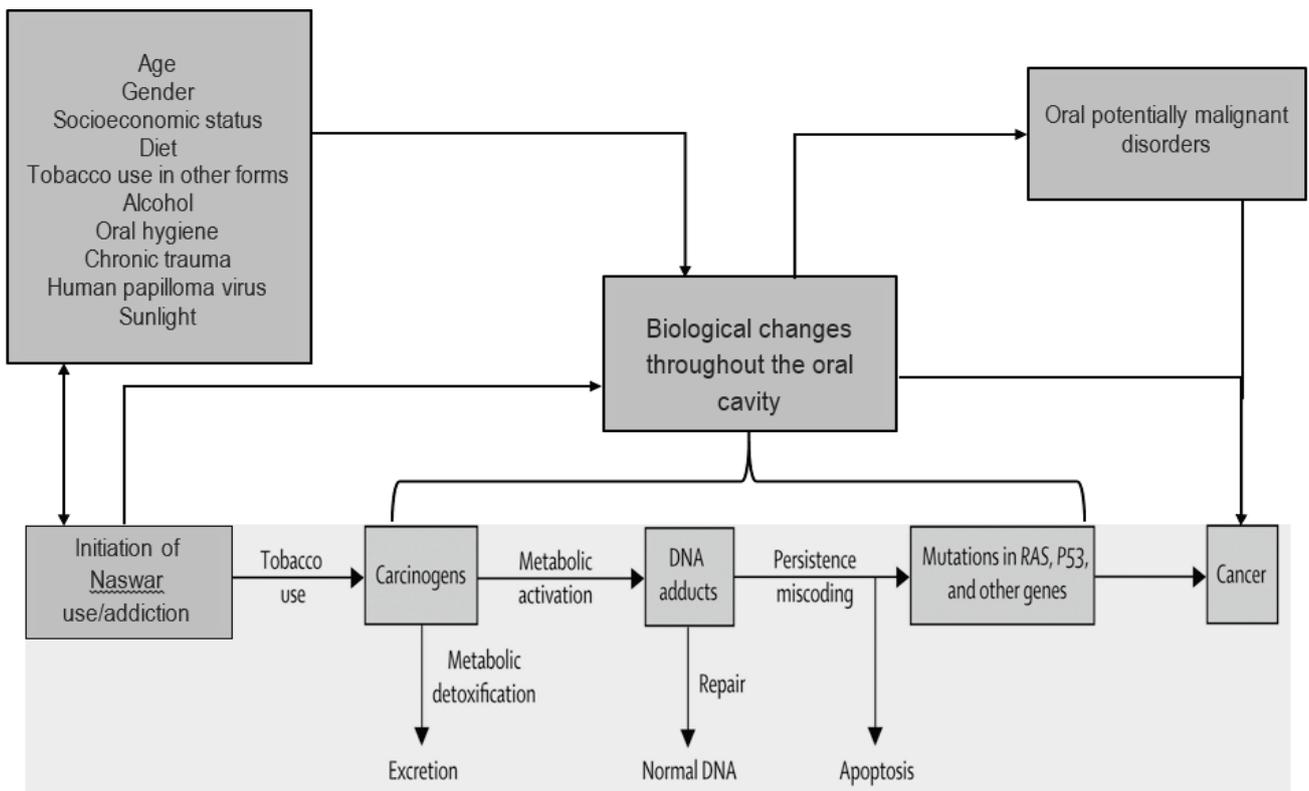


Figure 3.2. Bio-epidemiological model of carcinogenesis associated with *Naswar*. Adapted from Hecht, 2003 [126]

4 CONCEPTUAL FRAMEWORK: AT THE INTERSECTION OF CAUSALITY AND CHRONIC DISEASE PREVENTION.

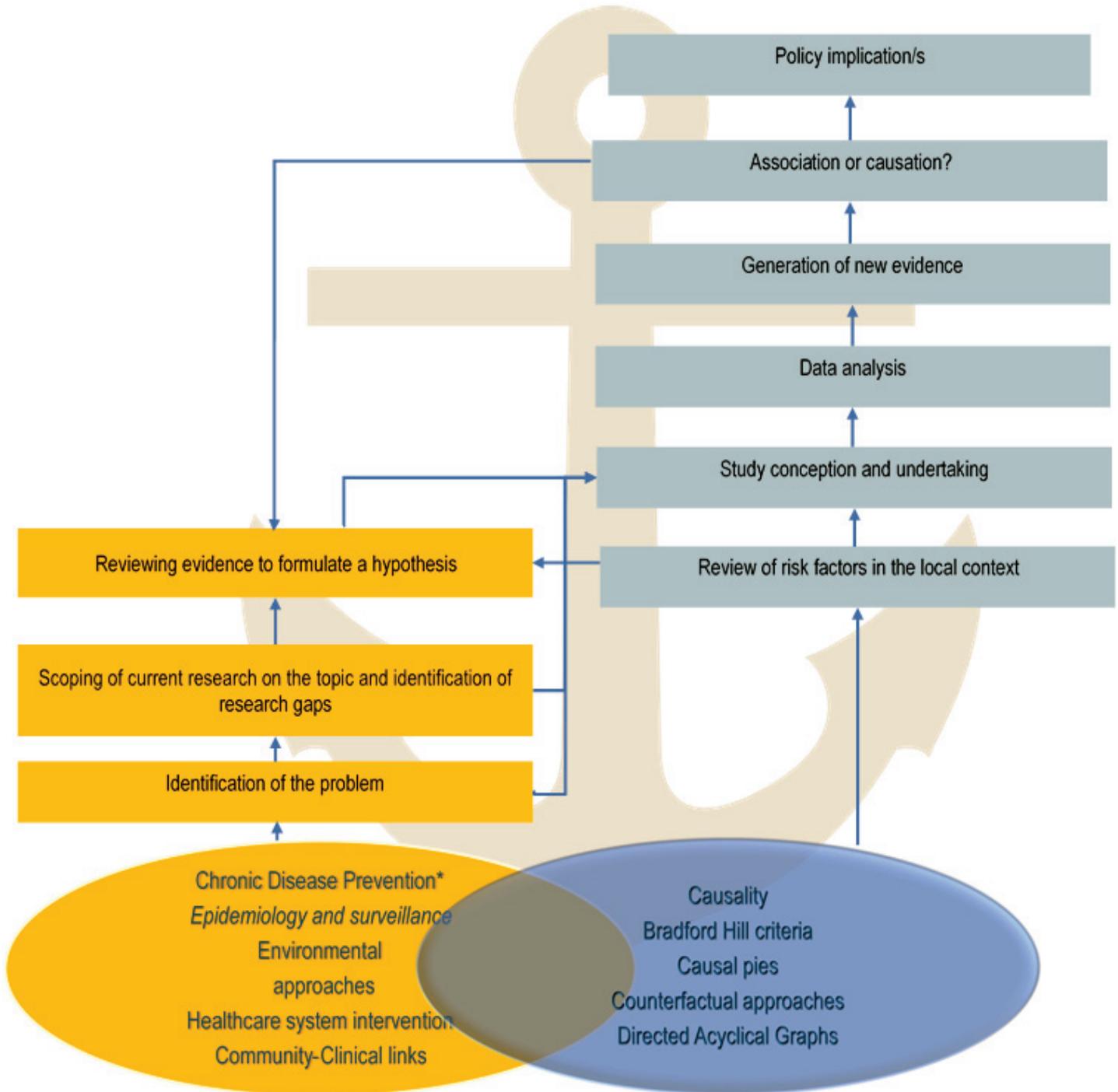
“We may never understand illnesses such as cancer. In fact, we may never cure it. But an ounce of prevention is worth more than a million pounds of cure”

- David Agus

This doctoral research is anchored in two separate but often over-arching public health concepts/domains of chronic disease prevention and causality (Fig 4.1). This chapter will focus on the different models of chronic disease prevention, and explore the classical and contemporary aspects of causality. This will be followed by a brief description of how these concepts have guided the rationale and operationalization of the research carried out in the context of this body of work.

4.1 CHRONIC DISEASE PREVENTION

Chronic or non-communicable diseases (NCDs) are the largest cause of death globally [131, 132]. According to the WHO, the expected number of deaths attributable to NCDs will rise from 30.8 million in 2015 to 41.8 million by 2030 [133]. Cancers constitute a major part of NCDs, with recent reports suggesting more than 14 million new cases diagnosed each year [134, 135]. Low and middle-income countries (LMIC) suffer from a double burden of disease i.e. both communicable and NCDs [136]. In 2012, more than three-quarters of the global NCD burden was contributed by the LMIC [137]. In Pakistan, NCDs account for more than 25% of all deaths annually [138]. Cancer alone is responsible for more than 100,000 annual deaths in the country [7].



*The four domains of chronic disease prevention-Center for Disease Control, Atlanta, USA [B9].

Figure 4.1. The conceptual flow diagram for this dissertation

The rapidly increasing global incidence of NCDs has become a major challenge for most countries [140]. In 2011, the member states of the United Nations agreed to adopt an integrated approach to combat and prevent chronic disease [141]. Research plays an important role in chronic disease prevention [142]. To tackle public health problems effectively, practice and policy should be based on sound research evidence [143]. Although LMICs suffer from the highest burden of NCDs, there is a scarcity of NCD research evidence generated from these countries [144]. The importance of NCD research has been emphasized upon in a number of key NCD control documents, relative to Pakistan. Table 4.1 refers to some of these key documents and how the issue of research has been addressed in each one of these.

From the review of the key documents and the cancer prevention models reported in the literature [145-152], it is evident that research on the risk factors for cancer and other NCDs is imperative to the prevention and control of these diseases. This theoretical construct served as a rationale for carrying out epidemiological research on oral cancer in Pakistan, commencing with a scoping review of oral cancer research output, and culminating in the analysis of the cumulative evidence of the risk of oral cancer associated with *Naswar*. The scoping review [13], identified research gaps, and potential areas for future research, while the systematic reviews [19, 153], established SLT as a major player in the etiology of oral cancer in South Asia. The reviews also identified some potential future SLT research areas e.g. *Naswar* use, which has not been researched extensively.

4.2 CAUSALITY, CAUSAL INFERENCE, AND EPIDEMIOLOGY

“de nihilo nihil” (nothing can be born of nothing)

– *Lucretius*

In epidemiology, a cause can operationally be defined as a factor that alters the occurrence of a disease [154]. Causality has always been a major point of debate in both science and philosophy [155, 156]. Epidemiological studies typically focus on the examination of associations between an outcome and an exposure/intervention. This association, though, may not be termed as a causal one because of the potential presence of alternative explanations e.g. i. It could be a random variation or play of chance, ii. The outcome may be

Table 4.1. Importance of research in the prevention and control of NCDs and oral cancer

Organization	Name of the document	Domain	Context	Relevant section	Recommendation/s providing a conceptual basis for this dissertation.
WHO	Global action plan for prevention and control of non-communicable disease (2013-2020)	NCDs control & prevention	Global	Objectives # 5 and # 6.	i. To promote and support national capacity for high-quality research and development for the prevention and control of non-communicable diseases. ii. To monitor the trends and determinants of non-communicable diseases and evaluate progress in their prevention and control.
WHO (EMRO)*	Plan of action for the prevention and control of non-communicable diseases in the Eastern Mediterranean Region.	NCDs control & prevention	Regional including Pakistan.	Objectives # 4 and # 6.	i. Promote research for the prevention and control of non-communicable diseases. ii. Monitor non-communicable diseases and their determinants and evaluate progress at the national, regional and global levels.
WHO (Pakistan)**	National action plan for NCD control	NCD control & prevention	Pakistan	Section 2.2.3	Research should focus on identifying causal associations for risk factors that have implications for setting targets for preventive interventions.
WHO and partners***	“Crete Declaration on oral cancer prevention 2005-a commitment to action”	Oral cancer control & prevention	Global	Section a and b.	i. Provision of systematic epidemiological information on the prevalence of oral cancer and cancer risks in countries, particularly in the developing world. ii. Promotion of research into understanding biological, behavioral and psychosocial factors in oral cancer, emphasizing the interrelationship between oral health and general health.

*WHO office of the Eastern Mediterranean Region, **WHO country office Pakistan, ***Hellenic Cancer Society, International Congress on Oral Cancer, Hellenic Association for the Treatment of Maxillofacial Cancer.

a cause of the exposure (reverse causality), iii) It could be a result of the systematic error, iv. The chronic diseases usually have a multi-factorial, as opposed to a single factor etiology, so there might be confounding. Until all these alternative explanations have been ruled out, an association cannot be deemed as causation [157, 158]. To assess the causal nature of the association between two or more variables, epidemiologists have used various models grounded in philosophy, epidemiology, and even computer science [159]. Models of causality like the Miasma theory, the Germ theory, and Robert Koch's postulates have played a significant role in reducing the burden of communicable disease [160]. However, with the coming to fore of the NCDs in the post second world war era, these models were considered inadequate to answer the multi-factorial etiology of chronic disease [159]. In 1965, Sir Austin Bradford Hill came up with nine different criteria [161], which have ever since been used extensively in epidemiology to assess associations for causality [162]. Rothman in 1976 proposed another model, popularly known as "Causal Pies", which deem multiple sufficient causes, each made up of several component causes, to be responsible for disease causation. He suggested that each sufficient cause acts like a pie made of the component causes and when these component causes join to complete the pie; the outcome is a disease [163]. More recently, models embedded in counterfactual thinking have been proposed to assess causality in epidemiology. The scientific ground for the counterfactual causality is based on the difference between outcomes in the presence of one set of conditions, and the presence of an alternative set of conditions i.e. What would be the outcome if the conditions were altered from the actual conditions that were observed ?. These models have an inclination towards the process of scientific inquiry by experimentation [164, 165]. Causal diagrams or "Directed Acyclical Graphs" are rooted in artificial intelligence and are becoming increasingly popular among epidemiologists [166]. These diagrams describe causal pathways based on uni-directional relationships between different measured and unmeasured study variables [167, 168]. Robins [169-172], and Greenland [173], introduced causal diagrams in epidemiology, providing a simple method to visually assess epidemiological associations between outcomes and predictor variables [174]. These diagrams aid researchers in data collection by identifying potential confounders that have to be conditioned during the

analysis. Moreover, they also identify variables, whose adjustment could potentially introduce bias, where none previously existed [175].

Although randomized experiments accompanied by causal diagrams, provide a robust method to assess causal associations, it is not always possible to conduct experimental studies in humans, for practical and ethical reasons [176]. Hence, well-designed observational studies still play a major role in the assessment of causality. There have been recent calls for a more pluralistic approach to determine causality, rather than following just one model, as the main aim of all the epidemiological models is to prevent disease [177].

4.3 CAUSALITY IN CANCER EPIDEMIOLOGY

Cancer risk assessment can sometimes be a controversial issue due to the inherent limitations of observational studies, which are by far the most used designs in cancer epidemiology [154, 178]. Nonetheless, evidence from these studies remains as one of the primary influencers of cancer control policy and practice [176, 179]. The International Agency for Research on Cancer (IARC) assesses the carcinogenicity of various agents in humans [180]. The evidence for this assessment comes from epidemiological studies in human beings, laboratory studies on animals, and mechanistic considerations [176]. Owing to the difficulties of carrying out randomized studies in humans [172], epidemiological evidence for establishing a causal association between a putative risk factor and cancer, usually comes from observational studies [181, 182]. Even though experimental studies can be, and are carried out on animals, to study associations between risk factors and cancer, observational studies of humans are often considered superior to the animal studies. The argument being that sound conclusions about normal or pathological phenomenon in humans can only be made by studying humans [183, 184].

Cohort and case-control designs are predominantly used in cancer epidemiology to assess associations. These studies are prone to the issues of bias and confounding [185], particularly in the case of case-control designs [186, 187]. Despite these issues, case-control studies are usually the first design choice in etiological research owing to their speed and efficiency and are especially indispensable in research of a rare disease like cancer [188]. In general, a

careful design, stringent quality control, thorough exposure assessment, and a sound analytical approach can all help in minimizing bias and confounding in case-control studies [185]. More specifically, an explicit definition of the selection criteria, and case ascertainment, full or at least partial blinding of the investigators to the case/control status of study participants/ study hypothesis, avoiding differential exposure assessment, and addressing confounding during study design or analysis are some of the measures that can be taken to increase confidence in the results of case-control studies [186, 187, 189]. In the absence of the evidence of confounding and bias, etiological inferences can be made from the results of adequately powered observational studies that address specific hypothesis [176]. Criteria such as the strength of association and its consistency, a dose-response relationship, and most importantly the evidence of temporality, can strengthen the argument for causality [186].

The aforementioned concepts of “causality” in cancer epidemiology have guided the various stages e.g. development of the study questionnaire, recruitment of the study subjects, data collection, and analysis (Fig 3.2), of a case-control study, carried out in the context of this body of work. Data collection methods, regarding the correct quantification and assessment of exposure at increasing levels of its duration, frequency, and intensity, were intended to establish the strength, temporality, and an exposure-response relationship, for a causal inference. Efforts to minimize bias and confounding in the study included recruitment of study participants from a single base population, partial blinding of interviewers and interviewees to the case/control status, and the use of causal diagrams.

Conceptual framework

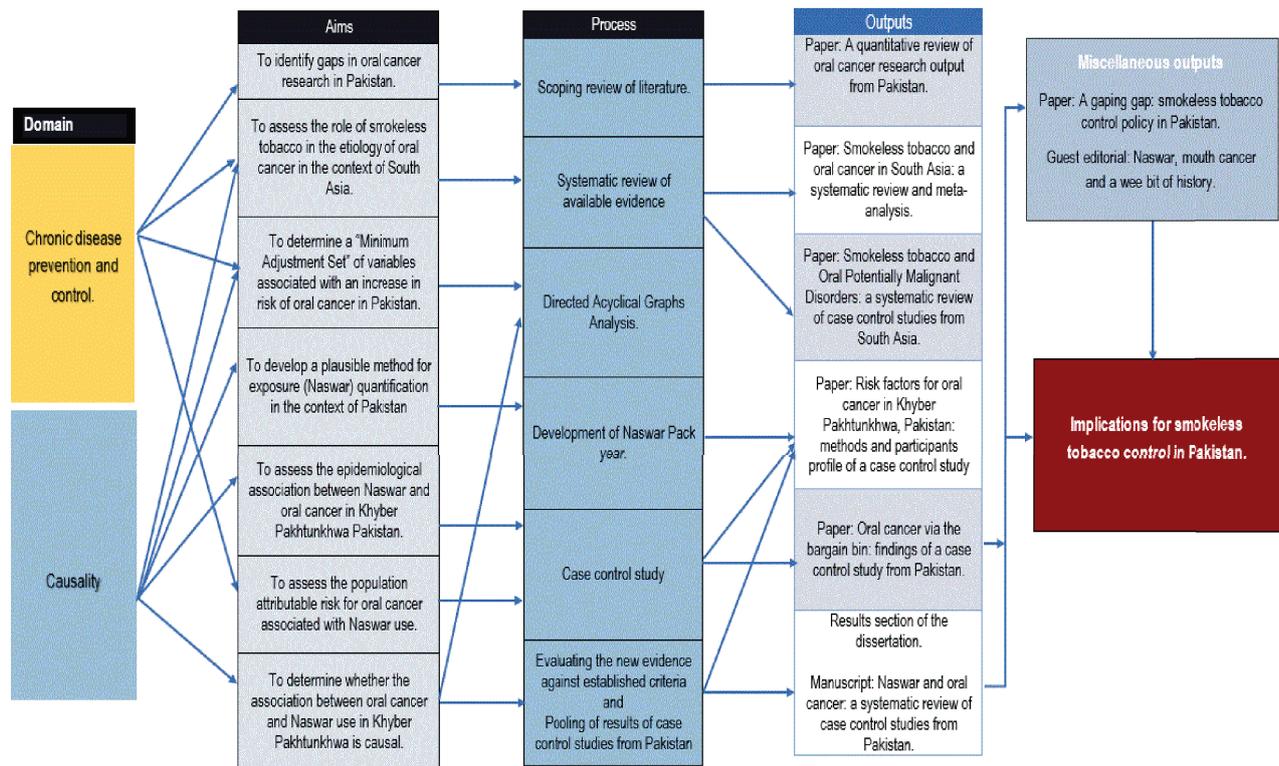


Figure 4.2. The conceptual framework for this dissertation

5 METHODS

“To put everything in balance is good; to put everything in harmony is better.”

- Victor Hugo

This chapter provides a description of the research methods that were used to address the primary and secondary objectives of this body of work. The methods of the “Oral Cancer epidemiology in Khyber Pakhtunkhwa, Pakistan” (OraCEP), study (Article V and VI), are described in detail. The methods used to address the other objectives of this dissertation (Articles I-IV and VI-VIII) are described briefly as the detailed description of these, is given in the corresponding manuscripts. (See annexure).

5.1 SPECIFIC OBJECTIVE I - *NASWAR* USE AND THE RISK OF ORAL CANCER IN KHYBER PAKHTUNKHWA, PAKISTAN- (ARTICLE V)

5.1.1 Study design, setting, and participants.

To assess the association between *Naswar* use and the risk of oral cancer in the Khyber Pakhtunkhwa province of Pakistan, we conducted a matched case-control study from September 2014 until May 2015, in two major cities of the province. Peshawar is the capital city of the province, while Abbottabad is a comparatively smaller city, serving as an educational hub for the whole of Pakistan. The Khyber Pakhtunkhwa province has an area of 74,521 km² and a total population of 17.5 million. The population of Peshawar is 3,575,000, while Abbottabad has 118,200 inhabitants. The majority of the population lives in rural areas, and agriculture and trade are the main earning resources [190]. A detailed description of the study centers is provided in “Article V”.

Since primary and secondary healthcare facilities in the province do not have adequate means to diagnose and/or manage oral cancer patients, the included study centers are mainly responsible for the provision of both diagnostic and curative services for oral cancer. Moreover, the services provided by the study centers are not limited to oral cancer or dentistry, these centers are a part of large multi-specialty tertiary care hospitals. The catchment area of the study centers comprises the whole province, as well as the federally

administered tribal areas (FATA) of Pakistan. All study centers were selected based on expert opinion of local cancer physicians and dentists.

A case was a person attending any of the study centers, who was clinically and histologically diagnosed with oral cancer, within the study period. For the purpose of this study, “oral cancer” was defined as, “squamous cell carcinoma of the buccal mucosa, lip, tongue and the oropharynx”. The ICD-10 classification was used to designate oral cancer sites to be included in the study. The eligible sites included lip, the base of tongue, other and unspecified parts of the tongue, gum, floor of mouth, palate, other and unspecified parts of the mouth, tonsil, and oropharynx (C00 - C06, C09 - C10 and C14).

The recruitment of controls was guided by the principles of causal inference in observational epidemiology, i.e. (a.) the cases and controls should come from the same base population, that is to say, if a control in the study would have acquired the disease of interest, he/she would probably attend the same hospital as the cases, (b.) controls should be recruited irrespective of their exposure status [162]. The basic goal is to recruit subjects who are representative of the source population [191]. For the purpose of this study, a control was defined as “a subject attending any of the study centers during the study period, and having any disease with the exception of cancer, pulmonary disease, cardiovascular disease, gastrointestinal disease, and periodontal disease”. Given the breadth of conditions that are treated at the selected study centers, we excluded certain diagnoses, because they are related to different forms of tobacco use and might have produced biased results [192, 193]. It has been suggested in the literature that hospital controls being treated for conditions positively or negatively associated with the exposure should be excluded [194]. Detailed Eligibility criteria for both cases and controls are reported in “Article V”. We employed a frequency-matching scheme based on variables, which have an established confounding role in the etiology of oral cancer, i.e. age and sex [195, 196]. Two matched controls were recruited per case from the outpatient and in-patient departments of the study centers.

5.1.2 Sample size and recruitment of participants

We calculated the minimum number of cases and controls to satisfy a power of 90% with a two-sided confidence interval of 95%, using the previous research findings on the

prevalence of *Naswar* use (exposure of interest) in oral cancer cases, and the general population of Khyber Pakhtunkhwa [96, 197-202]. Two sample sizes based on the case to control ratio of 1:1 and 1:2 were calculated, with the prevalence of exposure in cases set to 35%, and that in controls set to 15%. The Fleiss method, with continuity correction factor [203], was used to calculate the sample size with the “StatCalc” function of Epi Info 7 [204]. The estimated sample size of our study was 107 cases and 107 controls for a 1:1 case/control ratio or 78 cases and 156 controls for a 1:2 case/control ratio. The study started with a 1:1 case to control ratio, but in December 2014, Peshawar was subjected to a deadly terrorist attack that resulted in the death of more than 130 schoolchildren [205]. A state of emergency was declared in the province, with a very tight security situation in the following months. The strict measures taken by the armed forces as a part of an anti-terrorism operation led to a decrease in patient in-flow at most hospitals in Peshawar city, as both inter- and intra-city movement came to a halt. This decrease in patient in-flow hampered recruitment of cases in Peshawar, making it difficult to recruit the desired number of 107 cases for the study, within the stipulated time. Therefore, in February 2015, we decided to recruit two controls per case in order to be able to achieve the desired power for the study.

5.1.3 Ethical approval

Ethical approval for the study was granted by the ethical review board of Khyber Medical University, and by the ethical review committee of Khyber College of Dentistry (Appendix XI). Written consent on a printed form was taken from each study participant before recruitment (Appendix XII). All study participants had the option to withdraw their consent at any stage of the study if they did not want to continue as a part of the study. To ensure maximum participation, histology charges for cases were paid from the study funds. These charges are normally paid out of pocket, by the patients.

5.1.4 Data collection

The data collection for this study was guided by apriori “DAG” analysis to identify important study variables for which data had to be collected. The main outcome variable of our study was dichotomous, i.e. absence or presence of oral cancer. The predictor variables, as

identified by the DAG analysis i.e. The Minimum Adjustment Set (MAS), included *Naswar* use, tobacco smoking, alcohol use, socioeconomic status (SES), age, and sex.

The data collection tool for the study was adapted from the “Alcohol-related cancers and genetic susceptibility in Europe (ARCAGE)” study [1]. Additionally, questions from other previously validated questionnaires on sunlight exposure and SES [206, 207], were also incorporated in the study questionnaire (Appendix X). We conducted face-to-face interviews with the study subjects, abstracted data from their medical records and laboratory results, collected *Naswar* samples from the market, and *Naswar* pellet samples from the study subjects. We also collected biological samples for the detection of Human Papilloma Virus. The collection of blood, saliva, and the resection of tumor tissue for the biopsy was carried out by trained doctors and phlebotomists, as per the study hospital protocols. A detailed description of the study questionnaire, data collection, and variables is provided in “Articles V and VI”.

5.1.5 Statistical Methods

The data was double entered in Epi Info 7 [201], and then transferred to SAS 9.3. Cary, NC: SAS Institute. Inc. [208] for analysis. Random frequency checks, identification of any missing or incomplete data, and outlier detection was carried out to clean the data. Due to the quality assurance measures observed during data collection, the data set was virtually devoid of any missing data. Descriptive analysis of the data was carried out in Epi Info 7, while more complex statistical analyses were carried out in SAS 9.3. Frequency distribution of the variables in the MAS, as well as socio-demographic variables was computed. We carried out both univariate and multivariate analysis to assess the association of the different risk factors in the MAS, with oral cancer. For the multivariate analysis, we conducted a conditional (conditioned for age and sex) logistic regression analysis using the “PROC LOGISTIC” function in SAS 9.3. We also conducted a simple logistic regression analysis, to compare its results with the conditional model, as there have been recent suggestions that an unconditional logistic regression analysis can occasionally be more efficient for the analysis of some matched case-control studies [209]. The outcome was defined as the presence or absence of oral cancer, and age, sex, any tobacco use, alcohol use, and

socioeconomic status were set as the predictor variables in the model. Proxy variables were used to categorize continuous variables, e.g. socioeconomic status, and *Naswar* pack-year. The main analysis included estimation of odds ratio (OR) for “Ever-users” compared to “Never-users”, of *Naswar*, other tobacco, and alcohol. ORs were also calculated for the exposure-response relationship between *Naswar* and oral cancer. Cumulative exposure to *Naswar* (*Naswar* pack-years) was used to assess this relationship. Both crude and adjusted odds ratios with their 95% confidence intervals were calculated. We also carried out a stratified analysis by sex using simple logistic regression. A more detailed description of the data analysis is provided in articles V and VI.

5.2 SPECIFIC OBJECTIVE II - ORAL CANCER RESEARCH IN PAKISTAN (ARTICLE I)

An electronic search in “Medline” via “PubMed”, “Science Citation Index” via “Web of Science” and “PakMedinet” databases, supplemented by a google search, was carried out in January and February 2014. Publications were included in the review based on preset criteria. Data from the included articles were recorded and analyzed in Microsoft Excel. The analysis included estimation of yearly, as well as total research output in terms of publications. We computed the frequency of publications based on research study types, exposures, and outcomes, publication in indexed compared to non-indexed journals. We also performed a stratified analysis based on the research institute of the first author of the included studies, and by geographical region in Pakistan.

5.3 SPECIFIC OBJECTIVE III – SMOKELESS TOBACCO AND ORAL CANCER IN SOUTH ASIA (ARTICLE II)

An electronic search was carried out in “Medline” via “PubMed” and “Science Citation Index” via “Web of Science”, in August 2013 using a combination of MeSH terms. This search was supplemented by a google search. Articles were included/excluded in the review using pre-specified inclusion/exclusion criteria. The included articles were assessed for their quality using the “Effective Public Health Practice Project’s Quality Assessment Tool for Quantitative Studies” [210]. Data from the included articles were abstracted on a pre-designed spreadsheet and later transferred the Cochrane RevMan 5.0 software [211], for

analysis. We pooled the log risk estimates from the included studies to calculate a summary risk estimate, using the “inverse variance” method through a random effects meta-analysis. We also carried out a subgroup, and sensitivity analyses, to determine the effects of individual studies on the summary effect. Heterogeneity was quantified using the I^2 statistic. Publication bias was visually assessed via funnel plots.

5.4 SPECIFIC OBJECTIVE IV - SMOKELESS TOBACCO AND ORAL POTENTIALLY MALIGNANT DISORDERS IN SOUTH ASIA (ARTICLE III AND IV)

Article III reports the protocol of a systematic review. The protocol was guided by and was in accordance with, the “Preferred Reporting Items for Systematic Reviews and Meta-Analysis - Protocol” (PRISMA-P) guidelines [212]. In Article IV, a systematic review and meta-analysis of the association of SLT with Oral Potentially Malignant Disorders (OPMDs) is reported. We undertook a systematic literature search, using MeSH terms and keywords in Medline via PubMed, the Science Citation Index (SCI) via Web of Science, Scopus, and CINAHL databases. Articles were excluded from or included in the review based on preset inclusion/exclusion criteria. The quality of the included studies was assessed by the “Effective Public Health Practice Project’s Quality Assessment Tool for Quantitative Studies” [210]. Data were abstracted and recorded on a spreadsheet. Effect estimates were calculated for the studies that had not explicitly reported them but had sufficient data to calculate them. Statistical analyses were carried out in Rev Man 5.3. Log effect estimates were combined using the inverse variance method in a random effects model, to obtain a meta-odds ratio (mOR). Subgroup analyses included estimation of an mOR for: (1) the risk of developing individual sub-type of OPMD associated with the use of any SLT; (2) the risk of developing any OPMD with the use of different subtypes of SLT; (3) the risk of developing OPMDs based on intensity and duration of SLT use; and (4) the risk of developing OPMDs between male and female SLT users. Country-specific estimates (Sri Lanka and India) were also calculated. We conducted sensitivity analyses to assess the causes of heterogeneity. Publication bias was assessed through the visual inspection of funnel plots.

5.5 SPECIFIC OBJECTIVE V - CUMULATIVE EVIDENCE OF THE ASSOCIATION OF *NASWAR* USE AND ORAL CANCER IN PAKISTAN (ARTICLE VIII)

We conducted a systematic electronic search in “Medline” via “PubMed”, “Science Citation Index” via “Web of Science”, and “PakMedinet” using a combination of keywords and MeSH terms. Selection of articles was carried out according to a pre-set criteria. Data were abstracted from the included articles on a pre-designed spreadsheet. Adjusted odds ratios with their 95% confidence intervals were recorded. When these were not available, crude odds ratio were recorded or calculated from the given data. If possible, a stratified effect estimate (OR_{MH}), using the Mantel-Haenszel method [213] was calculated. A pooled analysis was carried out using both random and fixed effects model in STATA. Heterogeneity was assessed by using the I^2 statistic and publication bias was assessed by a visual inspection of funnel plots. The Population attributable fractions of oral cancer with the use of *Naswar* were calculated for Pakistan as a whole and for the constituent provinces.

5.6 SPECIFIC OBJECTIVE VI – GAPS IN SMOKELESS TOBACCO CONTROL POLICY IN PAKISTAN (ARTICLE VI)

A systematic electronic search of documents pertaining to the Government of Pakistan’s tobacco control policies and strategies was carried out using keywords. The included documents were appraised for the presence or absence of laws, rulings, state orders, and statutes, regarding smokeless tobacco control in Pakistan. Data from the documents were recorded on a spreadsheet and the findings were presented in a research communication/short review.

5.7 SPECIFIC OBJECTIVE VII – POLICY RECOMMENDATIONS (ARTICLE VII)

Manuscript VII is a guest editorial on the use of *Naswar* in the Khyber Pakhtunkhwa Province of Pakistan. It reviews the evidence gathered from the other manuscripts in this dissertation and presents a case for policy revision.

6 KEY RESULTS

“I pass with relief from the tossing sea of Cause and Theory to the firm ground of Result and Fact.”

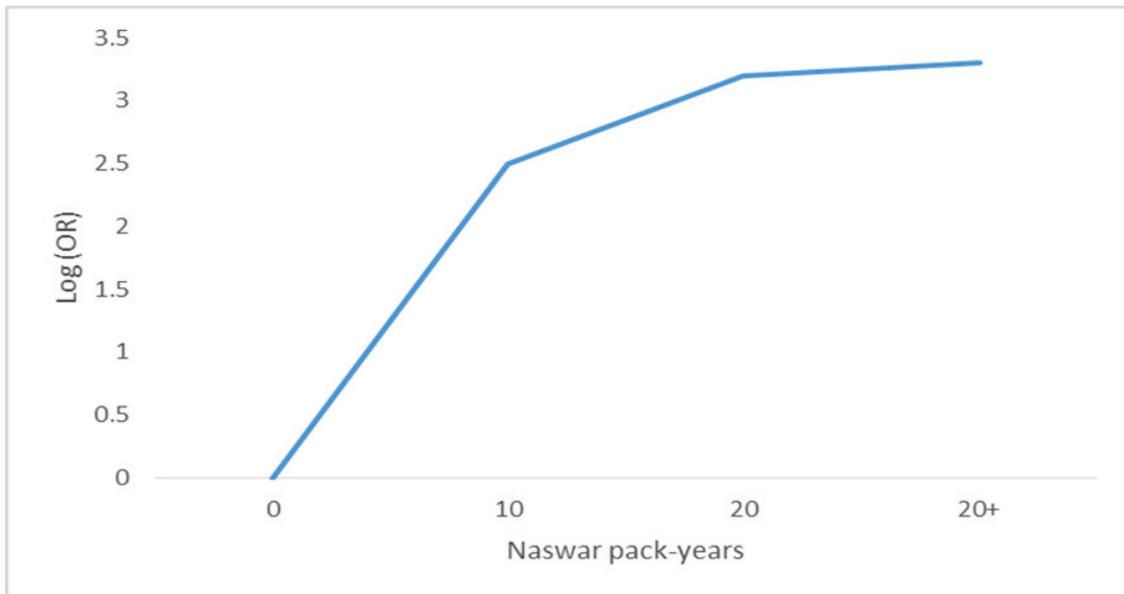
- **Sir Winston S Churchill** on facing an uprising, in what is the current Khyber Pakhtunkhwa province. ‘The Story of the Malakand Field Force’, 1897.

This chapter summarizes the key results pertaining to the objectives that guided this body of work. These results have been described in more detail in the related manuscripts (see appendices I - VIII).

6.1 RISK OF ORAL CANCER ASSOCIATED WITH *NASWAR* USE IN KHYBER PAKHTUNKHWA, PAKISTAN. (ARTICLE V)

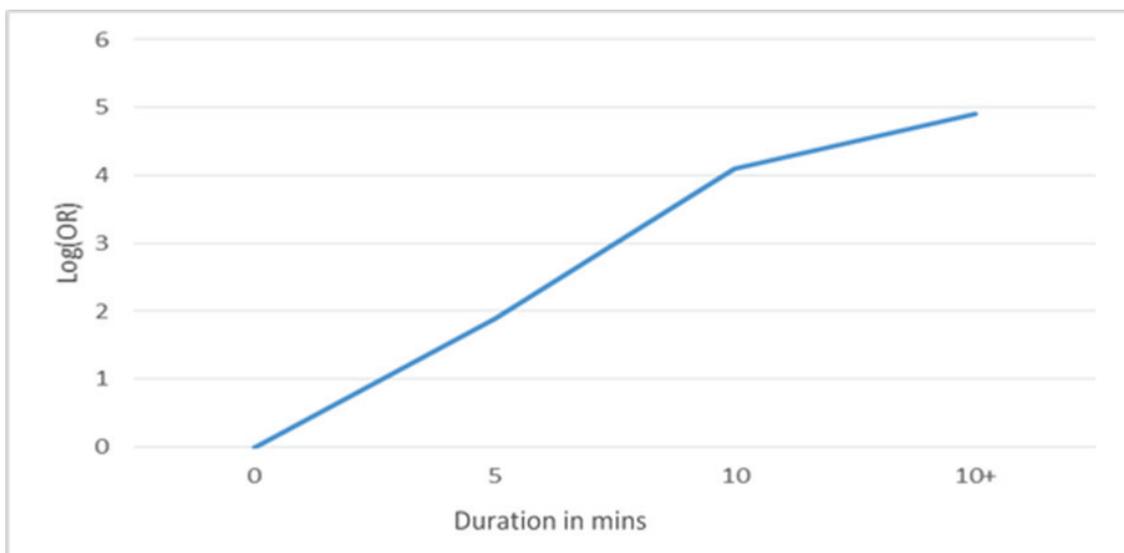
We found a strong association between the use of *Naswar* and oral cancer in the Khyber Pakhtunkhwa province of Pakistan. “Ever-users” of *Naswar* had a more than 20-fold higher risk of oral cancer compared to “Never-users”, OR=21.2 (95% CI, 8.4-53.8). Compared to “Never-users”, the risk was highest for “Current-users” of *Naswar*, OR=27.4 (95% CI, 10.0-74.7). “Past-users” had a 14-fold higher risk of developing oral cancer compared to “Never-users”, OR=14.3 (95% CI, 4.9-41.2). The risk of oral cancer for “Ever-users” compared to “Never-users” of *Naswar* was higher among women, OR=29.0 (95% CI, 5.4-153.9), compared to men, OR=21.0 (95% CI, 6.1-72.1) There was no difference in the risk of oral cancer incidence between the “green” and “black” varieties of *Naswar*. Spitting the saliva after *Naswar* use was associated with a lower risk for oral cancer compared to swallowing saliva, OR=0.4 (95% CI, 0.1-1.4). Figures 6.1 and 6.2 describe the exposure-response relationship between *Naswar* use and oral cancer among both sexes. More than 72% of the primary tumors originated either from the gingiva (Alveolus) or from the buccal mucosa, which are the primary anatomical sites coming in direct contact with a *Naswar* pellet. Tobacco smoking was also significantly related to oral cancer with “Ever-smokers” having a two fold elevated risk of oral cancer compared to “Never-smokers”, O.R=2.2 (95% CI, 1.4-4.9).

Alcohol (p-value=0.18) and socioeconomic status (p-value=0.36) were not significantly associated with oral cancer in Khyber Pakhtunkhwa, Pakistan.



Y-axis: Natural log of Odds Ratio, X-axis: *Naswar* pack-years

Figure 6.1. Relationship between the cumulative exposure to *Naswar* (*Naswar* pack-years) and the risk of oral cancer in Khyber Pakhtunkhwa, Pakistan



Y-axis: Natural log of Odds Ratio, X-axis: Intensity of *Naswar* use (duration of single use in minutes)

Figure 6.2. The relationship between the intensity of *Naswar* use and the risk of oral cancer in Khyber Pakhtunkhwa, Pakistan.

6.2 ORAL CANCER RESEARCH IN PAKISTAN (ARTICLE I)

166 publications were included in the review. Important characteristics of the included studies along with their references are given in the Supplementary table 1 (Annexure). 13% (n=22) of the publications were published before the year 2000, 38% (n=64) were published between 2000 and 2009, while 48% (n=80) were published from 2010 onwards. The majority of the studies were descriptive and case series was the most common study type (36%). There were only six epidemiological case-control studies to assess the association between lifestyle risk factors and oral cancer in Pakistan and none of them was carried out in the Khyber Pakhtunkhwa province.

6.3 SMOKELESS TOBACCO AND ORAL CANCER (ARTICLE II)

6.3.1 Chewing tobacco

15 publications were included in this meta-analysis. Studies adjusting for alcohol and smoking, when combined, provided a pooled OR of 4.3 (95% CI, 3.1-5.8). The pooled OR from combining only case-control studies was 5.4 (95% CI, 4.1-7.1). Cohort studies, when combined, provided a pooled OR of 2.9 (95% CI, 1.1-8.3). Studies carried out in men only when combined provided a pooled OR of 4.0 (95% CI, 2.9-5.7). The risk among women (2 studies) was in the range of 6.4 (95% CI, 3.3-9.0) to 25.3 (95% CI, 11.2-57.3). Studies that had adjusted for smoking and alcohol use, reported ORs varying from 2.0 (95% CI, 1.0-3.8), for chewing tobacco or chewable products containing tobacco for less than 5 times a day, to 13.9 (95% CI, 7.1-27.2), for more than 10 times a day, compared to non-chewers.

6.3.2 Betel quid

Nine studies were included in the meta-analysis of the risk of oral cancer associated with Betel-quid use. Studies that adjusted for alcohol and/or smoking, when pooled, provided an mOR of 6.3 (95% CI, 3.9-10.20). Betel quid chewers who chewed for less than 5 times/day had a 3-fold higher risk of oral cancer compared to non-chewers, the risk increased to 15-fold with a chewing frequency of 10 times/day. The OR for chewing habit duration varied

from 3.4 for a chewing habit of fewer than 10 years to 14.6 for a chewing habit persisting for 20 years or more.

6.4 SMOKELESS TOBACCO AND ORAL POTENTIALLY MALIGNANT DISORDERS (ARTICLE IV)

A total of 18 studies was included in this review. The pooled risk for OPMDs with the use of SLT was 15.5 (95% CI, 9.9-24.2). Exclusion of studies that had not reported an effect estimate adjusted for alcohol and smoking provided a pooled OR of 13.1 (95% CI, 8.3-20.7). The adjusted meta-risk for the development of Submucous fibrosis with the use of “all SLT” was 20.0 (95% CI, 12.3-32.5). Lower risks were observed for Leukoplakia, mOR=4.33 (95% CI 1.4-13.2). The pooled risk for the development of “all OPMDs” with the use of betel quid with tobacco was 16.1 (95% CI, 7.8-33.5). The corresponding risk, with the use of Gutkha, was much lower at 4.9 (95% CI, 2.6-9.4). Compared to non-users, the pooled risk for developing an OPMD with up to 20 years of SLT use was 29.3 (95% CI, 20.3-42.1). The risk increased to 41.9 (95% CI, 27.4-64.1), for an SLT habit of up to 40 years. The Population attributable fraction of SLT use for oral cancer in India was 74% and that for Sri Lanka was 69%.

6.5 NASWAR USE AND THE RISK OF ORAL CANCER: POOLED EVIDENCE FROM PAKISTAN (ARTICLE VIII)

Five studies were eligible for the review. Two studies each were carried out in Sindh and Khyber Pakhtunkhwa provinces and one study was from Punjab. Three studies had a moderate rating and two were rated low, based on the “Effective Public Health Practice Project’s Quality Assessment tool for Quantitative studies” [214]. Gender specific estimates were provided by two studies, and two studies provided data on the exposure-response relationship between *Naswar* use and the risk of oral cancer.

The overall risk [Meta OR (mOR)] (five studies) for oral cancer associated with *Naswar* use was 13.5 (95% CI, 9.5-19.2) and 11.9 (95% CI, 6.3-22.3) with fixed and random effects, respectively. The I^2 statistic had a value of 56%. When only the studies with a moderate quality were included in the meta-analyses, the mOR was 17.0 (95% CI, 11.4-25.3) in both random and fixed effects analysis, with an I^2 of 0%. Females [mOR 18.8 (95% CI, 12.5-28.2)]

had a comparatively higher risk of oral cancer compared to males [mOR 16.4 (95% CI, 10.7-24.1)] but the confidence interval overlaps.

6.6 SMOKELESS TOBACCO-RELATED GAPS IN TOBACCO CONTROL POLICY OF PAKISTAN (ARTICLE VI)

Sale of “cigarettes” and other “smoking” substances to minors is banned in Pakistan, but smokeless tobacco has not been addressed specifically in the policy, on a national level. There is a selective prohibition, regarding some forms of SLT, in just one province of Pakistan, i.e. a ban on the sale of Gutkha in the Sindh province. On the national level, public sector buildings, educational institutions and hospitals have been declared “smoke-free” but not “tobacco-free”.

The adjuvant laws that inform the tobacco control policy in Pakistan, ban children under the age of 14 years from selling or getting involved in the manufacture of tobacco related products. However, there are no such provisions for adolescents (14-18 years), except in Khyber Pakhtunkhwa province. There are no provisions in the national laws concerning regulation, taxation, or health warnings for smokeless tobacco.

7 DISCUSSION: A DISCOURSE ON CAUSALITY AND PREVENTION

“New opinions often appear first as jokes and fancies, then as blasphemies and treason, then as questions open to discussion, and finally as established truths.”

- George Bernard Shaw

This chapter discusses the methods and findings pertaining to this dissertation, in the light of the theoretical constructs of “Chronic disease prevention” and “Causality”. More specifically, the focus will be on the triangulation of these methods and findings, and how they coalesce, to address the broader general objective of this body of work.

7.1 SMOKELESS TOBACCO AND ORAL CANCER IN SOUTH ASIA (CONSIDERATIONS FOR CAUSALITY)

7.1.1 Evidence from the systematic reviews

7.1.1.1 Main findings

Betel quid with tobacco (Paan) and other forms of SLT used in South Asia were associated with an elevated risk of oral cancer. Paan users had a seven-fold increase in the risk of oral cancer compared to non-users. The risk for oral cancer with the use of other forms of SLT was approximately five times higher compared to non-users. Compared to men, women had a higher risk of oral cancer associated with the use of SLT products. We found a direct exposure-response relationship between SLT use and oral cancer. Users of any SLT products had a 15-fold higher risk of developing OPMDs, compared to non-users. Women had a twice-higher pooled risk of OPMDs associated with SLT use, compared to men. Betel quid with tobacco had the highest associated risk of OPMDs, among all SLT products. Submucous Fibrosis had the highest risk associated with SLT use, among all OPMDs.

7.1.1.2 Interpretation

The results from the two reviews point towards a potentially causal nature of the relationship between the use of SLT and oral cancer and OPMDs in South Asia. In both the reviews, we report a high magnitude of the pooled risk estimate and the presence of an exposure-response relationship between SLT use and oral cancer and OPMDs. Our results were in accordance with the systematic reviews of SLT use and the associated risk of oral cancer in

South Asia carried out parallel to, or after the publication of our review [16-18]. An elevated risk of oral cancer associated with the use of different SLT products in South Asia was reported by all the reviews. Similarly, all the reviews found an exposure-response relationship between SLT use and the incidence of oral cancer. Moreover, our findings were in accordance with the findings of the reviews from other parts of the world [105, 106, 215, 216]. The findings of the systematic review on OPMDs that an elevated risk of OPMDs is associated with SLT use and that an exposure-response relationship exists between the two, are comparable to individual studies on OPMD risk factors carried out around the world [217-229].

7.1.1.3 Strengths and limitations

The systematic reviews reported in this dissertation were the first reviews of South Asian literature on the risk of oral cancer and OPMDs, associated with the different forms of SLT products. Efforts were made to identify and include all the relevant publications, but some publications in local journals, which are not indexed in mainstream databases, may have been missed. The reviews were based on observational studies and hence bias e.g. selection and recall bias, as well as bias linked to retrospective exposure assessment in the included studies, may have influenced our reported risk estimate. We observed a high heterogeneity between the included studies. Efforts were made to minimize this through subgroup and sensitivity analysis. There was also a lack of studies addressing the less common OPMDs and as such, our findings are only applicable to the common OPMDs conditions such as Leukoplakia, Submucous Fibrosis, and Erythroplakia.

7.1.2 Naswar use and the risk of oral cancer in Pakistan

7.1.2.1 Main findings

The case-control study (Article V and VI) carried out in Peshawar reports a 21 fold elevated risk of oral cancer in Ever-users of *Naswar* compared to Never-users. An increasing cumulative exposure to *Naswar* i.e. *Naswar* pack-years was associated with an increasing risk of oral cancer and vice versa. A similar relationship was observed between the intensity of *Naswar* use i.e. duration of each use and the risk of oral cancer. About 70% of incident oral cancers in Khyber Pakhtunkhwa are attributable to *Naswar* use. The systematic review

(Article VIII) Ever-users of *Naswar* had a 12-fold higher risk of oral cancer compared to Never-users. An increase in the frequency and duration of *Naswar* use was associated with a corresponding increase in oral cancer risk.

7.1.2.2 Interpretation

We observed an elevated risk of oral cancer associated with the use of *Naswar* in the case-control study and the systematic review of Pakistani literature on *Naswar* use and the associated risk of oral cancer. The high magnitude of the risk estimate in both sexes, the presence of an exposure-response relationship, and the existing evidence of the potential carcinogenicity of *Naswar* due to its biochemical composition [230], all point towards a causal nature of the association between *Naswar* use and the elevated risk of oral cancer. This is further substantiated by the fact that approximately 72% of the primary tumors, reported in our study, developed from the gingival or the buccal mucosal tissue. These are the primary anatomical sites coming in direct contact with a *Naswar* pellet.

Risk factors for oral cancer are an under-researched area in Pakistan [13]. The case-control study at the core of this body of work is the first adequately powered epidemiological study carried out in the Khyber Pakhtunkhwa province, to assess the risk of oral cancer associated with *Naswar* use. A previous study from the same region reported a high biochemical risk of cancer associated with the constituents of *Naswar* [230], adding to the plausibility of our findings. The prevalence of *Naswar* use among both cases and controls at 79% and 27% respectively, was comparable to the previously reported prevalence of *Naswar* use, in oral cancer cases from this region and the general population of Peshawar [197, 198, 202, 231]. The prevalence of *Naswar* use in controls, though, was much higher compared to the national prevalence of *Naswar* in Pakistan i.e. 8% [96]. The difference can be explained by the difference in tobacco consumption practices between the different regions of Pakistan [96, 232]. The national figures are based on a representative sample of all the provinces of the country, our study sample is limited to Khyber Pakhtunkhwa province only, where *Naswar* use is observed as a cultural practice [233].

The high magnitude of the risk of oral cancer associated with the use of *Naswar*, observed in our study, is consistent with the existing literature on the risk of oral cancer associated

with the use of SLT products such as Gutkha and Betel quid, from India and Pakistan, [17, 18, 115, 125]. However, in our study, the observed risk estimates are even higher compared to these SLT products. This can be explained by the higher amounts of “Tobacco-Specific Nitrosamines”, nicotine, and a higher alkalinity (pH) of *Naswar*, compared to the other SLT products used in India and Pakistan [81]. The high pH of *Naswar* facilitates rapid absorption of high amounts of Nicotine, leading to a nicotine dependence [81, 230]. A positive feedback cycle then ensues, with more frequent and longer uses of *Naswar* to curb the nicotine urge, and hence, a greater exposure to the carcinogens present in *Naswar* [79]. Additionally, there are suggestions that *Naswar* causes local tissue trauma by erosion [234], and chronic trauma is an independent risk factor for oral cancer [235].

A previous case-control investigation by Merchant et al. [236], from the South of Pakistan, reported an OR of 9.5 (95% CI, 1.7-52.5), for the risk of oral cancer associated with *Naswar* use. Although of a high magnitude, this estimate is considerably lower than the risk estimate reported by us. A difference between SLT products used in different parts of Pakistan may be a possible explanation for the difference in the reported risk estimates. As noted earlier, Betel quid and Gutkha are the favored SLT products in the south of Pakistan, while in northern Pakistan and especially in the Khyber Pakhtunkhwa province, *Naswar* is the most common type of SLT [96, 237]. Betel quid was not significantly associated with an elevated risk of oral cancer in our study but Merchant et al reported a higher risk of oral cancer associated with the use of betel quid. This implies that the risk factor profile for oral cancer might be different in different regions of Pakistan, owing to varied tobacco use practices. Our findings though are comparable to a large case-control study (1192 cases, 3562 controls) carried out in the city of Karachi in the 1970's [238]. That study reported a 20-fold increase in the risk of oral cancer associated with the use of “Nass”, which is essentially the same product as *Naswar*. Although Karachi is also in the south of Pakistan, the large size of the study may have enabled the investigators to calculate more precise estimates regarding Nass, compared to Merchant et al., who had a much smaller sample size.

In the case-control study, current users of *Naswar* had a higher risk for oral cancer compared to past users, similar to the findings reported by a cohort study from India [239]. The

exposure-response relationship between *Naswar* and oral cancer in our study are in accordance with the systematic reviews of SLT and the risk of oral cancer i.e. the risk of oral cancer increases with increasing frequency, duration and intensity of SLT products use [16, 18]. Females had a higher risk of oral cancer associated with *Naswar* use in our study, which is similar to other findings from the Indian subcontinent [17, 115, 240]. This can be attributed to a lower background risk for oral cancer in women i.e. lower prevalence of smoking and alcohol use among women in the Indian subcontinent, compared to men.

7.1.2.3 Strengths and Limitations

A case-control study design was chosen to address one of the core objectives of this body of work. Although prospective study designs are better suited to establish epidemiological associations between putative risk factors and disease, they are sometimes not feasible to carry out, particularly when the outcome of interest is a rare disease like oral cancer [182]. Case-control studies are a suitable alternative in such situations, providing an efficient and cost-effective approach to study epidemiological associations between disease and their risk factors [181]. However, case-control designs due to their retrospective nature may be susceptible to a variety of biases [186, 192]. Our study was also susceptible to the biases inherent to case-control designs. This subsection provides an overview of the strengths and the limitations of our case-control study, which are necessary for a correct interpretation of our findings.

Strengths

- We were able to recruit the required number of participants despite unfavorable conditions in the wake of a deadly terrorist attack and the subsequent military operations against terrorism in Peshawar. Patient flow to the hospitals was severely hampered in the wake of these events, and as a result, we had to change our initial design of a 1:1 case-control ratio to 1:2. We were successful in recruiting the required number of participants in a nine months period and could have possibly recruited more cases and controls, if not for the events that took place in Peshawar during the study period.

- Only incident cases of oral cancer were included in the study to avoid incidence-prevalence bias [241]. Recruitment of incident cases can also potentially lead to a more accurate assessment of the pre-morbid exposure compared to prevalent cases [242].
- Interviews were carried out prior to the definitive diagnosis of oral cancer in the cases. This might have helped in avoiding the issue of differential recall between the cases and controls to some extent, as the potential cases were not aware of their disease status. The same holds true for interviewer bias as the Interviewers were not certain about the case or control status of the interviewee.
- The hospitals that were chosen for this study have a catchment area that includes most of the Khyber Pakhtunkhwa province and the Federally Administered Tribal Areas. This can be validated by the district wise distribution of our study participants, with all but two of the 23 districts of the province being represented in our study sample. This adds to our confidence in the representativeness of the study sample, to the source population.
- Reduction of bias was the focus while recruiting controls for the study. A variety of diagnoses was eligible to be recruited as controls to have a diverse selection of participants. To detect an effect estimate that is closer to the true effect, cancers and tobacco-related diagnoses were excluded,
- The high response rates achieved by the study helped in minimizing selection bias, further adding to the representativeness of our study sample. It also ensured minimal missing data.
- Exposure assessment was carried out with temporality as a consideration. Questions regarding lifestyle risk factors were aimed at assessing exposures over the life course rather than the near past, which might have been influenced by the present illness. This helped us in avoiding temporal ambiguity regarding exposure recall.
- An effort was made to quantify the exposure of interest in the form of *Naswar* pack-years. As has been suggested earlier (Chapter 5), exposure assessment regarding SLT products is very tricky, as their production is often unregulated i.e. various sizes and

compositions. Additionally, the serving size of *Naswar* is dependent on the user's personal preference. Given the circumstances and the available resources, the novel exposure metric of *Naswar* pack-years facilitated a more precise calculation of the cumulative exposure to *Naswar*, than would have been possible otherwise.

- We used a simple poverty scorecard that has been specifically tailored to the Pakistani context. To the best of our knowledge, the scorecard has never been used in health research before. The scorecard provides an efficient way to assess SES through ten simple questions. This considerably reduces the interview time with each participant, while providing a valid estimate of SES.
- Appropriate statistical analyses, guided by apriori causal diagram analysis, were carried out to address the aims of the study.

Limitations

- Potential interviewer bias can be anticipated in our case-control study. Not all the interviewers were blinded to the main hypothesis of the study. Due to limited resources, independent interviewers could not be hired and the researcher (doctoral candidate), who was aware of the study hypothesis, was a part of the interviewing team.
- Theoretically, it can be assumed that the interviewers were not familiar with the case-control status of the participants, but the presence of the lesion in the oral cavity is sometimes obvious to the naked eye, and hence the “case” status.
- While efforts were made to address the issue of differential recall and temporal ambiguity between the cases and controls, these cannot be ruled out completely.
- Even though a representation of most districts of Khyber Pakhtunkhwa province was observed in our study sample, findings from a hospital-based study may not be generalizable to the whole population (of Khyber Pakhtunkhwa). The same is true for the *Naswar*-pack years metric, which was developed by using samples from the study participants.

- Although we were able to recruit more than the estimated number of participants, numbers were small for the subgroup and exposure-response analysis and are reflected in the precision of the risk estimates.
- The risk estimates reported in our study have considerably wide confidence intervals. We carried out an unconditional analysis to compare the results with those of the conditional model and found out very similar results i.e. elevated ORs with wide confidence intervals, implying the absence of serious errors or an instability of the conditional model.
- The imprecision of the risk estimates reported by our study point towards potential bias in these estimates. However, even if we assume the presence of residual and/or unmeasured confounding, the high magnitude of the risk estimates implies that a potential causal association between *Naswar* and oral cancer cannot be ruled out.
- There may have been an underreporting of the prevalence of tobacco smoking, alcohol drinking, and *Naswar* use in the study participants, particularly the female controls. This is due to a social stigma attached to these habits in the Pakistani society.

7.1.3 Smokeless tobacco (*Naswar*) and oral cancer, a causal association?

In this subsection, I analyze the findings of the case-control study (Article V) in the light of the Bradford Hill criteria for causality [161], Rothman’s causal pies [162], and causal diagrams [173]. I do so, with the view that fulfillment of these criteria does not ESTABLISH or REFUTE causality between a risk factor and an outcome in absolute terms, but merely supports or opposes it [243], as Greenland and colleagues put it “...all causal inference is based on assumptions that cannot be drawn from observations alone” [173].

7.1.3.1 Bradford Hill criteria

Although oral cancers have a multifactorial etiology, in the context of South Asia, smokeless tobacco plays perhaps the most important role in the causation of oral cancer [17, 244, 245]. We report a 21-fold increase in the risk of oral cancer associated with the use of *Naswar* among “Ever-users” compared to “Never-users”. While, the confidence interval around this risk estimate is wide, the whole interval lies well above unity, signifying a strong association

between *Naswar* use and incidence of oral cancer. In our study, an increase in the frequency of use, the total duration of the *Naswar* habit, and the duration of a single use, were associated with a corresponding increase in the risk of oral cancer. The risk estimates reported by our study have been adjusted for other known risk factors such as age, sex, alcohol use, tobacco smoking, and socioeconomic status, hence pointing towards a high specificity of oral cancer causation related to the use SLT products in South Asia.

Although case-control studies are not ideal for the analysis of the temporal aspects of an association between two variables, the mean duration of *Naswar* exposure among the cases in our study (27.4 years), suggests that *Naswar* use among these cases may well have preceded their current illness (oral cancer). The biological model of *Naswar* related oral carcinogenesis presented in chapter 3 further validates this assertion (Fig 3.2). The model provides a plausible explanation, and the possible temporal sequence, of the events involved in the incidence of oral cancer associated with *Naswar* use. Our finding that more than 70% of the cases had a primary tumor arising from either the gums or the cheeks (sites of *Naswar* use), also provides evidence of a temporal succession of oral cancer to *Naswar*.

“Article VIII” describes the results of previous case-control studies on the use of *Naswar* in other areas of Pakistan [236, 238, 246, 247]. These results show a strong association between the incidence of oral cancer and the use of *Naswar*. Our findings are thus, consistent with the previous findings. The pooled risk estimate reported in article VIII highlights the high risk of oral cancer associated with the use of *Naswar*. Our finding that a high risk of oral cancer is associated with the use of *Naswar* is coherent with previous findings from systematic reviews on SLT products used in South Asia [16, 115]. Pooled risk estimates reported in these reviews are of a high magnitude, which is in accordance with the risk estimates that we have reported. The risk of oral cancer associated with the use of *Naswar* and other SLT products is analogous to the risk of lung cancer associated with tobacco smoke i.e. the anatomical site primarily responsible for the absorption of the bioactive ingredients in tobacco, is the most susceptible to the development of a tumor [248].

Zakiullah et al. reported very high levels of carcinogens in the *Naswar* samples available in the Pakistani market [249]. The model of *Naswar* related carcinogenesis presented in

Chapter 3 (Figure 3.2) outlines the bio-epidemiological processes that are potentially involved in the causation of oral cancer associated with *Naswar* use. Experimental studies in humans for assessing health risks related to the use of *Naswar* are not practical due to ethical considerations. Experiments on animals are also seldom informative given the long latency period of cancers and a short life span of experimental animals, as few survive until the appearance of tumors [250, 251]. The IARC evaluated experimental studies carried out in animals, in the monograph on smokeless tobacco [101], the reviewed literature revealed no tumors at the site of application of *Naswar* but a variety of tumors developed in other organs.

7.1.3.2 *Smokeless tobacco (Naswar) as the component of a sufficient cause*

Rothman defines a **sufficient cause** as "...a complete causal mechanism" that "inevitably produces disease." Consequently, a "sufficient cause" is not a single factor, but a minimum set of factors and circumstances that, if present in a given individual, will produce the disease [162]. In the view of the available literature, the proposed bio-epidemiological model of *Naswar* related carcinogenesis in chapter III, and the research studies carried out in the context of this dissertation, a sufficient cause for oral cancer in the context of South Asia can be presented as the following pie chart

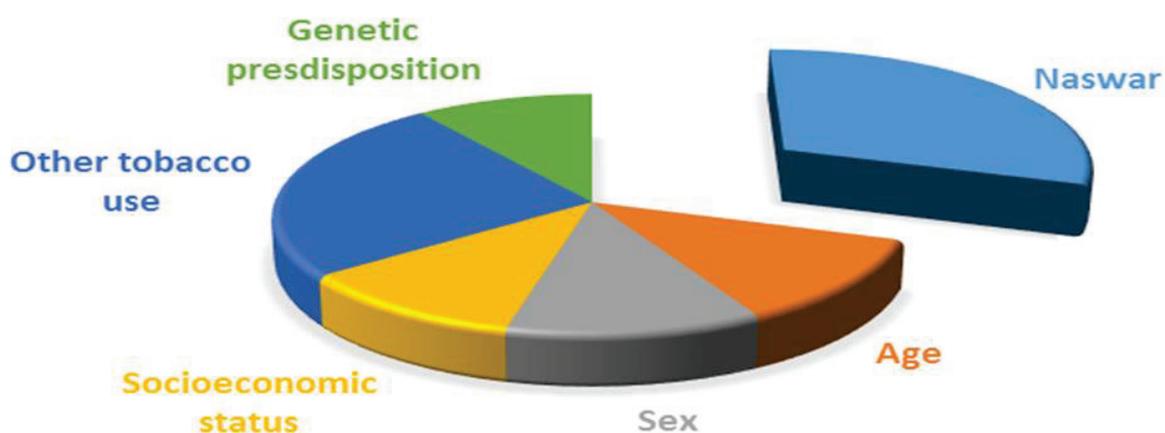


Figure 7.1. *Naswar* as the component of a sufficient cause for oral cancer.

It is evident that smokeless tobacco (*Naswar*) plays an important role in the causation of oral cancer in the context of Pakistan, as a component sufficient cause. Other sufficient causes for oral cancer may also exist, especially in the presence of emerging risk factors such as HPV. Smokeless tobacco thus cannot be termed as a “necessary” component of all causal pies for oral cancer in South Asia, but when present, contributes to a large chunk of the pie. The role of *Naswar* in oral cancer causation in Pakistan cannot be underpinned enough, as is suggested by the high *Naswar* associated attributable fractions in articles V and VIII, and the *Naswar* attributable incident cases reported in article VIII.

7.1.3.3 *Naswar, oral cancer, and causal diagrams*

Data collection and the subsequent statistical analysis for the case-control study conducted in the context of this dissertation were guided by apriori DAG analysis (Article V). A “minimum adjustment set (MAS)” of variables was identified through this analysis. We observed both direct and indirect unblocked causal paths between *Naswar* use and the incidence of oral cancer. This implies that the relationship between *Naswar* and oral cancer can possibly be a causal one and that this association may be confounded by other variables, which would need adjustment during the analysis. Data regarding all the MAS variables was collected and the adjustment for the confounding variables was carried out in the logistic regression model to compute an unconfounded effect estimate.

7.1.4 Confidence in cumulative evidence

The evidence that informs this body of work is produced by systematic reviews of observational studies and a case-control study. Evidence generated by systematic reviews is usually placed high in the evidence hierarchy [252]. The validity and applicability of systematic reviews are often dependent on the quality of the included studies [253]. In theory, this could imply that systematic reviews of observational studies are less valid and applicable compared to systematic reviews of experimental studies. Observational studies have multiple biases inherently related to them, which can affect the quality of a study and may produce spurious results [254]. In practice, though, this is not the case, systematic reviews of observational studies, particularly those related to disease etiology are increasingly becoming popular, and are considered useful in informing policy and practice

[255]. Etiological observational studies are usually small and examining several such studies simultaneously can give us a deeper insight into real and spurious associations between disease and their risk factors [256]. Systematic reviews of observational studies are usually carried out to answer questions which cannot be answered by experimental studies due to ethical or practical concerns like an outcome being very rare or when there is a lack of adequate experimental evidence [257]. In such scenarios, evidence from observational studies constitutes the “Best available evidence” [258], that can be used to inform public policy and clinical practice [257, 259].

Case-control studies form an integral part of cancer epidemiology because of their speediness, efficiency and cost-effectiveness [260]. Case-control studies are used to establish an association between an exposure and a disease (outcome) [261], and are invaluable in the studies of rare outcomes, where the causal pathway may span decades [262]. It is a refined method of observation that allows us to look back in time and establish associations between disease and their risk factors [193]. The case-control study designs are particularly important in the context of developing countries, where longer prospective studies are not feasible due to a lack of resources [193, 263, 264]. A well-designed and properly conducted case-control study can provide valid, informative and unbiased effect estimates, which may be comparable with those produced by cohort studies [193, 262].

In the context of the pooled analysis of evidence from Pakistan (Article VIII), two independent reviewers assessed the quality of the case-control study (Article V), that informs the core dissertation of this body of work, by using a validated quality assessment tool for quantitative studies [214], and found that the study was of “moderate” quality. The reason it did not get a “strong” rating was that the data collection tool for the study was not shown to be valid nor reliable. The data collection tool for our study was adapted from a large multicenter case-control study carried out in Europe under the umbrella of the IARC [1]. Owing to limited resources and time constraints, validity and reliability of the tool could not be established in the local context of Pakistan. However, the contents of the questionnaire were assessed by senior dentists at the study centers in Pakistan, who unanimously agreed that all the questions were valid and exhaustive, to get the necessary information needed

to address the aims of the study. Detailed results of the quality assessment of the case-control study are provided in as appendix VIII.

In the light of the above discussion, it can be concluded that although the findings reported by this body of work are based on observational research designs, in the absence/infeasibility of experimental studies, it represents the “best available evidence”. It can be inferred from the evidence generated by this body of work that smokeless tobacco products are strongly associated with an increased risk of oral cancer and oral potentially malignant disorders in South Asia. It can also be inferred that *Naswar* use is strongly associated with a high risk of oral cancer in the Khyber Pakhtunkhwa province, Pakistan. The application of the Bradford Hill criteria to the association between *Naswar* use and the risk of oral cancer suggests that this association may potentially be a causal one.

7.2 ORAL CANCER PREVENTION IN PAKISTAN

7.2.1 The lack of epidemiological research

The importance of research in tackling NCDs has been outlined in chapter 4. In the context of this dissertation, we carried out an electronic search of local (Pakistani) and international research databases to identify oral cancer literature produced from Pakistan (Article 1). The electronic search was supplemented by google searches and hand searches of bibliographies of the included publications. Oral cancer research in Pakistan is lagging in terms of quantitative output. Although the output has doubled post the year 2000, as compared to the output from 1972 until 2000, the growth is only relative. A meager 166 publications from Pakistan, addressing oral cancer, were found through searches of national and International databases. Only six studies were epidemiological investigations fit to assess the modifiable risk factors for oral cancer. Our results are supported by previous findings from Mushtaq and colleagues, who observed a general lack of a research culture in Pakistan, which can be confirmed by its less than 0.1% share of the worlds’ research output [265]. To put it into perspective, Pakistan is the sixth most populous country in the world [266]. The pattern (not numbers) of oral cancer literature growth in Pakistan is comparable to findings from India [267, 268]. Both countries have seen a rapid growth in oral cancer research output in

recent years [269]. The lack of epidemiological studies from Pakistan on relevant risk factors for oral cancer is evident by an under-representation in the systematic reviews on the risk of oral cancer associated with smokeless tobacco products used in South Asia [16, 18, 115]. Although efforts were made to make our electronic searches as exhaustive as possible, studies published in journals with no or minimal presence on the world-wide-web might not have been included in our review. Citation analysis could not be carried out due to the unavailability of suitable data required for such an exercise.

7.2.2 Gaps in tobacco control policy

In the context of this body of work, I carried out a review of smokeless tobacco control policies in Pakistan (Article VII). Pakistan is a signatory to the WHO's "Framework Convention for Tobacco control". To date, the focus of tobacco control in Pakistan has primarily been on tobacco smoking. In the reviewed policy documents of the Tobacco Control Cell of Pakistan, SLT is seldom mentioned, and usually in vague terms, such as "other tobacco". There are no formal regulations regarding the production of the various SLT products in Pakistan, neither are these products covered by the tobacco taxation net. Unlike cigarettes, there are no health related warnings on the packaging of SLT products produced in Pakistan. One of the most alarming gaps in tobacco control policy is perhaps the absence of any provisions regarding the prohibition of sale to, and by adolescents (14-18 years), an age group which is usually the most vulnerable to take up tobacco habits.

The findings of the review were in accordance with a previous review of smokeless tobacco control policies in South Asia [270]. The finding that smokeless tobacco sale to and by minors is not explicitly prohibited by the tobacco control policy of Pakistan, is, however, in contrast to the findings of Khan et al. We have provided the grounds for this disagreement in the related manuscript (Article VI). Efforts were made to include all the related policy documents available on the national and provincial Government websites but there is a possibility that unpublished documents which have been recently approved or are in the process of getting approvals, and not yet available on the world-wide-web, might not have been included in this review.

8 CONCLUSIONS AND PERSPECTIVES

“We are not going in circles, we are going upwards. The path is a spiral; we have already climbed many steps.”

-Hermann Hesse

Non-communicable diseases pose a great challenge to public health in the 21st century [132]. A key aspect in the prevention of NCDs is the generation of research evidence regarding the causes and risk factors of these diseases [142]. Sound research evidence, synthesized through rigorous methods, has a potential to drive both policy and practice [143]. It is imperative that such evidence is produced in the local context of the countries, as risk factor profiles and genetic make-up of the people differ in various geographical regions [142]. Findings from industrialized countries may not be applicable to developing countries and vice versa.

The incidence of oral cancer is on the rise in Pakistan [7]. The excessive burden of oral cancer in Pakistan necessitates research into the causes and risk factors of this disease. A lack of a research culture and the non-availability of resources are obvious reasons for the scarcity of oral cancer research in Pakistan. However, researchers should also be shouldering the responsibility, their motivation for doing research should be the prevention and elimination of disease, rather than a satisfaction of the minimum number of publications required for promotion.

Smokeless tobacco use presents a unique public health dilemma. The IARC has labeled SLT as a Group I carcinogen in humans [15], but aided by evidence from a few research studies conducted on Swedish SLT products, some tobacco control experts vouch for SLT use as means of tobacco harm reduction [271]. This further emphasizes the point made earlier about the importance of research in a local milieu. The two systematic reviews carried out in the context of this dissertation, show that the use of SLT is associated with an elevated risk of oral cancer and OPMDs in South Asia. The findings of this dissertation imply that approximately 60-70% of the oral cancers and about the same proportion of OPMDs in India, Pakistan, and Sri Lanka can be prevented with the elimination of SLT products in these countries. The review of tobacco control policies in Pakistan outlined a negligence

towards SLT control in the country. If Pakistan aims to relieve its public health system from the burden of oral cancer then SLT control policies need to be in place. The rising cigarette prices in Pakistan further necessitates this, as more people may potentially take up SLT products as cheaper substitutes to cigarettes.

On a more local level i.e. Khyber Pakhtunkhwa Province, the results of our case-control study suggest a strong association between oral cancer and the use of *Naswar*. The strength of the association, coupled with the exposure-response relationship, and the biochemical evidence of *Naswar*'s carcinogenicity, points towards the possibility of a causal relationship between *Naswar* and oral cancer. It certainly warrants larger studies to assess this association in more detail. *Naswar* is cheap, easily available all over the province and a lack of research on its deleterious effects means that potentially many *Naswar* users might not be aware of its health consequences. To add more insult to injury, there are no official regulations regarding the manufacture of *Naswar*, there are no health warnings, and it largely evades the tobacco tax-net. The Government of Khyber Pakhtunkhwa province needs to address these issues and in the light of the findings of this body of work and formulate a tobacco control policy for the province, which specifically targets *Naswar*.

8.1 POLICY IMPLICATIONS

“Smokeless Tobacco” is a recognized term by the WHO and hence, should be used as such in the public policy documents, rather than the use vague terms like “other tobacco”, which can be misleading and may be misinterpreted. The use of smokeless tobacco, explicitly citing “*Naswar*”, “*Gutkha*”, and “*Paan*”, shall be prohibited in public places, offices, and educational institutions, akin to the prohibition of tobacco smoking. Sale of all forms of tobacco (smoking and smokeless), to and by, a person who is younger than 18 years of age shall be prohibited. Health warnings should be made mandatory on the packaging of *Naswar* and other SLT products. As opposed to an absolute ban on SLT products, the government should try to introduce regulatory legislation regarding the composition of these products. The content of carcinogenic agents in *Naswar* and other SLT products could be reduced using the “Swedish Snus” model. This will ensure a security of the livelihoods and jobs of the

workers in the smokeless tobacco industry while reducing the harmful potential of the SLT products. These policy provisions should be supplemented by media, and school awareness campaigns on the deleterious effects of SLT products.

9 AFTERWORD

A cancer specialty hospital in Peshawar and two tertiary care dental teaching hospitals in two other large cities of Khyber Pakhtunkhwa have started working since the conclusion of the case-control study presented in this dissertation. This presents an ideal opportunity to carry out a larger study, involving more study centers and a larger number of participants, to confirm the findings presented in this dissertation.

At the time of writing, detection of Human Papilloma Virus DNA in the blood and tissue samples of our study participants is underway at the Khyber Medical University, Peshawar. The Human Papilloma Virus analysis can potentially provide us new insights into the changing risk factor spectrum of oral cancer, as has been seen in developed countries.

The Tobacco control cell of the Government of Khyber Pakhtunkhwa province has shown a keen interest in our study findings and have asked them to be shared. We intend to write a policy brief in the light of our findings with the aim that these findings would influence tobacco control policies in the Khyber Pakhtunkhwa province. As a practice implication, we intend to further refine the *Naswar* pack-year exposure metric, so that it can be used clinically akin to the smoking pack-years. This could involve the replication of our original methods on a larger scale and a randomly selected sample from the population of Khyber Pakhtunkhwa.

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“... And above all those who know is the One Who truly knows”

- (Yusuf 12:76)

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12 APPENDICES

- I. A quantitative review of oral cancer research output from Pakistan.
- II. Smokeless tobacco and oral cancer in South Asia: a systematic review and meta-analysis.
- III. Smokeless tobacco and oral potentially malignant disorders: a protocol for a systematic review and meta-analysis.
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ARTICLE I

A QUANTITATIVE REVIEW OF ORAL CANCER RESEARCH OUTPUT FROM PAKISTAN

First author: Zohaib Khan

Order of authors: Zohaib Khan, Steffen Mueller, Shahzad Ahmed, Justus Toennies, Faryal Nadir, Florence Samkange-Zeeb.

Author's contributions: ZK developed the concept for the study, conducted the literature search, assessed studies for inclusion in the review and extracted data, also prepared drafts and undertook edits. S.M was involved in the study conceptualization and draft preparation and revision. S.A was involved in the development of the study concept, literature search, and draft reviews. J.T was involved in data extraction, and paper editing. F.N was involved in data analysis and draft preparation. F.S.Z was involved in reviewing the initial draft and write up of the pre-submission final draft

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RESEARCH ARTICLE

Quantitative Review of Oral Cancer Research Output from Pakistan

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Abstract

Background: Oral cancer is the most common cancer among men and second only to breast cancer among women in Pakistan. For the effective control and prevention of oral cancer, Pakistan needs to recognize the importance of research and generation of the evidence-base which can inform policy making and planning and implementation of intervention programs. The objective of this review was to quantify oral cancer research output in Pakistan. **Materials and Methods:** A systematic electronic search in “Medline”, “ISI-Web of Science” and “Pakmedinet”, supplemented by a Google search, was carried out in January and February, 2014, to identify literature from Pakistan relevant to oral cancer. The selection of publications for the review was carried out according to preset criteria. Data were recorded and analyzed using Microsoft Excel. **Results:** A total of 166 publications comprising 62 case series, 36 cross sectional, 31 case control, 10 basic laboratory research, eleven reviews and two trials, were included in this review. Some 35 % of the publications focused on risk factors for oral cancer. COMSATS Institute of Information Technology was the institution with the highest contribution. **Conclusions:** There is a lack of research in the field of oral cancer research in Pakistan. Focused efforts should be put in place to improve both quality and quantity of oral cancer research in the country.

Keywords: Oral cancer - research - Pakistan

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Introduction

Oral cancer affects around 14.1 million people, making it one of the most prevalent cancers in the world (Warnakulasuriya, 2009). Developing countries, especially those from the South Asian region, have a higher burden of oral cancer compared to developed countries (Cancela et al., 2010; Krishna Rao et al., 2013; Mishra and Meherotra, 2014). With an estimated increase of 13,000 new cases each year, oral cancer is the most common cancer among men and second only to breast cancer among women in Pakistan. It also has the second highest cancer related mortality rates in the country (IARC, 2012). Oral cancer thus warrants immediate public health attention and evidence based concerted efforts for its control and prevention in Pakistan.

Research into non-communicable oral disease such as oral cancer is high on the agenda of the World Health Organization's (WHO) “Oral health program” (Petersen, 2005). Research is considered to be a central component of any cancer control strategy (Sullivan et al., 2014) and efforts made to reduce cancer burden involve plans and actions based on sound intervention and surveillance research, which are important for knowledge synthesis (Best et al., 2003). The application of such knowledge

and current results of research can help in tackling cancer mortality and morbidity in low and middle income countries (Sankaranarayanan and Boffetta, 2010). Global cancer research priorities exist (National Cancer Institute, 2012) and new trends are emerging in oral cancer research in developed countries. However, for developing countries it is imperative that they set their own cancer research priorities, based on their needs rather than following an agenda set up by high income countries (Sullivan et al., 2014). The setting up of research priorities and agenda is a process that should be founded on available evidence and information. However, often it is a problem for policy makers to identify and collect such information (Nuyens, 2007).

The aim of this paper is to provide an overview, including a quantitative analysis of published literature from Pakistan in the field of oral cancer. The specific objectives are: *i*) to analyze the growth pattern of oral cancer literature from Pakistan, *ii*) to examine the types of research studies, *iii*) to assess the focus of oral cancer publications in Pakistan, and *iv*) to identify institutions contributing literature on oral cancer and the core journals publishing this literature. This information can be useful for policy makers, future researchers and other stakeholders.

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Materials and Methods

Literature search

A systematic search was conducted in the following electronic databases: “PubMed”, “ISI-Web of Science” and “Pak Medinet”, using various combinations of the words “oral cancer”, “oral carcinoma”, “head and neck cancer”, “oral neoplasia”, “squamous cell carcinoma of the oral cavity” and “Pakistan”, from January 7, 2014 till February 29, 2014. No filters were used for the search process. A supplementary search in the web search engine www.google.com.pk and choosing the option “Pages from Pakistan” was also carried out to minimize the possibility of missing potential literature. Bibliographies of the selected publications were additionally searched to identify any further relevant studies.

For the purpose of this literature search, oral cancer was defined as “cancer that forms in tissues of the oral cavity (the mouth) or the oropharynx (the part of the throat at the back of the mouth)” (National Institute of Health, 2014)

Inclusion criteria

Publications were included in the review if they fulfilled the following criteria: *i*) With the exemption of review articles, the research described in the publication was carried out in Pakistan or was in the context of Pakistan. *ii*) Oral cancer was the main focus or one of the foci of the publication. *iii*) Manuscript published in an indexed or non-indexed journal up to 29.02.2014

Exclusion criteria

iv) Publications focusing solely on salivary glands or laryngeal or oesophageal cancers. *v*) Publications (except review articles) by authors affiliated to an institution in Pakistan but not carried out in Pakistan or in the context of Pakistan.

Selection of publications

The selection of the publications for this review was carried out in three stages: *i*) Screening of titles of publications identified through the electronic search, *ii*) scrutiny of abstracts of the publications selected after the first step and acquisition of the full texts of the selected abstracts and *iii*) selection of publications to be included in this review based on the scrutiny of full texts (Figure 1).

Data abstraction

Two authors (Z.K and J.T) separately abstracted the following data from the selected publications on a spreadsheet in Microsoft Excel: first author, journal name, year of publication, study type, sample size, main focus of the study and the first authors’ institutional affiliation. The data were later compared and any discrepancies or differences were assessed and dealt with by mutual agreement.

The assessment of institutional research output was done using the first author’s institutional affiliation as stated in the publication. Research output on the basis of geographical location (District or Division taken as an administrative unit) was ascertained by the district or

division where the institution to which the first author is affiliated, is located.

Publications were also divided into two groups: those which were published in indexed journals and those in non-indexed journals. Journals indexed in “Medline” or “Embase” were classified as “indexed journals”. In addition, the WHO database “Index Medicus for the Eastern Mediterranean region” was searched to identify journals that are indexed with it. However, these were only classified as “Indexed” if they were also indexed in Medline or Embase.

Data analysis

Analyses involving the calculation of frequencies and percentages were carried out using Microsoft Excel. The institutions and geographical administrative units in Pakistan with the most number of publications, as well as the journals in which the identified publications were published, were identified. Foreign collaboration was also assessed on the basis of the presence of an author belonging to an institute/body outside Pakistan. A quantitative summation of the different research foci and study designs of the included publications was carried out. The cumulative number of research publications was plotted against the corresponding year to analyze research productivity over time. To identify the core journals, Bradford-Zipf plotting (Tsay and Yang, 2005) was carried out.

Results

The search in the three electronic databases returned a total of 1692 publications, including duplications. 1196 publications were left after the exclusion of duplications. After application of the selection criteria, a total of 151 publications were eligible to be included in the final review. A further 12 full text publications were identified through the google search and three more were identified through a search of the bibliographies of the selected papers. In total 166 publications were included in the final review (Figure 1). Important characteristics of the included studies, such as author and publication year, along with their references are given in the supplemental Table 1. Full texts/abstracts of a further six papers selected after the first step of publication selection could not be retrieved. The titles of these papers were however suggestive of oral cancer being the main focus and thus have been included in the supplemental table for reference. These publications were also incorporated in the “Journal, geographical and author affiliation” analysis as the data pertaining to these could be extracted.

Oral cancer literature growth in Pakistan

The first oral cancer publication from Pakistan was published in 1972, while the latest publication at the time of writing this review was from April 2014 (“Article in press” version was available at the time of our electronic search). The cumulative growth of oral cancer research over time is shown in fig 2. 13% (n=22) of the publications were published before the year 2000, 38% (n=64) were published between 2000 and 2009, while 48% (n=80)

Table 1. Top Ranked Journals According to the Number of Oral Cancer Publications from Pakistan

Rank	Journal Name	Number of publications	Cumulative number of publications (%)	Impact factor of Journal	Indexed with Medline	Indexed with Embase	Subject area
1	J Pak Med Assoc	18	18 (11)	0.4	✓	✓	Medicine and Dentistry
2	Asian Pac J Cancer Prev	15	33 (19)	1.5	✓	✓	Cancer
3	J College of Physic and Surg, Pakistan	12	45 (26)	0.3	✓	✓	Medicine and Dentistry
4	J Pakistan Dental Assoc	9	54 (31)	N/A	X	X	Dentistry
5	Pakistan J Otolaryngol, Head and Neck Surg	8	62 (36)	N/A	X	X	Otolaryngology and head and neck surgery
5	J Ayub Med College	8	70 (41)	0.1	✓	✓	Medicine and Dentistry
5	Pakistan Oral and Dent J	8	78 (46)	N/A	X	X	Dentistry
5	Ann King Edward Med University	8	86 (51)	N/A	X	X	Medicine and Dentistry

were published from 2010 till February, 2014. There were no publications in the years 1973 to 1975, 1978 to 1985, 1988 to 1991 and 1993. The most productive year, in terms of publications, was 2013. The average number of publications per year after the first publication from 1972 up to 2000 was less than one. The average number of publications increased to 7.5 per year between 2001 and 2010, and to 20 per year between 2011 and 2013.

Type of research

36% (n=60) were case series studies, 21% (n=36) were cross sectional surveys of which five studies were of a comparative type and one was a national pathfinder survey. 18% (n=31) were case control studies, of which only 6 were about modifiable lifestyle risk factors while the rest were molecular, chemical, viral and genetic epidemiology studies. 11% (n=19) were reviews while five (Six)% (n=10) were basic laboratory research studies. Four% (n=7) were case reports. There were two clinical trials among the included studies.

Foci of research

57 (34%) of the studies focused on different risk factors for oral cancer, among which 31 studies were of case control and two of cross sectional comparative design. Among these studies 12 focused on genetic risk factors, 13 on viral, chemical or molecular risk factors and six on lifestyle risk factors. Most of the publications focusing on genetic epidemiology were published from 2010 onwards. There was only one epidemiological case control study focusing on lifestyle risk factors post year 2000.

34 (20%) of the selected publications focused on the distribution and hospital/clinic based frequencies of oral cancer. 24 (15%) studies focused on treatment modalities for oral cancer. 16 (9%) were based on diagnostic procedures while 10 (5%) studies focused on histopathological characteristics of oral cancer cases. Seven studies assessed knowledge and/or attitudes of different population groups regarding oral cancer. Four studies reported on follow up outcomes of oral cancer. Three studies were about quality of life in oral cancer patients and two studies focused on cancer care.

Core journals

The included publications were published in a total of 65 journals, 39 of which were indexed with "Medline" and/or "Embase". 66% (n=111) of the articles included

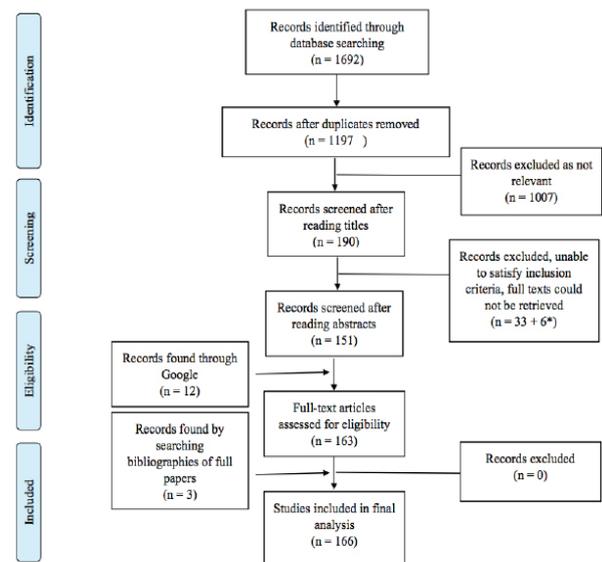
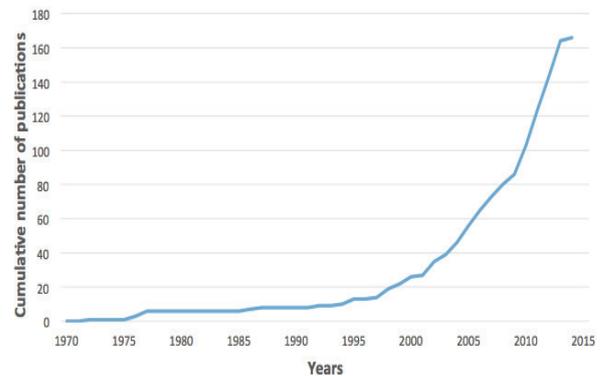
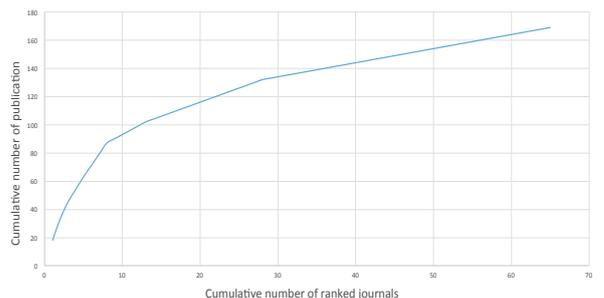
**Figure 1. Flow Chart of Publication Selection Process****Figure 2. Cumulative Growth of Oral Cancer Literature in Pakistan from 1972 till February 2014****Figure 3. Distribution of Oral Cancer Publications from Pakistan Over Research Journals**

Table 2. Major Oral Cancer Research Producing Institutions in Pakistan

Institution	City	No. of publications
Comsats institute for information technology	Islamabad	16
Jinnah post graduate medical centre	Karachi	12
Karachi cancer registry	Karachi	11
Aga Khan university	Karachi	11
King Edward medical university	Lahore	10
Dow university of health sciences	Karachi	10
Khyber college of dentistry	Peshawar	8

in this review were published in journals indexed with these two databases, while 33% (n=54) were published in journals that are currently not indexed with them. However, 44 of the latter were published in journals that are currently indexed with the WHO Index Medicus for the Eastern Mediterranean region. Three journals were indexed with all three databases, while 18 of the “non-indexed” journals were indexed in the WHO database.

More than half of the included articles were published in eight of the 65 journals (Figure 3), with three journals publishing more than a third of the articles: the Journal of Pakistan Medical Association (n=18), the Asian Pacific Journal of Cancer Prevention (n=15), and the Journal of College of Physicians and Surgeons, Pakistan (n=12) (table 1). These select few journals can be termed as “Core journals” for oral cancer publications from Pakistan.

Geographical distribution of included publications

Karachi has the highest number of publications with 65 (38%), followed by Lahore with 27 (15%), Islamabad with 24 (14%), Rawalpindi with 16 (9%), and Peshawar with 11 (6%). Other notable contributors include Jamshoro and Abbottabad with 5 publications each. Only two publications were from Quetta. Provincially, Sindh has contributed 42% of the total publications. Punjab has 30%, Khyber Pakhtunkhwa 10% and Baluchistan 1%. The federal capital Islamabad contributed 15% of the total included studies. There were no publications from Gilgit/Baltistan or the federally administered tribal areas.

Institutional output

A total of 63 institutions contributed to oral cancer research output in Pakistan. Table 2 includes the major institutional oral cancer research producers in Pakistan. Other notable contributors include Liaquat University of Medical and Health Sciences and Armed Forces Institute of Pathology (five publications each), Shaikat Khanum Memorial Cancer Hospital, Ayub Medical College and University of Karachi (four publications each).

Discussion

Historically, in Pakistan, Government policies including those in the field of health, have neither emphasized on, nor clearly defined its research priorities (Government of Pakistan, 2001; Government of Pakistan, 2011), thus underscoring the importance of research. This, among many factors, has contributed to a lack of research culture in the country, evidenced by Pakistan's poor performance in the field of research, where it has

contributed less than 0.1% of the world's research output, including health research (Mushtaq et al., 2012). Our study shows that the scarcity of research output in Pakistan (Akhtar, 2004) also holds true in the field of oral cancer, one of the most prevalent non-communicable diseases in the country.

At first glance, oral cancer research output in Pakistan appears to have grown exponentially over time. This growth is however relative rather than absolute, since publication numbers were very small at the beginning. The growth trend in oral cancer research is in contrast to the field of clinical radiology in Pakistan, the only medical field in which research output analysis has been carried out. No differences in clinical radiology research output were seen before or after the year 2000 (Akhtar et al., 2009). The general pattern of oral cancer literature growth in Pakistan however is comparable to the Indian cancer research output (Sullivan et al., 2014; Lewison and Roe, 2012), where oral cancer is one of the most researched cancers due to its huge burden of disease. Oral cancer research output in both countries has seen a rapid growth post year 2000 (Ghaffar et al., 2013).

The numerical increase in research output in Pakistan post year 2000 can be attributed to the emergence of the Higher Education Commission (HEC) in the early 2000s (Qureshi et al., 2013). The commission issued a directive that educational institutions be more research oriented and also introduced schemes for students to pursue research degrees such as Master and Doctoral programs, where publications are a requirement. Additionally, a minimum number of research publications are now required for promotion to a higher post in academia. The latter was implemented in the medical/dental colleges and universities by the Pakistan Medical and Dental Council (PMDC) and the HEC (Ghaffar et al., 2013). Although these steps have contributed to the total number of publications, there is a lack of good quality output with meaningful impact.

An essential aim of cancer research in low and middle income countries should be to understand the social, environmental, behavioral and biological determinants of the disease in a local context, so as to be able to assess efficacy of treatment protocols and interventional research (Sankaranarayanan and Boffetta, 2010). Epidemiological case control and cohort studies are effective research approaches to understand various risk factors or determinants of disease (Song and Chung, 2010). Our study data, however, shows a significant lack of such epidemiological studies in Pakistan. Although an increase in genetic epidemiological studies was observed, we did not find any studies on lifestyle and environmental risk factors published after the year 2000. More importantly, all the case control studies on lifestyle risk factors were carried out in one single city and are therefore most likely not generalizable to the whole of Pakistan. Further, the majority of the included case control studies were laboratory based and investigated molecular and genetic risk factors. The practical implementation of such studies into public health policy is however difficult, considering Pakistan's restricted resources. Overall, case series studies were the primary study form, reporting simple

descriptions of oral cancer cases or histo-pathological findings from some tumor samples. These studies hardly added new knowledge to, nor aided the prevention and control of oral cancer in Pakistan. There is also a distinct lack of clinical trials on oral cancer in Pakistan, with just two trials being conducted, both of which were not randomized (Shaharyar et al., 2006; Masud et al., 2007). Trials are an important tool for establishing cost effective treatment and prevention measures, and should be the priority of cancer research in low and middle income countries (Sankaranarayanan and Boffetta, 2010; Magrath, 2010). In contrast, in India cancer research, including oral cancer, comprises of a number of case control studies. Although these studies might not be up to the standards of those in developed countries, this is the right approach to assess risk factors (Sullivan et al., 2014). In addition to these, focusing on established lifestyle factors (Gupta and Johnson, 2014), a few cohort and interventional studies have also been well documented (Gupta et al., 1986; Muwonge et al., 2008; Jayalekshmi et al., 2009; Jayalekshmi et al., 2011).

Our finding that almost 50% of the total publications included in this review were published in just eight of the 65 journals which published oral cancer research from Pakistan, is comparable to similar studies in cancer research and other fields of health research around the world (Tsay and Yang, 2005; Patra and Bhattachariya, 2005). These journals can therefore be regarded as core journals which form the literature basis for oral cancer research in Pakistan (Garfield, 2006). The previously mentioned directive by the governing bodies of medicine and education on having a certain number of publications for promotion purposes resulted in authors publishing in certain journals, which are recognized by the HEC and PMDC, but are often non-indexed with the globally recognized Indices (Ghaffar et al., 2013; Mushtaq et al., 2012). These journals have limited circulation and often very little web presence, making it difficult to reach potential stakeholders and policy makers. In conducting this review, we were faced with difficulties in finding articles from some of these non-indexed journals. This highlights the fact that the research findings published in these journals may have little impact if they fail to reach the intended audience.

Karachi is the biggest metropolis in Pakistan (World Population Statistics, 2013) and has the highest concentration of medical universities in Pakistan. Hence it is not surprising that it has the highest research output among all the Pakistani cities. The point of concern however is the lack of oral cancer research output from the bigger cities such as Quetta and Peshawar, which, despite having a heavy burden of oral cancer, have contributed very little to its research (Begum et al., 2009; Roohullah et al., 2012). Also of concern is the lack of research from the FATA and Gilgit Baltistan provinces. There are no epidemiological data whatsoever available on the prevalence or incidence of oral cancer in these areas. There appears to be a gradient of inequality in oral cancer research carried out in Pakistan, with less research being done in areas with poorer access to healthcare. This scenario as a whole is comparable to oral health

improvement and disease prevention on a global level, where marked inequalities exist both inter and intra regionally (Sgan-Cohen et al., 2013). Oral cancer research output appears to be associated with the regional human development index of Pakistan (Jamal and Khan, 2007), with, as observed in our study, districts which score high on the human development scale having more research output and vice versa.

With regards to institutions, the trend is similar to that of the geographical distribution, with institutions based in larger cities producing more research publications than those in smaller, less developed cities. The Comsats institute of information technology is a relatively new academic institution which is principally non-medical. Despite this, it has produced the most number of publications, and along with Karachi cancer registry and King Edward medical university, has collaborative publications with authors from other countries. A finding of this review which is of great concern is the lack of recent publications from the Karachi cancer registry. A possible explanation could be the death of its founder who was the principal author in most studies produced from the registry. This is a possible indication that research in Pakistan is generally not institutionalised, but rather depends on personal motivation, and in some cases, is an activity imposed by the respective authorities (15).

A further finding of concern is the lack of research output from the cancer specialty hospitals of the Pakistan atomic energy commission (PAEC) and the biggest cancer hospital in the country, Shaukat Khanum memorial cancer hospital (SKMCH). There are just three publications by first authors affiliated to the PAEC hospitals, and four publications from the SKMCH.

In conclusions, oral cancer is a big public health problem in Pakistan and as such needs a serious commitment and a holistic approach to tackle it. The lack of timely and quality research informing policy and practice can be a hindrance to such an approach. This study highlights the fact that oral cancer research output from Pakistan is lacking in both relative and absolute terms, and also that the type of research studies carried out may not be in line with the cancer research needs of Pakistan.

Cancer is a heterogeneous disease. Hence, the strategies needed for its prevention as well as the research needed to devise such strategies should be heterogeneous. Although the publications on oral cancer from Pakistan reviewed in this paper do tackle various topics regarding oral cancer, much of the research done is of a very basic level. Most publications are based on institutional records and there is a lack of population based studies.

To our knowledge, this research work is the first effort which has been made to collect and summarize all the oral cancer research done in Pakistan for future use by researchers and other relevant stakeholders. This review was based entirely on electronic search, and though we tried to include all relevant studies, it cannot be ruled out that we missed some of the literature. Citation analysis, which is sometimes used in such publications could not be carried out due to the incompleteness of suitable data required for such an exercise. This was due to the presence of a considerable number of studies that are unfortunately

not included in ISI-Web of Science or Scopus databases, where data is available for more complex bibliometric analysis.

The government needs to look into setting up a national oral cancer research agenda based on local needs. This should be done in collaboration with the various stake holders such as the Pakistan Medical Research Council, PAEC, academia, SKMCH, the Karachi and Punjab cancer registries, the pharmaceutical industry and provincial health departments. Once an agenda and research priorities are set, research projects in the area of need, should be commissioned to the educational and research institutes. These in turn should carry out these projects in collaboration with clinicians and hospital staff, who otherwise are unable to conduct research on their own, due to a heavy patient load.

Although communicable diseases are often on the priority list of policy makers in Pakistan, the importance of non-communicable diseases such as oral cancer need to be realized and more concerted efforts should be made for their prevention and control. Strategies should be devised to combat the high incidence of oral cancer in the country and such strategies should be based on sound scientific research. A holistic approach to cancer research, bringing together both medical and non-medical institutions with relevant expertise, should be implemented, so that researchers with different skills complement each other. Research linkages between institutes working on oral cancer research should be established. The Pakistan medical research council (PMRC), with offices in all major cities of Pakistan, can act as a liaison among these institutions. The Offices of research, innovation and commercialization (ORIC) at the medical and general universities, which are responsible for research related activities in these universities and also coordinate with the HEC on research related funding, can help PMRC in bringing together these institutions.

At an institutional level, efforts should be made to forge links with international cancer research institutes, which can provide technical assistance in carrying out novel research projects. Collaborative research with these partners and the resultant publications can help increase the research understanding of local researchers, resulting in well executed research projects and high quality publications having an impact on oral cancer prevention, treatment and control.

At the individual level, authors should strive to publish research which has a direct impact on disease prevention and outcomes, and to come up with efficient and effective methods for the control of oral cancer, keeping the local context in mind. In choosing journals for publication, authors should try and publish their research work in journals which have a good scientific standing among the cancer research community and are easily accessible to potential stake holders, so that their research reaches its intended audience.

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Supplementary table: Salient characteristics of the included studies

Study Reference	Year of Publication	Journal	Type of Study	Location	Institutional affiliation	Sample size (n)	Primary focus of the study
(Vahidy et al., 1972)	1972	Surgical oncology	Case series	Karachi	Jinnah postgraduate medical centre	1192	Diagnosis / Accuracy of toluidine blue
(Jafarey & Zaidi, 1976a)	1976	Tropical doctor	Case control	Karachi	Jinnah postgraduate medical centre.	1,192/3,562*	Lifestyle risk factors for oral cancer
(Jafarey & Zaidi, 1976b)	1976	Journal of Pakistan medical association	Cross sectional	Karachi	Jinnah postgraduate medical centre	24,784	Distribution of oral cancer
(Jafarey et al., 1977)	1977	Journal of Pakistan medical association	Case control	Karachi	Jinnah postgraduate medical centre	1,192	Lifestyle risk factors for oral cancer
(Frequency of malignant tumours in seven centres of pakistan. pakistan medical research council cancer study group.1977)	1977	Journal of Pakistan medical association	Cross sectional	Islamabad	PMRC	-	Distribution of oral cancer
(Ibrahim et al., 1977)	1977	Clinical oncology	Case control	Karachi	Jinnah postgraduate medical centre	203/112*	Nutritional risk/protective factors for oral cancer
(Aziz-ur-Rehman & Jafarey, 1986)	1986	Journal of Pakistan medical association	Case control	Karachi	Jinnah postgraduate medical centre	50 / 100*	Viral risk factors for oral cancer
(Jafarey & Zaidi, 1987)	1987	Journal of Pakistan medical association	Cross sectional	Karachi	Jinnah postgraduate medical centre	6,192	Distribution of oral cancer

Supplementary table: Salient characteristics of the included studies

(Shah & Khan, 1992)	1992	Journal of environmental, pathology, toxicology and oncology	Case series	Abbottabad	Ayub medical college	186	Risk factors for oral cancer
(Khan & Ahmad, 1994)	1994	British dental journal	Report	Karachi	-	-	Report of a dental elective
(Ahmed & Jafarey, 1995)	1995	Journal of environmental, pathology, toxicology and oncology	Case control	Abbottabad	Ayub medical college	56/156 *	Viral risk factors for oral cancer
(Khan et al., 1995)	1995	Cancer	Case control	London	St. Thomas Hospital Fatima	24 /24*	Lifestyle risk factors for oral cancer
(Khurshid & Baseer, 1995)	1995	Pakistan armed forces medical journal	Case series	Lahore	Jinnah medical college	50	Risk factors for oral cancer
(Mirza, 1996)**	1996	Pakistan journal of otolaryngology and head and neck surgery	-	Karachi	Dow university of health sciences	N/A	Distribution of oral cancer
(Khan et al., 1997)	1997	Journal of Pakistan medical association	Cross sectional	Peshawar	Institute of radiotherapy and nuclear medicine	13,359	Distribution of oral cancer
(Mirza et al., 1997)**	1997	Pakistan journal of otolaryngology and head and neck surgery	-	Karachi	Dow university of health sciences	N/A	Protective/risk factors for oral cancer
(Mirza, 1997)**	1997	Pakistan journal of otolaryngology and head and neck surgery	-	Karachi	Dow university of health sciences	N/A	Molecular risk factors for oral cancer
(Bhurgri et al., 1998)	1998	Journal of Pakistan medical association	Cross sectional	Karachi	Karachi cancer registry	3,500,000	Oral cancer incidence/distribution

Supplementary table: Salient characteristics of the included studies

(Haider, 1998)	1998	Journal of Baqai medical university	Review article	Karachi	Baqai medical university	N/A	Care for cancer patients
(Mirza et al., 1998)	1998	Pakistan journal of otolaryngology and head and neck surgery	Case control	Karachi	Dow university of health sciences	130/25	Viral risk factors for oral cancer
(Mirza, 1998)**	1998	N/A	Basic laboratory research	Karachi	University of Karachi	N/A	Viral risk factors for oral cancer
(Ali et al., 1998)	1998	Biomedica	Case series	Lahore	Mayo hospital	202	Distribution patterns and characteristics of the cases.
(Bhurgri et al., 1999)	1999	Journal of Pakistan medical association	Cross Sectional	Karachi	Karachi cancer registry	1253	Oral cancer incidence/Distribution
(Latif et al., 1999)	1999	Journal of Rawalpindi medical college	Case control	Rawalpindi	Rawalpindi medical college	60/30*	Chemical risk factors
(Shah & Malik, 1999)**	1999	Annals of King Edward medical university	Case series	Lahore	Shaukat Khanum memorial cancer hospital	-	Treatment/Surgical techniques
(Latif et al., 1999)	1999	Journal of Rawalpindi medical college	Case control	Rawalpindi	Rawalpindi medical college	60 /30*	Chemical risk factors
(Bhurgri et al., 2000)	2000	International journal of cancer	Cross sectional	Karachi	Karachi cancer registry	4268	Cancer incidence including oral cancer/Distribution
(Merchant et al., 2000)	2000	International journal of cancer	Case control	Karachi / U S A	Harvard school of public health	79/149*	Lifestyle risk factors for oral cancer
(Latif et al., 2000)	2000	Pakistan armed forces medical journal	Case control	Rawalpindi	Rawalpindi medical college	60/30*	Chemical risk factors
(Asif & Muzafar, 2000)	2000	Journal of college of Physicians and surgeons Pakistan	Case series	Abbottabad	Ayub medical college	10	Patterns and characteristics of the cases/ Distribution

Supplementary table: Salient characteristics of the included studies

(Shah, 2001)	2001	Annals of King Edward medical university	Review	Lahore	De' Mont Morency college of dentistry	N/A	Care for cancer patients
(Cheema & Cheema, 2001) **	2001	Annals of King Edward medical university	Case series	Lahore	Mayo Hospital Lahore	-	Treatment/ Surgical technique
(Aziz et al., 2001)	2001	Journal of college of Physicians and surgeons Pakistan	Case series	Lahore	Allama Iqbal medical college	375	Histopathological patterns and characteristics of the cases.
(Ali et al., 2002)	2002	Journal of Ayub medical college	Review	Lahore	Shaukat Khanum cancer memorial hospital Karachi	N/A	Treatment/ Radiation technique
(Bhurgri et al., 2002)	2002	Journal of Pakistan medical association	Cross sectional	Karachi	Karachi cancer registry	14,243	Cancer incidence including oral cancer/ Distribution
(Zakai et al. , 2002)	2002	Annals of Abbasi Shaheed hospital and Karachi medical and dental college	Case series	Karachi	Abbasi Shaheed hospital	66	Pattern of oral cancer /Distribution
(Husein & Husein, 2002)	2002	Journal of Pakistan dental association	Review	Karachi	Fatima Jinnah dental college.	N/A	Role of general dentists in oral cancer prevention.
(Malik, 2002)	2002	Pakistan journal of pathology	Review	Rawalpindi	Armed forces institute of dentistry	N/A	Treatment / Screening methods for oral cancer
(Ghani, 2002)	2002	Journal of Pakistan association of dermatologists	Case report	Karachi	Liaquat national postgraduate centre	One	Lip cancer with verrucoid features
(Kadar & Maher, 2002)	2002	Journal of surgery Pakistan	Basic laboratory research	Karachi	Jinnah postgraduate medical centre	30	Diagnosis / Histological grading of oral cancer developing from sub mucous fibrosis

Supplementary table: Salient characteristics of the included studies

(Bhurgrī et al., 2003)	2003	Asian Pacific journal of cancer prevention	Case series /Overview	Karachi	Karachi cancer registry	1153	Review of risk factors
(Zakai et al., 2003)	2003	Annals of Abbasi Shaheed hospital and Karachi medical and dental college	Case series	Karachi	Abbasi Shaheed hospital	60	Risk factors for oral cancer
(Isaac et al., 2003)	2003	Journal of Pakistan dental association	Case series	Jamshoro	Liaquat university of medical and health sciences	43	Patterns and characteristics of the cases / Distribution
(Memon et al., 2003)	2003	Pakistan journal of surgery	Case series	Jamshoro	Liaquat university of medical and health sciences	76	Treatment modalities for oral cancer
(Isaac et al., 2004)	2004	Pakistan oral and dental journal	Case series	Jamshoro	Liaquat university of medical and health sciences	27	Histopathological features of oral cancer.
(Haider & Mehdi, 2004)	2004	Journal of Pakistan dental association	Case series	Karachi	Abbasi Shaheed hospital	70	Diagnosis /Ascertainment of referral source.
(Rashid et al., 2004)	2004	Journal of college of physicians and surgeons Pakistan	Case series	Rawalpindi	Combined military hospital Margalla	63	Treatment techniques
(Khurram et al., 2004)	2004	Journal of Pakistan dental association	Review article	Islamabad	institute of health sciences	N/A	Molecular risk factors
(Kadar & Hashmi, 2004)	2004	Medical channel	Case series	Karachi	Jinnah postgraduate medical centre	25	Patterns and characteristics of the cases/ Distribution
(Lehri et al., 2004)	2004	Journal of Pakistan dental association	Basic laboratory research	Quetta	Bolan medical college	40	Diagnosis / Evaluation of a diagnostic technique

Supplementary table: Salient characteristics of the included studies

(Khan et al., 2004)	2004	Federation dentaire international report	Pathfinder cross sectional survey	Nationwide	N/A	N/A	Distribution of oral disease
(Anwar et al., 2005)	2005	Journal of clinical and experimental cancer research.	Case series	Japan	Kagoshima University	56	Viral risk factors for oral cancer
(Bhurgri et al., 2005)	2005	Asian Pacific journal of cancer prevention	Cross sectional	Hyderabad	Karachi cancer registry Pakistan	2,840,653	Oral cancer age standardized rates / Distribution
(Hussain et al., 2005)	2005	Journal of college of Physicians and surgeons Pakistan	Cross sectional comparative	Islamabad	institute of medical sciences Ayub medical college	60	Risk factors for oral cancer
(Wahid et al., 2005)	2005	Journal of Ayub medical college	Case series	Abbottabad	Combined military hospital Khyber college of dentistry	60	Pattern of oral cancer/ Distribution
(Khan et al., 2005)	2005	Journal of college of physicians and surgeons Pakistan	Case report	Kohat	Rawalpindi General hospital	One	Treatment Modality for oral cancer.
(Khan & Khitab, 2005)	2005	Pakistan oral and dental journal	Case series	Peshawar	Margalla institute of health sciences.	80	Diagnosis / Histopathological grading of oral cancer
(Khawaja et al., 2005)	2005	Asian Pacific journal of cancer prevention	Letter to the editor/Review	Rawalpindi	Postgraduate medical institute Hayatabad medical complex Karachi cancer registry	N/A	Review of risk factors and preventive measures
(Khurram, 2005)	2005	Journal of Pakistan dental association	Basic laboratory research	United Kingdom/Islamabad		N/A	Molecular risk factors for oral cancer
(Jamil et al., 2005)	2005	Annals of King Edward medical university	Case control	Lahore		50/50*	Molecular risk factors for oral cancer
(M. Tahir, Ullah, & Khan, 2005)	2005	Journal of postgraduate medical Institute	Case series	Peshawar		20	Treatment / Surgical technique
(Bhurgri, Pervez et al., 2006)	2006	Asian Pacific journal of cancer prevention	Cross sectional	Karachi		1,724,915	Oral cancer age standardized rates / Distribution

Supplementary table: Salient characteristics of the included studies

(Bhurgrri et al., 2006)	2006	Asian Pacific journal of cancer prevention	Cross sectional	Karachi	Karachi cancer registry	552,509	Oral cancer age standardized rates / Distribution
(Najeeb, 2006)	2006	Journal of college of physicians and surgeons Pakistan	Case series	Islamabad	Pakistan institute of medical sciences	67	Patterns of oral cancer and treatment / Distribution
(Khan et al., 2006)	2006	Pakistan oral and dental journal	Case series	Peshawar	Khyber college of dentistry	23	Diagnosis / Histopathological grading of tongue cancer
(Jamal et al., 2006)	2006	Pakistan armed forces medical journal	Case series	Rawalpindi	Armed forces Institute of pathology	723	Patterns and characteristics of the cases / Burden of different types of cancers in Pakistan including oral cancer / Distribution
(Bhurgrri et al., 2006)	2006	Journal of Pakistan medical association	Review	Karachi	Karachi cancer registry	N/A	Treatment modalities for oral cancer
(Ahmed, 2006)	2006	Journal of Pakistan dental association	Review	Karachi	Jinnah postgraduate medical centre	N/A	Treatment modalities for oral cancer
(Shaharyar et al., 2006)	2006	Pakistan journal of medical sciences	Clinical trial	Lahore	King Edward medical college	39	Treatment modalities for oral cancer
(Ikram et al., 2006)	2006	Journal of Pakistan medical association	Case series	Karachi	Aga Khan University	80	Follow up / Recurrence of oral cancer
(Jamil et al., 2006)	2006	Annals of King Edward medical university	Case control	Lahore	Ghurki hospital	50/50*	Molecular risk factors for oral cancer
(Ahmed et al., 2007)	2007	Journal of college of Physicians and surgeons Pakistan	Case series	Wah	Wah medical college	37	Diagnosis / Occult metastasis in oral cancer
(Z. Ahmed et al., 2007)	2007	Journal of Ayub medical college	Cross sectional	Karachi	Aga khan university	20,000	Oral cancer prevalence and decade of life / Distribution

Supplementary table: Salient characteristics of the included studies

(Ahmed et al., 2007)	2007	Journal of surgery Pakistan	Case series	Wah	Wah medical college	714	Patterns and characteristics of the cases / Distribution
(Khan et al., 2007)	2007	Journal of Pakistan dental association	Case series	Peshawar	Khyber college of dentistry	100	Niswar as a risk factor for oral cancer
(Masud et al., 2007)	2007	Professional medical journal	Clinical Trial	Multan	Nishtar hospital	200	Treatment modalities for oral cancer
(Akhtar et al., 2007)	2007	Journal of Pakistan medical association	Case series	Karachi	Aga Khan university	32	Treatment / Surgical technique
(Butt et al., 2007)	2007	Annals of King Edward medical university	Review article	Lahore	King Edward medical university	N/A	Viral risk factor for oral cancer.
(Chaudhry et al., 2008)	2008	Asian Pacific journal of cancer prevention	Cross sectional	Lahore	Sheikh Zayed federal post graduate institute	41,4017	Institution based prevalence of head and neck cancers / Distribution
(Ali et al., 2008)	2008	Oral surgery	Case series	Karachi	Aga Khan University	140	Viral risk factors for oral cancer
(Sharif et al., 2008)	2008	Journal of college of Physicians and surgeons Pakistan	Case report	Rawalpindi	Armed forces institute of pathology	One	Rare biphasic synovial sarcoma of oral cavity
(Mirza et al., 2008)	2008	Pakistan journal of otolaryngology and head and neck surgery	Case control	Karachi	Dow university of health sciences	130/65*	Diagnosis
(Ahmed et al., 2008)	2008	Rawal medical journal	Case series	Islamabad	Pakistan institute of medical sciences	37	Treatment / Surgical technique
(Panjwani & Sadiq, 2008)	2008	Pakistan journal of medical sciences	Basic laboratory research	Karachi	Jinnah postgraduate medical centre	423	Genetic risk factors for oral cancer
(Abid et al., 2008)	2008	Annals of King Edward medical university	Case series	Lahore	King Edward medical university	30	Treatment / Surgical technique

Supplementary table: Salient characteristics of the included studies

(Musani et al., 2009)	2009	Journal of Ayub medical college	Case series	Karachi	Abbasi Shaheed hospital	45	Patterns of oral (cheek) cancer / Distribution
(Khan et al., 2009)	2009	Journal of Dow university of health sciences	Case series	Karachi	Civil hospital	50	Prevalence of risk factors for oral cancer among cases.
(Begum et al., 2009)	2009	Journal of postgraduate medical Institute	Case series	Peshawar	Institute of radiology and nuclear medicine	628	Patterns of oral (cheek) cancer / Distribution
(Hanif et al., 2009)	2009	Asian pacific journal of cancer prevention	Case series	Karachi	University of Karachi	16351	Cancer trends including head and neck cancers / Distribution
(Isaac et al., 2009)	2009	Journal of Liaquat University of medical and health sciences	Case series	Jamshoro	Liaquat university of medical and health sciences	106	Histopathological grading of oral cancer
(Rizvi et al., 2009)	2009	Journal of college of Physicians and surgeons Pakistan	Cross sectional	Rawalpindi	Combined military hospital	37	Quality of life with surgical prostheses
(Cancela et al., 2010)	2010	Head and Neck	Cross sectional	Worldwide including Pakistan	Karachi cancer registry	108,780	Oral cancer age standardized rates/ Distribution
(Alamgir et al., 2010)	2010	Pakistan journal of otolaryngology and head and neck surgery	Review	Karachi	Bahria University	N/A	Genetic risk factors
(Bile et al., 2010)	2010	Eastern Mediterranean health journal	Cross sectional	Islamabad	World health organization regional office	29,307	Prevalence and risk factors for oral cancers
(Fadoo et al., 2010)	2010	Journal of Pediatric Hematology Oncology	Case report	Karachi	Aga Khan university	One	Squamous cell carcinoma in young age.
(Zaidi et al., 2010)	2010	Journal of Pakistan medical association	Review/Short communication	Karachi	Aga Khan university	N/A	Prevention / Head and neck cancer control

Supplementary table: Salient characteristics of the included studies

(Kazi et al., 2010)	2010	Journal of hazardous material	Case control	Jamshoro	University of Sindh	96/110*	Chemical risk factor for oral cancer
(Kazi et al., 2010)	2010	Human and experimental toxicology	Case control	Jamshoro	University of Sindh	92/78 *	Chemical risk factor for oral cancer
(Liaqat et al., 2010)	2010	Journal of Ayub medical college	Case series	Rawalpindi	Armed forces institute of dentistry	24	Treatment technique
(Nosheen et al., 2010)	2010	Asian Pacific journal of cancer prevention	Case control	Islamabad	Comsats institute of information technology	388/150*	Genetic risk factors for head and neck cancer
(Shah et al., 2010)	2010	Journal of Ayub medical college	Case series	Rawalpindi	Armed forces Institute of dentistry	334	Stages of oral cancer at diagnosis
(Shahid et al., 2010)	2010	Journal of basic and applied sciences	Basic laboratory research	Karachi	Dow university of health sciences	518	Diagnostic procedure
(Akram et al., 2010)	2010	Pakistan journal of otolaryngology and head and neck surgery	Basic laboratory research	Karachi	Dow university of health sciences	301	Histopathological features of oral cancer.
(Shafique et al., 2010)	2010	Journal of Pakistan dental association	Case series	Karachi	Civil hospital	60	Patterns and characteristics of the cases. / Distribution
(Ali et al., 2010)	2010	Oral surgery	Case series	Karachi	Aga Khan university	140	Histopathological characteristics of oral cancer.
(Khan et al., 2010)	2010	Anesthesia, pain and intensive care	Case report	Lahore	Shaukat Khanum memorial cancer hospital	One	Follow up/Glossopharyngeal neuralgia after radiation treatment for tongue cancer
(Riaz & Warriach, 2010)	2010	Journal of Ayub medical college	Cross sectional	Lahore	King Edward medical university	30	Quality of life with surgical prostheses

Supplementary table: Salient characteristics of the included studies

(Ayaz et al., 2011)	2011	Biomedica	Case series	Karachi	Armed forces institute of pathology	268	Patterns and characteristics of the cases. / Distribution
(Nazir et al., 2011)	2011	Asian Pacific journal of cancer prevention	Case series/Lab	Islamabad	Comsats institute of information technology	120	Genetic risk factors
(Mehboob et al., 2011)	2011	Pakistan oral and dental journal	Cross sectional	Peshawar	Khyber college of dentistry	236	Oral cancer knowledge assessment.
(Urooj et al., 2011)	2011	Journal of Dow university of health sciences	Cross sectional	Karachi	Dow university of health sciences	399	Frequency of oral cancer/ Distribution
(Bashir et al., 2011)	2011	Journal of Ayub medical college	Cross sectional comparative	Lahore	Shaukat khanum memorial cancer hospital	28	Diagnosis / Reliability of MRI to gauge thickness of tumor
(Bhurgri, 2011)	2011	IARC scientific publications	Case series	Karachi	Karachi cancer registry	413	Five year survival rate for oral cancer cases / Follow up
(Castillo et al., 2011)	2011	World journal of gastroenterology	Cross sectional	Multiple countries including Pakistan/ Lahore	Kagoshima university/ King Edward medical university	71	Viral risk factors for oral cancer
(Mahjabeen et al., 2011)	2011	Asian Pacific journal of cancer prevention	Case control	Islamabad	Comsats institute of information technology	300/ 300*	Genetic risk factors for head and neck cancer
(Masood et al., 2011)	2011	Asian Pacific journal of cancer prevention	Case control	Islamabad	Comsats institute of information technology	228/150*	Genetic risk factors for head and neck cancer
(Rana et al., 2011)	2011	Head and neck oncology	Cross sectional comparative	Lahore	Hannover medical school / King Edward	398	Treatment / Retrospective comparison between surgical techniques for Ca tongue.

Supplementary table: Salient characteristics of the included studies

(Sobani et al., 2011)	2011	Journal of Pakistan medical association	Cross sectional comparative	Karachi	medical university Aga khan university	29	Care / Comparison of feeding methods in oral cancer patients
(Sobani et al., 2011)	2011	Journal of Pakistan medical association	Case report	Karachi	Aga khan university	One	Mucoepidermoid carcinoma of tongue
(Kazmi et al., 2011)	2011	Pakistan oral and dental journal	Cross sectional KAP survey	Lahore	University of Lahore	760	Knowledge about oral cancer, its prevention and screening etc.
(Haq et al., 2011)	2011	Journal of college of Physicians and surgeons Pakistan	Case series	Lahore	King Edward medical university	50	Histopathological findings.
(Riaz, 2011)	2011	Pakistan journal of medical health sciences	Case series	Lahore	King Edward medical university	237	Treatment / Surgical technique
(Khan et al., 2011)	2011	Professional medical journal	Case series	Peshawar	Khyber college of dentistry	25	Treatment / Surgical technique
(Malik et al., 2011)	2011	Pakistan journal of medical health sciences	Case series	Lahore/	Allama Iqbal medical college/ King Faisal Hospital Riyadh	25	Treatment / Surgical technique
(Ahmed et al., 2011)	2011	Journal of college of Physicians and surgeons Pakistan	Case report	Lahore	University of Faisalabad	One	Treatment modalities for oral cancer
(Riaz & Warraich, 2011)	2011	Annals of King Edward medical university	Case series	Lahore	King Edward medical university	64	Patterns and characteristics of the cases. / Distribution
(Masood et al., 2011)	2011	Asian Pacific journal of cancer prevention	Review	Rawalpindi	Comsats institute for information technology	49/49*	Genetic risk factors for head and neck cancer

Supplementary table: Salient characteristics of the included studies

(Masood & Kayani, 2011)	2011	Genetics and molecular biology	Case control	Islamabad	Comsats institute for information technology	388/150*	Genetic risk factors for head and neck cancer
(Gillani et al., 2012)	2012	Asian Pacific journal of cancer prevention	Case series	Rawalpindi	Armed forces institute of pathology	40	Diagnosis / Diagnostic accuracy of a technique
(Janjua & Qureshi, 2012)	2012	Journal of skin cancer	Cross sectional	Faisalabad	Punjab medical college	171	Basal cell carcinoma of head and neck / Distribution
(Junaid et al., 2012)	2012	World Journal of surgical oncology	Basic laboratory research	Karachi	Jinnah medical and dental college	280	Diagnosis / Diagnostic accuracy of toluidine blue
(Kazmi et al., 2012)	2012	Journal of Pakistan medical association	Case series	Karachi	Aga Khan university	72	Follow up/ Size of tumor as a prognostic factor for recurrence
(Kazmi et al., 2012)	2012	Pakistan oral and dental journal	Cross sectional	Lahore	University of Lahore	75	Knowledge about oral cancer, its prevention and screening etc.
(Roohullah. et al., 2012)	2012	Annals of Punjab medical college	Cross sectional	Quetta	CENAR	270	Cancer Prevalence including oral cancer / Distribution
(Zaib et al., 2012)	2012	Pakistan oral and dental journal	Case series	Islamabad	Islamic international medical and dental college	114	Histopathological findings
(Mangi et al., 2012)	2012	Pakistan journal of surgery	Case series	Karachi	Jinnah postgraduate medical centre	28	Treatment modalities for oral cancer
(Khan, 2012)	2012	Webmed central	Review	Abbottabad	NIMS medical college	N/A	Oral cancer prevention

Supplementary table: Salient characteristics of the included studies

(Makhdoom & Ullah, 2012)	2012	Journal of Khyber college of dentistry	Cross sectional Knowledge assessment survey	Peshawar	Khyber college of dentistry	200	Knowledge about oral cancer, its prevention and screening etc.
(Rehman & Khan, 2012)	2012	Pakistan oral and dental journal	Cross sectional Knowledge assessment survey	Peshawar	Khyber college of dentistry	100	Knowledge about oral cancer, its prevention and screening etc.
(Rehman et al., 2012)	2012	Journal of khyber college of dentistry	Case series	Peshawar	Khyber college of dentistry	102	Assessment of quality of life among oral cancer patients.
(Akhtar et al., 2012)	2012	Annals of Pakistan institute of medical sciences	Case series	Rawalpindi	Armed forces institute of pathology	240	Histopathological features of oral cancer.
(Ahmed et al, 2012)	2012	Pakistan journal of medical health sciences	Cross sectional	Multan	Combined military hospital	88	Diagnostic delay as a risk factor
(Masood et al., 2012)	2012	Asian Pacific journal of cancer prevention	Case control	Islamabad	Comsats institute of information technology	49/49*	Genetic risk factors
(Mahjabeen et al., 2012)	2012	Medical oncology	Case control	Islamabad	Comsats institute of information technology	50**	Genetic risk factors
(Sabir et al., 2012)	2012	Hereditary cancer in clinical practice	Case control	Islamabad	Comsats institute of information technology	380/350*	Genetic risk factors
(Maqsood et al., 2012)	2012	Medical forum monthly	cross sectional	Karachi	Dow university of health sciences	279	Diagnostic delay as a risk factor
(Mahjabeen et al., 2013)	2013	Mutagenesis	Case control	Islamabad	Comsats institute of information technology	300/300*	Genetic risk factors
(Junaid et al., 2013)	2013	Journal of Pakistan medical association	Basic lab research	Karachi	Jinnah medical	56	Diagnostic accuracy of toluidine blue.

Supplementary table: Salient characteristics of the included studies

(Sabir et al., 2013)	2013	International journal of biological markers	Case control	Islamabad	college hospital Comsats institute of information technology	380/350*	Genetic risk factors
(Akram et al., 2013)	2013	Pakistan journal of medical sciences	Case control	Karachi	Sindh medical college	100/100*	Multiple lifestyle risk factors for oral cancer
(Iqbal et al., 2013)	2013	Oral disease	Basic laboratory research	Lahore	King Edward medical university	80	Viral risk factors for oral cancer
(Mahjabeen et al., 2013)	2013	Pathology oncology research	Case control	Islamabad	Comsats institute of information technology	300/150*	Genetic risk factors for head and neck cancer
(Saleem et al., 2013)	2013	Oral oncology	Case control	Karachi	University of Karachi	260/260*	Genetic risk factors, related with smokeless tobacco use, for head and neck cancer.
(Tahir et al., 2013)	2013	Journal of cancer research and therapeutics	cross sectional comparative	Bhawalpur	Quaid e Azam medical college	37	Chemical risk factors for oral cancer
(Ikram et al., 2013)	2013	Journal of Pakistan medical association	Case series	Karachi	Aga Khan university	42	Treatment modalities for oral cancer
(Siddiqui, 2013)	2013	Pakistan journal of medicine and dentistry	Review	Karachi	Ziauddin University	N/A	Prevention of oral cancer.
(Ashfaq et al., 2013)	2013	Journal of Rawalpindi medical college	Case series	Rawalpindi	Combined military hospital	95	Patterns, characteristics and treatment procedures for carcinoma tongue
(Chaudhary et al., 2013)	2013	Rawal medical journal	Case series	Bhawalpur	Quaid e Azam medical college	184	Patterns and characteristics of the cases. / Distribution

Supplementary table: Salient characteristics of the included studies

(Rasool et al., 2013)	2013	Isra medical journal	Cross sectional	Islamabad	Islamic international university	140	knowledge about oral cancer, its prevention and screening etc.
(Hassan et al., 2013)	2013	Medical forum monthly	Cross sectional knowledge assessment survey	Jamshoro	Liaquat university of medical and health sciences	50	Knowledge about oral cancer, its prevention and screening etc.
(Alamgir et al., 2013)	2013	Pakistan journal of medicine and dentistry	Case series	Karachi	Bahria university	50	Patterns and characteristics of the cases / Distribution
(Zulfiqar et al., 2013)	2013	Biomedica	Case series	Lahore	University of health sciences	39	Histopathological patterns and characteristics of the cases.
(Janjua et al., 2013)	2013	Journal of college of Physicians and surgeons Pakistan	Case series	Faisalabad	Punjab medical college	319	Histopathological patterns and characteristics of the cases.
(Rubab et al., 2013)	2013	Journal of Pakistan medical association	Review	Karachi	Ziauddin university	N/A	Viral risk factor for oral cancer.
(Khyani et al., 2013)	2013	Pakistan journal of otolaryngology and head and neck surgery.	Review	Karachi	Dow university of health sciences	N/A	Diagnostic techniques.
(Tahir et al., 2013)	2013	Theoretical biology and medical modelling	Basic laboratory research	Islamabad	Islamic international university	N/A	The role of a specific tumor necrosis factor in development of an anti-cancer drug.
(Khaliq et al., 2013)	2013	Springer plus	Case series	Karachi	University of Karachi	5134	Ethnicity as a risk factor.
(Masood & Kayani, 2013)	2013	Pathology oncology research	Case control	Islamabad	Comsats institute of information technology	192	Genetic risk factors

Supplementary table: Salient characteristics of the included studies

(Sabir et al., 2013)	2013	Molecular biology reports	Case control	Islamabad	Comsats institute of information technology	300 / 270*	Genetic risk factors
(Masood et al., 2013)	2013	Asian Pacific journal of cancer prevention	Review	Islamabad	Comsats institute of information technology	N/A	Genetic risk factors
(Malik et al., 2014)	2014	International journal of clinical chemistry	Case control	Karachi	University of Karachi	45/30*	Chemical risk factors for oral cancers
(Masood et al., 2014)	2014	Molecular biology reports	Review article	Rawalpindi	Comsats institute of information technology	N/A	Genetic risk factors

*number of cases/number of controls, **abstract and/or full text not accessed, included in the supplemental table but not in the review, *** both cases and controls included, N/A: Not applicable.

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ARTICLE II

SMOKELESS TOBACCO AND ORAL CANCER IN SOUTH ASIA: A SYSTEMATIC REVIEW WITH META-ANALYSIS.

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Author's contributions: ZK developed the concept for the study, conducted the literature search, assessed studies for quality and for inclusion in the review and extracted data, also prepared drafts and undertook edits. JT was involved in quality assessment, data extraction, and editing. SM was involved in the development of the study concept, literature search, paper selection and narrative synthesis. All authors contributed to the editing of the drafts and have read and approved all versions of the manuscript.

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Review Article

Smokeless Tobacco and Oral Cancer in South Asia: A Systematic Review with Meta-Analysis

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Introduction. Smokeless tobacco is considered one of the major risk factors for oral cancer. It is estimated that over 90% of the global smokeless tobacco use burden is in South Asia. This paper aims to systematically review publications reporting epidemiological observational studies published in South Asia from 1984 till 2013. **Methods.** An electronic search in “Medline” and “ISI Web of Knowledge” yielded 734 publications out of which 21 were included in this review. All publications were assessed for quality using a standard quality assessment tool. Effect estimates (odds ratios (OR)) were abstracted or calculated from the given data. A random effects meta-analysis was performed to assess the risk of oral cancer with the use of different forms of smokeless tobacco. **Results and Conclusion.** The pooled OR for chewing tobacco and risk of oral cancer was 4.7 [3.1–7.1] and for paan with tobacco and risk of oral cancer was 7.1 [4.5–11.1]. The findings of this study suggest a strong causal link between oral cancer and various forms of smokeless tobacco. Public health policies in affected countries should consider SLT specific cessation programs in addition to campaigns and activities incorporated into smoking cessation programs.

1. Introduction

Oral cancer is one of the most common noncommunicable diseases worldwide with an estimated increase of 275,000 new cases each year [1]. Oral cancer is the term used for cancers that form in tissues of the oral cavity (the mouth) or the oropharynx (the part of the throat at the back of the mouth) [2]. These along with other head and neck cancers are the sixth most prevalent type of cancer in the world [3, 4] and one of the leading causes of death in developing countries [5]. The countries of South Asian region including India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Bhutan, Nepal, Iran, and Maldives [6] are particularly affected, with oral cancer ranking either first or second with regard to different types of cancer prevalence in these countries [7].

The reasons for the high prevalence of head and neck cancers in South Asia have been investigated to some extent but, as is the case with most developing countries, a lack of research infrastructure has put constraints on studying the epidemiology of these conditions in the context of

South Asia [8]. One of the major risk factors associated with the high prevalence of head and neck cancer and oral potentially malignant diseases (OPMD) in this region is smokeless tobacco (SLT) [9]. It is estimated that over 90% of the global smokeless tobacco use burden is in South East Asia [10]; around 100 million people use smokeless tobacco in India and Pakistan alone [11]. SLT is used in many forms varying from chewing tobacco not mixed with any other ingredient to a mixture of tobacco with other ingredients such as in betel quid, areca nut with tobacco, Naswar, paan-masala with tobacco, Gutkha, Khaini, and Mishri [12, 13]. Smokeless tobacco contains around 28 known carcinogens. These include the nonvolatile alkaloid-derived tobacco-specific N-nitrosamine and N-nitrosamino acids as the major group while volatile tobacco-specific nitrosamines, volatile aldehydes, and some poly nuclear agents have also been shown to be present in smokeless tobacco [14].

With such a high prevalence of both SLT use and oral cancer in the South Asian region, it is of utmost importance that epidemiological research is carried out to carefully

assess their detailed relationship. Two published reviews coauthored by IARC researchers have focused on overall associations found in studies worldwide [15, 16]. Several overviews originating from South Asia have been published on oral cancer and smokeless tobacco [15, 17–26] but to date no systematic review of the published literature on association of oral cancer with different forms of smokeless tobacco focusing specifically on South Asia has been conducted. This paper aims to address the issue by systematically reviewing publications reporting epidemiological observational studies carried out/published in the South Asian region during the last 30 years, that is, published after 1984 on the use of all forms of SLT and its relationship with oral cancer.

2. Methods

2.1. Search Strategy. An electronic search was carried out in “Medline” and “ISI Web of Knowledge” in August 2013. Various combinations of the terms “oral premalignant disease,” “oral precancer,” “leukoplakia,” “erythroplakia,” “submucous fibrosis,” “lichen planus,” “oral cancer,” “oral carcinoma,” “mouth neoplasm,” “head and neck cancer,” “squamous cell carcinoma of the oral cavity,” “carcinoma lip,” “carcinoma tongue,” “oral neoplasms,” and “head and neck neoplasms”; “smokeless tobacco,” “Naswar,” “paan,” “snuff,” “oral snuff,” “chewing tobacco,” “betel quid,” “areca nut,” and “Gutkha”; “Pakistan,” “India,” “Bangladesh,” “Iran,” “Sri Lanka,” “Afghanistan,” “Bhutan,” “Nepal,” “Maldives” were used. The terms for different OPMDs were included in the search process as sometimes these conditions are studied together with oral cancer. No filters were used during the electronic searches.

2.2. Publication Selection. The following selection criteria were applied to all the publications returned by the electronic searches to be included in the review.

2.2.1. Inclusion Criteria. Inclusion criteria are as follows:

- (i) papers published after 1984,
- (ii) epidemiological observational study in humans of cohort or case-control design,
- (iii) studies carried out in “South Asia” according to the United Nations geographical region classification (including the following countries: India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Bhutan, Nepal, Iran, and Maldives),
- (iv) reported outcome or one of the reported outcomes is oral cancer or head and neck cancer, and
- (v) exposure to paan, Gutkha, betel nut, areca nut, or any other type of smokeless tobacco.

2.2.2. Exclusion Criteria. Exclusion criteria are as follows.

- (i) Studies reporting oesophageal, base of the tongue, and salivary glands cancers were excluded.
- (ii) Studies involving laboratory research and molecular/genetic epidemiology were excluded.

The selection process was done in three steps: first, the titles of all publications were scanned and relevant publications selected. The next step involved reading the abstracts of the publications selected in the first step. Full text of the publications identified during step two were then obtained. The selected publications were then divided into three groups according to their reported outcomes: (1) OPMD as an outcome, (2) oral cancer as an outcome, and (3) OPMD and oral cancer as an outcome; publications reporting only OPMDs as an outcome were excluded at this stage. Reference lists of the selected publications were scanned to identify any additionally relevant publications.

2.3. Quality Assessment. All selected publications were assessed for their quality on the basis of the “Effective Public Health Practice Project Quality Assessment Tool for Quantitative Studies” [27]. Studies were ranked as “strong,” “moderate,” and “weak” after being assessed on six parameters, that is, selection bias, study design, confounding, blinding, data collection methods, and withdrawals and dropouts. Quality assessment was carried out by two authors independently and the results were later compared. Any differences were discussed in the presence of all three authors and a final decision was reached by mutual consensus.

2.4. Data Extraction. Data extraction was carried out between October and December 2013. First, data regarding the study type, location of the conducted study, sample size, year of publication, exposure, outcome, and the effect size were tabulated separately by two authors and later compared in the presence of all three authors. The data were then divided into two broad groups according to the difference in the type of SLT exposure, that is, “paan or betel quid with tobacco” and “chewable tobacco which included all types of smokeless tobacco other than paan.” Adjusted odds ratios (OR) along with their 95% confidence intervals (95% CI), if reported, were recorded. In studies, where OR were not reported but the data required to calculate them were available, OR were calculated using a Mantel-Haenszel (MH) approach, thus providing us with weighted OR across the different strata reported. However, if the paper did not report an adjusted OR and the data given were too scarce to calculate MH-OR, then the crude OR as reported in the paper or calculated from the given data was recorded. OR were also recorded or calculated for male and females separately, total duration of the habit in years, and frequency of daily use. Again efforts were made to record the most adjusted measure, whenever permissible. Standard errors of the natural logs of the OR were calculated either from the 95% CI of the respective log OR or by using the formula $SE(\ln OR_{MH}) = \sqrt{\sum (b_{1c_i}/N_i)^2 v_i / (\sum b_{1c_i}/N_i)^2}$ when a MH-OR was calculated.

2.5. Meta- and Heterogeneity Analysis. During the data extraction stage it had become obvious that there was major heterogeneity regarding methodological and other parameters among the selected publications. Nevertheless all data were entered into Rev Man 5.2 [28] and meta-analyses

performed across all exposure categories, and their effect on oral cancer separately and combined was recorded. This was done with the inverse variance method using both fixed and random effects. This also provided the I^2 estimates of statistical heterogeneity. The I^2 estimate was used to assess heterogeneity as it provides a better estimate for quantifying heterogeneity. Heterogeneity was considered low if the I^2 estimate was below 25%, moderate if it was between 25 and 50%, high if it was between 50 and 75%, and very high above the value of 75%. Due to a very high level of heterogeneity, random effect meta-analysis has been used for this review. Sensitivity and influence analysis were done by excluding one study at a time and checking its effect on the pooled estimate and the heterogeneity, but this had little effect on lowering the I^2 statistic.

Meta-analyses were performed for overall estimates, case-control studies, studies with hospital controls, cohort studies, studies from India only, studies from southern India only, studies for Maharashtra state, studies adjusting for smoking and/or alcohol, studies with moderate quality, and studies involving only men.

2.6. Narrative Synthesis. For the categories where the data was incomplete, unavailable, or calculated using different methods, for example, the exposure response categories, a narrative synthesis was done. The synthesis highlights the highest and lowest estimates in general, according to gender and for studies that had done adjustment for alcohol and/or smoking.

3. Results

A total of 734 publications were identified from both database searches (Medline, ISI Web of Knowledge) (Figure 1). One more paper was identified from a supplementary web search but it just reported the findings from one of the included studies and hence was excluded. After the first round of exclusion 137 publications remained; after reading the abstracts, 38 publications were selected and their full text versions obtained. 4 publications were excluded after examining the full paper. This left us with a total of 34 publications. 21 publications reported oral cancer as the outcome or one of the outcomes and 13 publications reported just OPMD as the outcome. The publications corresponding to OPMD were excluded at this stage.

The 21 publications [29–49] for oral cancer included in this review correspond to 19 different studies and three studies were of cohort design while the remaining were of case-control design (Table 1). Two studies were carried out in Pakistan and the rest in India. 13 publications were published in or after the year 2000 while the remaining publications were published before the year 2000, the oldest publication being from 1989.

11 of the selected publications reported or contained data on paan with tobacco (betel quid) as a risk factor whereas 14 publications reported or contained data on chewing tobacco other than paan or without specifying any particular type of

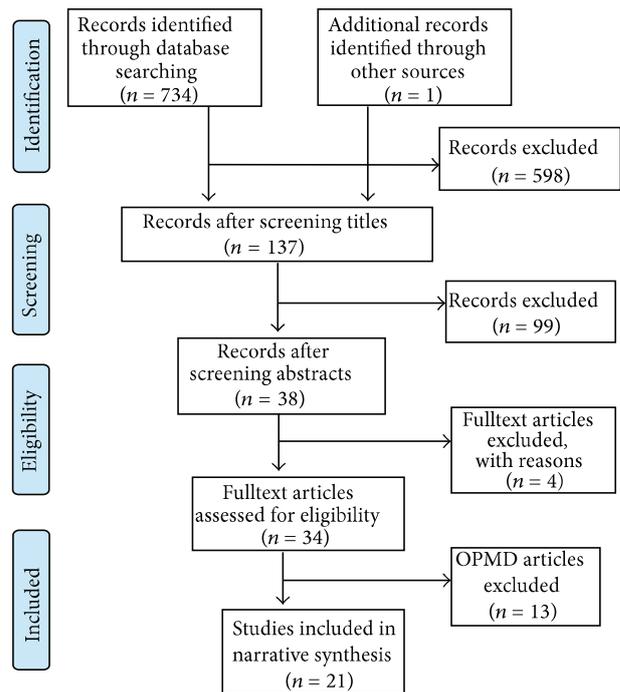


FIGURE 1: Flow chart of selection process of articles included in the review.

SLT. 11 publications reported or contained data stratified by sex.

Data regarding daily frequencies of smokeless tobacco use were reported in 14 publications, while data on the total duration of the habit was reported in 10 publications. Table 1 includes all selected studies for oral cancer and their features along with the quality assessment result for each study.

The values for I^2 statistic ranged from 77% to 96% when pooling studies across different strata. Core findings from the included publications are given in Table 1. Additional characteristics of the included studies are presented in the supplementary Table 1 available online at <http://dx.doi.org/10.1155/2014/394696>.

For the purpose of clarity and taking into consideration the considerable difference between the outcome estimates related with the use of betel quid and other forms of SLT, we reviewed the relationship of oral cancer with SLT in two groups: (1) chewing tobacco of all kinds excluding betel quid or paan with tobacco and (2) betel quid or paan with tobacco.

3.1. Chewing Tobacco and Oral Cancer. Overall 14 publications reported different forms of chewing tobacco, predominantly Gutkha and chewing tobacco leaves (Table 2). Five publications reported OR that had been adjusted for smoking among other confounders. The adjusted OR ranged from 3.6 [2.5–5.6] [34] to 8.3 [5.4–13] [48]. The OR ranged from 1.2 [1.0–1.4] [47] to 12.9 [7.5–22.3] [33] among the publications in which either crude odds ratios were mentioned or a MH-OR was calculated from the given data. The pooled OR for chewing tobacco and risk of oral cancer was 4.7 [3.1–7.1] (Figure 2). The studies where adjustment for alcohol and/or

TABLE 1: Characteristics of included studies on oral cancer.

Authors	Year	Location	Study type	Sample Size (cases/controls) (Cohort size/oral cancer cases)**	Quality assessment*	Mean age of cases	Adjustment for smoking and alcohol
Sankaranarayanan et al. [29]	1989	India	Case-control	228/453	Moderate	n/a	Smoking and alcohol
Sankaranarayanan et al. [30]	1989	India	Case-control	187/895	Moderate	n/a	Smoking and alcohol
Goud et al. [32]	1990	India	Case-control	102/102	Weak	53	No
Nandakumar et al. [33]	1990	India	Case-control	348/348	Moderate	54.8	No
Sankaranarayanan et al. [31]	1990	India	Case-control	414/895	Moderate	n/a	Smoking and alcohol
Rao et al. [34]	1994	India	Case-control	713/635	Moderate	50.35	Smoking and alcohol
Khan et al. [35]	1995	Pakistan	Case-control	24/24	Moderate	54	No
Wasnik et al. [36]	1998	India	Case-control	123/246	Moderate	n/a	No
Dikshit and Kanhere [37]	2000	India	Case-control	558/260	Moderate	n/a	Smoking
Merchant et al. [38]	2000	Pakistan	Case-control	79/149	Moderate	49	Smoking and alcohol
Balaram et al. [39]	2002	India	Case-control	591/582	Moderate	n/a	No
Znaor et al. [40]	2003	India	Case-control	1563/3638	Moderate	n/a	Smoking and alcohol
Subapriya et al. [41]	2007	India	Case-control	388/388	Moderate	50.85	No
Gangane et al. [42]	2007	India	Case-control	140/380	Weak	n/a	No
Basu et al. [43]	2008	India	Case-control	110/110	Weak	n/a	No
Muwonge et al. [44]	2008	India	Case-control	282/1410	Moderate	n/a	Smoking and alcohol
Jayalekshmi et al. [45]	2009	India	Cohort study	79593/92**	Moderate	n/a	No
Jayalekshmi et al. [46]	2011	India	Cohort study	66277/160**	Moderate	n/a	No
Pednekar et al. [47]	2011	India	Cohort study	87222/1267**	Moderate	n/a	No
Madani et al. [48]	2012	India	Case-control	350/350	Moderate	n/a	Smoking and alcohol
Ray et al. [49]	2013	India	Case-control	698/948	Weak	n/a	No

* Based on the "Effective Public Health Project Quality Assessment Tool for Quantitative Studies".

** Size of the cohort and the number of oral cancer cases in the cohort.

smoking had been done, when combined, provided a pooled OR of 4.3 [3.1–5.8]. The pooled OR from combining only case-control studies was 5.4 [4.1–7.1]. Case-control studies having hospitals as a source of controls when combined gave a pooled estimate of 4.2 [2.5–6.9]. Cohort studies when combined provided a pooled OR of 2.9 [1.1–8.3]. For studies carried out in India the pooled estimate was 4.8 [3.2–7.4]. For studies carried out in South India, which comprises of the states of Andhra Pradesh, Kerala, Karnataka, and Tamil Nadu, the pooled OR was 5.1 [3.3–8.1]. The pooled OR for studies carried out in the state of Maharashtra was 4.8 [1.7–13.5]. When studies of moderate quality were combined, the pooled estimate came out to be 4.5 [2.8–7.3]. The pooled estimate for studies ranked as "weak" was 5.2 [2.6–10.3].

3.1.1. Gender Differences. Three publications reported or contained data from which OR for men and/or women could be calculated separately (Table 2). Among men the OR ranged from 1.2 [1.0–1.4] [47] to 5.8 [3.6–9.5] [37]. Only two studies reported OR separately for women ranging

from 6.4 [3.3–9.0] [49] to 25.3 [11.2–57.3] [33]. Studies carried out with only men taken as study subjects when combined provided a pooled OR of 4.0 [2.9–5.7].

3.1.2. Exposure-Response Relationships

Intensity/Frequency. A total of seven publications provided dose response relationships according to the intensity of daily usage as exposure metric (Table 2). These OR varied from 1.1 [1.0–1.4] [47] for chewing tobacco or chewable products containing tobacco for less than 5 times a day to 20.0 [8.1–48.9] [36] for more than 10 times a day compared to nonchewers; among studies adjusted for smoking and/or alcohol the corresponding values were 2.0 [1–3.8] and 13.9 [7.1–27.2], both coming from the same study done by Diskshit et al. [37].

Duration of Use. Six publications described the effect of chewing tobacco on developing oral cancer in terms of the total duration of the habit (Table 2). The OR varied from

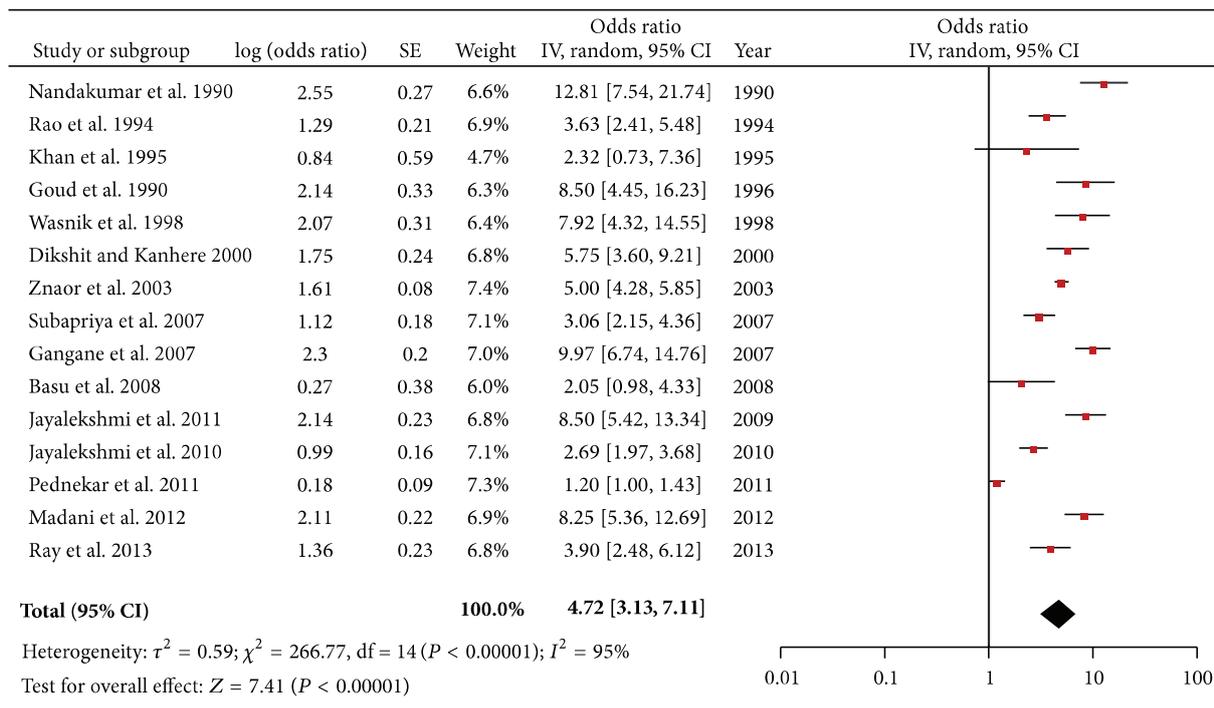


FIGURE 2: Forest plot of chewing tobacco and risk of oral cancer.

0.8 [0.4–1.7] [47] for the total duration of the habit being less than 10 years, compared to nonchewers, to 10.9 [5.9–20.0] [36] for a usage duration of 20 years or more compared to nonchewers.

3.2. Paan/Betel Quid (with Tobacco) and Oral Cancer. A total of nine publications included in this review reported OR or contained data from which OR could be calculated for the risk of chewing paan/betel quid and oral cancer (Table 3). Six publications [29–31, 38, 41, 44] reported overall OR which were adjusted for confounding factors such as smoking and/or alcohol. The adjusted OR varied from 3.1 [41] to 14.1 [7.4–26.5] [31]. Overall, the OR (both adjusted and unadjusted) varied from 3.1 [41] to 15.7 [11.0–22.1] [39]. The pooled OR for chewing paan/betel quid and risk of oral cancer was 7.1 [4.5–11.1] (Figure 3). The studies where adjustment for alcohol and/or smoking had been done, when combined, provided a pooled OR of 6.3 [3.9–10.2]. Case-control studies having hospitals as a source of controls when combined gave a pooled estimate of 7.4 [4.4–12.4]. For studies carried out in India the pooled estimate was 7.0 [4.4–11.1]. For studies carried out in South India the pooled OR was 7.4 [4.1–13.0]. Only one study was carried out in the state of Maharashtra where the OR was 9.3 [5.1–17.2]. When the one “weak” study, for which the OR was 3.9 [2.4–6.4], was excluded, the pooled estimate came out to be 7.6 [4.7–12.3]. Similarly the pooled risk estimates from studies carried out in South India were comparatively higher than the overall pooled estimate.

3.2.1. Gender Differences. Six studies reported or contained data from which OR could be calculated separately from men

and/or women (Table 3). For men the OR for chewing betel quid with tobacco ranged from 1.5 [0.75–3.02] [49] to 10.9 [31]; among women the OR ranged between 6.5 [29] and 45.8 [25–84.1] [39].

3.2.2. Exposure-Response Relationships

Intensity/Frequency. Five studies reported the effect of frequency of daily use of paan with tobacco on oral cancer (Table 3). The OR varied from 3.3 [1.6–6.9] [29] for chewing paan with tobacco, for less than 5 times a day compared to nonchewers, to 24.7 [12.5–48.7] [39] for someone chewing it more than 10 times a day compared to nonchewers; for studies adjusted for smoking and/or alcohol the corresponding values were 3.3 [1.6–6.9] [29] and 15.7 [31].

Duration of Usage. Four studies reported OR for the total duration of habit and oral cancer (Table 3, last column). The OR for chewing habit duration varied from 3.4 [30] for a chewing habit of less than 10 years to 14.6 [30] for a chewing habit persisting for 20 years or more; the corresponding values for studies adjusting for smoking and/or alcohol were 3.4 and 14.6 both from the same study by Sankarnarayanan et al. [30].

4. Discussion

The results of this systematic review suggest a strong link between different forms of smokeless tobacco (SLT) and oral cancer and further strengthens and supports the IARC’s take on SLT that it is a risk factor for oral cancer [15, 16]. Users

TABLE 2: Epidemiological studies of chewing tobacco and oral cancer.

Authors	OR (95% CI)	Men OR (95% CI)	Women OR (95% CI)	Frequency/day**			Duration of use in years***			
				Tobacco ≤ 5 OR (95% CI)	Tobacco 6-10 OR (95% CI)	Tobacco > 10/OR (95% CI)	Tobacco ≤ 10 yrs/OR (95% CI)	Tobacco 11-20 yrs/OR (95% CI)	Tobacco > 20 yrs/OR (95% CI)	
Goud et al. [32]	8.5 (4.3-16.5)	n/a	n/a	8.2 (3.0-22.3)	4.7 (2.0-10.7)	18.4*	n/a	n/a	4.2*	10.2*
Nandakumar et al. [33]	12.9 (7.5-22.3)	3.6 (1.7-7.9)	25.3 (11.2-57.3)	9.3 (4.9-17.5)	12.8 (6.6-25.0)	16.6 (6.3-44.3)	n/a	n/a	n/a	n/a
Rao et al. [34]	3.6 (2.5-5.6)	3.6 (2.5-5.6)	n/a	n/a	2.8 (2.2-3.5) ⁺	3.8*	n/a	1.2 (0.9-1.8)	3.9 (2.7-5.7)	4.1*
Khan et al. [35]	2.3 (0.7-7.4)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Wasnik et al. [36]	7.9 (4.1-13.5)	n/a	n/a	2.1*	8.1 (3.7-17.9)	20.0 (8.1-48.9)	n/a	n/a	n/a	n/a
Dikshit and kanhere [37]	5.8 (3.6-9.5)	5.8 (3.6-9.5)	n/a	2.0 (1.0-3.8)	6.7 (3.7-12.1)	13.9 (7.1-27.2)	n/a	n/a	n/a	n/a
Znaor et al. [40]	5.0 (4.2-5.9)	5.0 (4.2-5.9)	n/a	5.0*	11.9 (8.9-15.9) ⁺⁺	n/a	n/a	3.1 (2.5-3.8) ⁺⁺⁺	n/a	9.5*
Gangane et al. [42]	10.0 (6.7-14.8)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Subapriya et al. [41]	2.9*	n/a	n/a	n/a	n/a	n/a	2.9*	n/a	2.5*	2.7*
Basu et al. [43]	2.0 (0.9-4.4)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Jayalekshmi et al. [45]	5.5 (3.3-9.0)	n/a	5.5 (3.3-9.0)	3.3 (1.7-6.4)	7.8 (4.4-13.9)	9.2 (4.5-18.7)	n/a	n/a	n/a	n/a
Jayalekshmi et al. [46]	5.4 (3.0-9.0)	5.4 (3.0-9.0)	n/a	1.9 (1.2-2.8)	n/a	n/a	n/a	n/a	n/a	n/a
Pednekar et al. [47]	1.4 (1.0-2.1)****	1.4 (1.0-2.1)	n/a	1.1 (0.9-1.4)	1.1 (0.9-1.4)	n/a	0.8 (0.4-1.7)	1.0 (0.7-1.4)	n/a	1.1 (1-1.4)
Madani et al. [48]	8.3 (5.4-13.0)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Ray et al. [49]	3.9 (2.4-6.1)	2.8 (1.5-5.1)	6.4 (3.2-12.7)	n/a	n/a	n/a	n/a	n/a	n/a	n/a

OR: odds ratio, CI: confidence interval, *95% CI not reported and/or could not be calculated, **daily frequency in number of times tobacco is chewed in a day, ***total duration of habit in "years," **** for cancer of lip, oral cavity, and pharynx only, n/a: not available, ⁺1-10/day, ⁺⁺>5/day, ⁺⁺⁺0-19 years, and nonchewers taken as reference category. Frequency/intensity OR are for both genders.

TABLE 3: Epidemiological studies of chewing paan with tobacco and oral cancer.

Authors	OR (95% CI)	Men OR (95% CI)	Women OR (95% CI)	Daily frequency/intensity**			Total duration of use***		
				Paan ≤ 5 OR (95% CI)	Paan 6-10 OR (95% CI)	Paan > 10 OR (95% CI)	Paan ≤ 10 yrs OR (95% CI)	Paan 11-20 yrs OR (95% CI)	Paan > 20 yrs OR (95% CI)
Sankaranarayanan et al. [29] ^a	6.1 (3.2-5.7)	3.6*	6.5*	3.3 (1.6-6.9)	2.3 (1.2-4.6)	6.1 (2.8-13.2)	4.7*	2.4*	5.0*
Sankaranarayanan et al. [30] ^a	8.7 (3.5-21.4)	9.0*	11.3*	4.7 (2.2-10.0)	4.0 (1.9-8.4)	13.2 (6.2-27.8)	3.4*	4.0*	14.6*
Sankaranarayanan et al. [31] ^a	14.1 (7.4-26.5)	10.9*	7.3*	6.0*	9.5*	15.7*	7.1 (2.7-18.2)	4.4 (2.4-8.1)	n/a
Wasnik et al. [36]	9.4 (5.1-17.4)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Merchant et al. [38]	8.4 (2.3-30.6)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Balaram et al. [39]	15.7 (11.0-22.1)	6.1 (3.8-9.7)	45.8 (25.0-84.1)	8.5 (5.4-13.3)	19.4 (10.8-27.0)	24.7 (12.5-48.7)	n/a	n/a	n/a
Subapriya et al. [41]	3.1*	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Muwonge et al. [44]	5.4 (3.8-7.7)	3.4 (2.2-5.2)	11.8 (6.0-23.3)	3.7 (2.4-5.5)	5.8 (3.9-8.7)	7.8 (4.8-12.7)	n/a	n/a	5.6*
Ray et al. [49]	3.9 (2.4-6.4)	1.5 (0.7-3.0)	8.5 (4.6-15.5)	n/a	n/a	n/a	n/a	n/a	n/a

OR: odds ratio, CI: confidence interval, * 95% CI not reported and/or could not be calculated from given data, ** daily frequency in number of times paan is chewed in a day, *** total duration of habit in years, n/a: not available, and ^a the difference between overall and stratum specific OR is because the overall OR and some dose response OR are adjusted for smoking and alcohol while others were calculated using MH method. Nonchewers are taken as reference category. Frequency/intensity OR are for both genders.

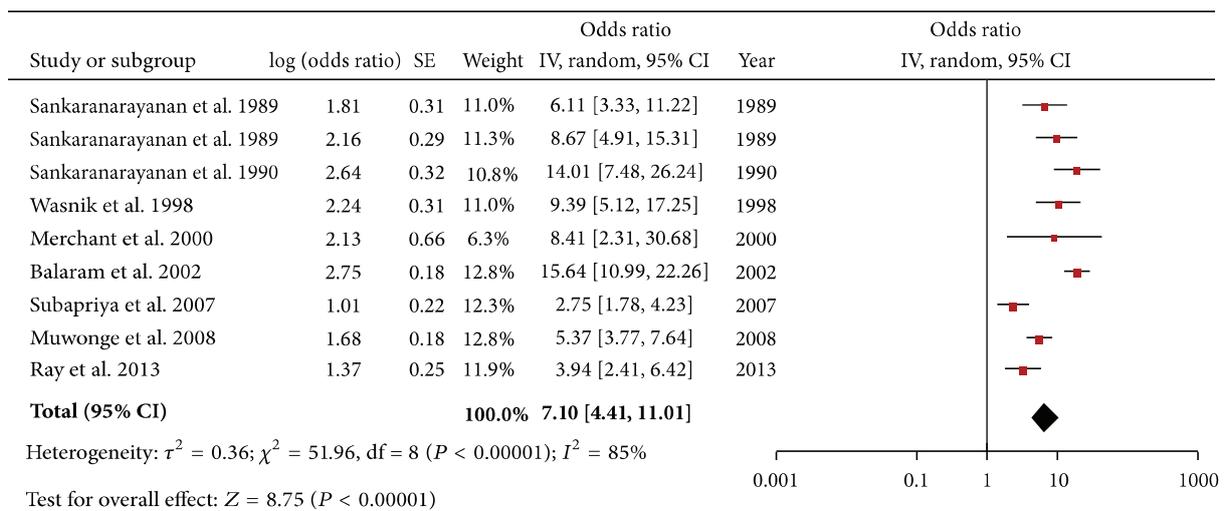


FIGURE 3: Forest plot of betel quid plus tobacco and the risk of oral cancer.

of betel quid with tobacco have a sevenfold higher risk for developing oral cancer as compared to nonchewers, OR 7.1 [4.5–11.1]. This finding is consistent with findings from the earlier reviews [15, 16]. Similarly, people using other forms of SLT than betel quid with tobacco have an almost five-time higher risk of developing oral cancer as compared to nonchewers, OR 4.7 [3.1–7.1]. These increased risks were consistently significant even after adjustment for other risks factors such as alcohol and smoking; that is, pooled OR for betel quid with tobacco after adjustment for alcohol and smoking was 6.3 [3.9–10.2] and the corresponding value for the chewing tobacco group was 4.3 [3.1–5.8]. These pooled estimates, however, should be dealt with caution because of the high levels of heterogeneity present among the studies but, despite indications of heterogeneity, even the lowest effect estimates among the individual studies are above the value of 1, pointing towards a causal link between SLT and oral cancer. The large variability of the effect estimates among individual studies may be attributed to differences in the composition of the products and population characteristics across the region. Additionally, although most studies are case-control design, there are differences between the sources and ratio of controls to the number of cases. In general, the three cohort studies provide relatively conservative estimates as compared to the case-control studies (Table 1).

For the chewing tobacco category, case-control studies provided a pooled estimate, OR 5.46 [4.1–7.1], which was significantly higher than that of the cohort studies, OR 2.9 [1.0–8.3]; albeit the pooled estimate for the cohort studies had an increased width of the confidence intervals. This finding is in contrast with the review done by Guha et al. [15], where they reported a higher pooled estimate for cohort studies as compared to the case-control ones. This may be explained by a difference in the selected cohort studies, as this review has only one cohort study in common with that review. They have included two cohort studies published prior to 1983, which might have reported considerably higher risk estimates. The source of controls had only a slight bearing on the pooled

estimates, with the pooled OR for combining studies where controls were taken from hospitals, being slightly lower as compared to studies where population controls were recruited. This is consistent with previous findings [15]. The pooled OR for studies carried out in South India and the state of Maharashtra are relatively higher than the overall pooled estimate and this might be explained by the relatively high prevalence of SLT use in these geographic locations [9, 13, 50] and incidence of oral cancer [51]. The quality of the combined studies had minimal effect on the overall summary estimate, that is, OR 4.5 [2.8–7.3], compared to the overall pooled OR of 4.7 [3.1–7.1]; however, in the chewing tobacco group exclusion of the weak studies ($n = 4$) lowered the pooled estimate, while in the betel quid group, where there was just one weak study, the overall estimate increased when the “weak” study was excluded. The studies which were ranked as “weak” did not play any role in the narrative synthesis either; most had not reported any results or suitable data for calculation of ORs, in the exposure response categories of frequency/intensity and duration.

Paan with tobacco appears to have a higher risk as compared to chewing other tobacco products; the overall pooled OR for paan as well as the pooled OR across different exposure strata are significantly higher in comparison with the other forms of chewing tobacco (Figure 3 and Tables 2 and 3). A possible reason for this could be the use of areca nut in paan, as it has been shown to have carcinogenic properties on its own [52] and thus might have a synergistic effect with the carcinogenicity of SLT, resulting in a higher risk of oral cancer as compared to other forms of SLT use. Similarly another ingredient, slaked lime, used in betel quid preparation has been shown to have carcinogenic potential. It facilitates the production of reactive oxygen species (ROS) in the saliva of chewers and also facilitates the hydrolysis of arecoline into arecaidine which in turn facilitates increased fibroblast proliferation and collagen synthesis, which are essential for premalignant and malignant transformation of the affected tissues [53]. The betel quid with tobacco group

analysis included only case-control studies and therefore a formal comparison of the risk estimates among case-control and cohort studies could not be done. However, similar to the chewing tobacco group, the studies which recruited hospital controls had a relatively higher pooled risk estimate, that is, OR 7.4 [4.4–12.2], compared to the overall estimate.

An interesting observation is the risk differences among males and females, with females being at a significantly higher risk of oral cancer from SLT use as compared to men (Tables 2 and 3). This may be attributed to increased susceptibility of the female oral mucosa to damage by tobacco products [39] and relative lack of education and poverty, all of which have been shown to be significant risk factors on their own [9, 22]. Also it may be due to a lower background risk for oral cancer among women of this region because of a lower prevalence of smoking and alcohol drinking [15]. Also a high prevalence of cervical cancer among women in India [54] may be suggestive of the presence of human papilloma virus (HPV) [55], which is an established risk factor for oral cancer as well. There is, however, significant inconsistency in effect estimates among the case-control studies regarding risks in women and also between the case-control and the cohort studies, which might have led to an overestimation of the risk estimates among women. In the study carried out by Jayalekshmi et al. where cohorts of men and women were analyzed separately [45, 46], the authors found that the risk estimates were almost similar among both sexes, which underscores the argument that the true effect size for the relationship between SLT and oral cancer in women may be overestimated. However, it should be clear that, regardless of the magnitude of effect size, all included studies that provide sex-specific estimates provide evidence that SLT is a major risk factor for oral cancer among women in the South Asian region. These results may warrant future research to specifically focus on sex differences and provide reliable risk estimates among men and women using SLT.

The results of our review suggest that there is an exposure-response causal relationship between SLT use and oral cancer, for both the intensity and duration of use. This effect is somewhat linear in case of the chewing tobacco group but for the betel quid group, though the data suggests a possible relationship, it is a nonlinear one. This result is consistent with the IARC reviews but differs from findings of some other reviews [56–58] carried out on published literature from North America and Europe. In these reviews no dose response relationships were identified. This may be explained by the difference in the types of SLT used in South Asia compared to North America and Europe. Differences in ethnicity and socioeconomic status and environmental differences may be additional reasons for these conflicting findings. It may also be noted that the effect sizes reported in the reviews of studies carried out on SLT and oral cancer in Europe and North America report significantly smaller observed effect estimates as compared to the studies included in the present review. The synthesis of the reviewed publications suggests that the total duration of exposure to SLT increases the risk of oral cancer; that is, subjects who used SLT (chewing tobacco, paan/betel quid) for more than 10 years were at a higher risk of oral cancer than those who used SLT for less than

10 years. Parallels can be drawn here with the habit of smoking and alcohol use, where the risk for developing oral cancer increases with an increase in the total duration of exposure to these substances [59]. Furthermore, the mean age of oral cancer cases in the included studies was mostly in the fifth decade of life (Table 1). Given that usually habits like SLT use are generally taken up in early adolescence, this might suggest that prolonged exposure to SLT increases the risk of oral cancer, although age itself has been shown to be a risk factor for oral cancer.

5. Limitations of the Review

Some of the limitations are inherent to the observational study designs included in this systematic review, such as recall and selection bias, under-/overreporting of exposure status, retrospective exposure assessment, and uncontrolled confounding. Our electronic search included terms for all countries comprised in the South Asian region, but only publications from India and Pakistan were included because no case-control or cohort studies could be found for other countries. Therefore the results may not be applicable to the entire region. Due to a lack of resources a meta-regression analysis could not be performed to identify the sources of heterogeneity; however, in the most recent review done by IARC researchers [15], which includes most of the studies included in our review, meta-regression analysis did not lower heterogeneity to moderate or low levels.

6. Policy Implications

Given the various types of SLT used in the Indian sub-continent and its increasing popularity in the neighboring countries [60], it is of great importance that the general public be made aware of SLT use as a major risk factor for oral cancer. Most of the tobacco control initiatives around the world have been aimed towards cessation of smoking, where the main strategy to decrease smoking prevalence is the high amount of taxes levied on smoking products. Although this might be productive for smoking cessation, this strategy may facilitate an unintentional push towards smokeless tobacco use and increasing prevalence because SLT is cheaper compared to smoking. Additionally, big tobacco companies revert to manufacturing smokeless tobacco products and advertising them as less harmful than smoking [61]. All these scenarios may potentially lead to a surge in the use of smokeless tobacco products and subsequent increased risks for oral cancer for the general public. The governments and general public should be made aware of the potential dangers related to such approaches and may consider new programs for smokeless tobacco cessation or incorporate the risks of SLT consumption into smoking cessation programs.

7. Conclusion

From the published literature it appears that various forms of smokeless tobacco used in South Asia should be considered as strong risk factors for oral cancer. Public health policies

in affected countries should consider SLT cessation programs in addition to campaigns and activities to inform the general public about SLT use and oral cancer risks.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Zohaib Khan developed the concept for the study, conducted the literature search, assessed studies for quality and for inclusion in the review, and extracted data. He also prepared drafts and undertook edits. Justus Tönnies was involved in quality assessment, data extraction, and editing. Steffen Müller was involved in the development of the study concept, literature search, paper selection, and narrative synthesis. All authors contributed to the editing of the drafts and have read and approved all versions of the paper.

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ARTICLE III

SMOKELESS TOBACCO AND ORAL POTENTIALLY MALIGNANT DISORDERS IN SOUTH ASIA: A PROTOCOL FOR A SYSTEMATIC REVIEW.

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Author's contributions: ZK wrote the first draft of the protocol. FSZ and OE provided comments on the first and subsequent versions of the protocol. All authors were involved in developing the technical aspects of the review protocol.

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PROTOCOL

Open Access



Smokeless tobacco and oral potentially malignant disorders in South Asia: a protocol for a systematic review

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Abstract

Introduction: Oral potentially malignant disorders (OPMDs) are chronic lesions or conditions characterized by a potential for malignant transformation. Apart from being possible pre-cursors to oral cancer, OPMDs themselves are usually painful and debilitating conditions having an influence on the quality of life, both in terms of pain and social disability. Smokeless tobacco (SLT) use is considered a major risk factor for OPMDs. SLT use is a culturally and socially acceptable habit in South Asia. According to a recent report, 90 % of the SLT burden of the whole world lies in the South Asian countries of Pakistan, India, Sri Lanka, Bangladesh, Bhutan, Nepal, Afghanistan, and Maldives. This review aims to assess the association between the use of various SLT products in South Asia and risk of OPMDs.

Methods: This review will focus on epidemiological studies on the use of SLT and risk modification for OPMDs, which have been carried out in the human population of South Asian countries. Articles reporting estimates of relative risk, e. g., odds ratio (OR) or relative risk (RR) with their 95 % confidence intervals (CI) for SLT users versus non-users. Articles reporting data from which these effect estimates can be computed will be included in the review. We will search MEDLINE, the Science Citation Index (SCI), Scopus, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) databases for relevant literature using a combination of keywords and MeSH terms, where applicable. Appropriate sources of gray literature will also be included in the search. The electronic searches will be supplemented by a hand search of the bibliographies of the included articles. The included studies will be assessed for their quality using an established quality assessment tool. All relevant data from the included articles will be recorded in an MS Excel spread sheet and then transferred to Rev Man 5.3 to carry out a meta-analysis. Heterogeneity among the estimates will be assessed through the I^2 statistic. Sensitivity and subgroup analysis will be carried out to see the effects of individual or group of studies on the pooled effect estimate. Results of the review will be reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Discussion: This review may have a potential limitation with regard to the designs of the studies included as we expect that most of the included studies will be of the observational types. We will however try to address this issue by conducting sensitivity and subgroup analysis of similar quality studies.

Systematic review registration: PROSPERO CRD42015029705.

Keywords: Oral potentially malignant disorders, Smokeless tobacco, Leukoplakia, Submucous fibrosis, Erythroplakia, Paan, Betel quid, Gutkha, South Asia

Abbreviations: AK, Actinic keratosis; DLE, Discoid lupus erythematosus; EP, Erythroplakia; LKP, Leukoplakia; OPMD, Oral potentially malignant disorders; OR, Odds ratio; RR, Relative risk; SLT, Smokeless tobacco; SMF, Submucous fibrosis

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Introduction

Oral potentially malignant disorders (OPMD) are chronic lesions or conditions characterized by a potential for malignant transformation. More specifically, "It is a group of disorders of varying etiologies, usually tobacco; characterized by mutagen associated, spontaneous or hereditary alterations or mutations in the genetic material of oral epithelial cells with or without clinical and histo-morphological alterations that may lead to oral squamous cell carcinoma transformation" [1]. Leukoplakia (LP), erythroplakia (EP), submucous fibrosis (SMF), lichen planus (LP), actinic keratosis (AK), discoid lupus erythematosus (DLE), and palatal lesions among reverse smokers constitute OPMD [2]. Rare, inherited syndromes, e.g., xeroderma pigmentosum and Fanconi's anemia, and immunodeficiency have also been linked with the development of oral cancer. In addition, patients suffering from chronic Graft Versus Host Disease after stem cell transplantation may also be at a risk of developing oral cancer [3]. It is generally believed that the prevalence of OPMD worldwide varies between 1 and 5 % [4]. The potential for malignant transformation among these conditions vary from less than 1 % to as high as 36 % [5] and is often influenced by the post-diagnosis cessation or continuity of the high risk behaviors, like tobacco and alcohol use, and clinical intervention [6, 7]. LP, EP, and SMF have a higher potential for malignant transformation as compared to other [8]. Apart from being possible pre-cursors to oral cancer, OPMD by themselves are usually painful and debilitating conditions having an influence on the quality of life, both in terms of pain and social disability [9].

Smokeless tobacco (SLT) use is considered as a major risk factor for OPMD [10, 11]. SLT refers to the forms of tobacco which are used without burning the product. It is estimated that SLT contains more than 30 carcinogenic agents [12]. SLT use is a culturally and socially acceptable habit in South Asia [13] according to a recent WHO report 90 % of the SLT burden of the whole world lies in the South Asian countries [14]. South Asia includes Pakistan, India, Sri Lanka, Bangladesh, Bhutan, Nepal, Afghanistan, and Maldives. Different forms of SLT products are used in these countries, often dictated by regional influences [15–18]. The most widely used products include Betel quid or PAAN with tobacco, Gutkha, Naswar, Chaini, Misri, and chewable tobacco leaves. The carcinogenic agents in tobacco act by inducing changes at both genetic level and locally by providing a conducive local environment for hyperplastic transformation of the buccal cells [19].

We conducted pre-review scoping searches to identify the current state of literature on OPMDs, specifically systematic reviews on OPMDs and literature pertaining to the risk of OPMDs associated with the use of SLT.

The majority of recent systematic reviews on SLT use have focused on the link between SLT and oral cancer [13, 20, 21], but we identified a knowledge gap with regard to the effects of SLT use and development of OPMDs, particularly in the context of South Asia. We therefore aim to conduct a systematic review on the relationship between SLT and OPMDs to provide evidence which would be beneficial for the scientific community, the tobacco industry, patients, and the general public. It could also help inform the tobacco control policies in South Asia, which have been shown to be underperforming and less orientated when it comes to smokeless tobacco.

Objectives

General

The objective of this study is to quantify the risk of developing OPMDs associated with the use of SLT (ever versus never users) by pooling effect estimates from epidemiological studies carried out in South Asia among both males and females of any age group.

Specific

- To assess the individual risk of different forms of SLT use associated with the development of any OPMD
- To assess the individual risk of development of different OPMDs with the use of SLT
- To assess the risk of developing OPMDs with the use of SLT separately among men and women
- To explore exposure-response relationships in terms of duration and intensity of use of SLT products and the risk of development of OPMDs
- To stratify the risk estimates for individual countries and/or if applicable regions
- To calculate the population attributable fraction (PAF) associated with the use of SLT and development of OPMDs

Methods

This protocol follows the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols (PRISMA-P) 2015, guidelines. The corresponding checklist is provided in Additional file 1.

Eligibility criteria

Study design

This review will focus on epidemiological studies investigating the use of SLT and risk modification for OPMDs, which have been carried out in the human population of South Asian countries. Although data generated by experimental research designs, e.g., randomized clinical or community trials, are considered the gold standard, we

expect that our search will mostly yield studies with an observational design, due to the inherent ethical issues regarding the potential harm of using SLT. This has been further substantiated through our preliminary scoping searches for the review and a perusal of previous reviews on SLT and oral cancer, where most of the published literature involves observational studies, mostly case-control and cohort designs [13, 20, 21]. However, we will not limit our search to observational designs and will also include experimental studies satisfying our inclusion criteria. Laboratory-based genetic epidemiological studies will not be eligible for inclusion.

Participants/population

Studies carried out among both males and females irrespective of age, socio-economic, physical, or dental status, who are residents of the countries of South Asia, will be included in the review. For studies to be eligible for the review, the reported cases must have been ascertained as having an OPMD through medical and/or histological records. Studies carried out among expatriate populations of South Asia will not be included, as there may be large differences between tobacco habits and products among the resident populations and the expatriate ones. Studies focusing on animals will not be included.

Exposure

Studies in which exposure to an SLT product has been ascertained through written records, e.g., medical history, structured interviews, or written self-reports will be included. For the purpose of this review, an “ever” exposed participant is defined as someone who might have used an SLT product at least once in life. Exposure will be quantified in years and daily frequencies for the assessment of the exposure-response relationship between OPMD and SLT. Only the studies reporting daily frequencies and total duration of exposure or reporting data to calculate these will be eligible for the exposure-response analysis. Since SLT products in South Asia are often produced unregulated [22], it is difficult to quantify the intensity of exposure and hence intensity will not be used as an inclusion/exclusion criteria for this review. Studies which exposure of interest is areca nut alone or betel quid without added tobacco, although often investigated together along with other SLT products, will not be eligible for this review as these might contribute to increased heterogeneity because of differences in carcinogenic potential among these and SLT products.

Comparator(s)/control

For case-control studies, the control group must have included subjects who have no history of OPMDs,

irrespective of their use of SLT. Matching for age, sex, and other potential confounders will not be used as inclusion criteria, in order to include maximum studies. The source of controls, i.e., hospital or community-based will not affect the inclusion/exclusion of a study for the review. For cohort studies, the use of a comparator will not be a requirement for inclusion.

Outcome

Studies which report an OPMD as the, or one of the, primary or secondary outcome/s will be included in the review. Studies where cases were recruited after verification through a medical record or a laboratory report or a clinical examination by a qualified person will be included. Articles reporting estimates of relative risk, e.g., odds ratio (OR) or relative risk (RR) with their 95 % confidence intervals (CI) for SLT users versus non-users (ever versus never) as well as those reporting data from which these effect estimates can be computed will be included in the review. In case there is a potentially eligible study, where effect estimates or data to calculate them is not reported in the manuscript, the authors of the manuscript will be requested to provide the relevant data.

Follow-up

In order to include maximum studies, the length of follow-up will not be used as an inclusion/exclusion criterion for studies. If applicable, studies having similar lengths of follow-up will be pooled together in a subgroup analysis.

Setting

Studies will be included irrespective of the study setting, i.e., hospital-based (private and public) or community-based. The only exception will be laboratory based genetic epidemiology studies.

Language

No filters will be used during the search process and hence studies published in any language will be eligible for inclusion in this review.

Search methods

We will search MEDLINE, the Science Citation Index (SCI), Scopus, and Cumulative Index to Nursing and Allied Health Literature (CINAHL) databases for relevant literature. A combination of keywords and MeSH terms, where applicable, will be used for the electronic search. A detailed search strategy, developed for this review by an information sciences specialist (LC), is provided in Additional file 2. In order to include all relevant literature, no filters will be used during the electronic search. The electronic search will be supplemented by a hand search

of the bibliographies of selected articles and previously published narrative reviews. Efforts will be made to find non-indexed and gray literature pertaining to the topic via an electronic search of regional electronic research repositories especially the World Health Organization's regional and global Index Medicus, which often contain local literature not indexed with the main stream research indices (search strategy provided as Additional file 3). Internet search engines, e.g., Google Scholar will also be used to identify any relevant literature. Search strategy for these databases is provided in Additional file 4.

Selection of studies

One author (LC) will create and run the search query in the databases and export the results to reference management software EndNote [23]. Duplicate records will then be removed via the duplicate search function of the reference management software. Two authors (SK and SR) will then independently go through the titles and abstracts of all the records and select the studies relevant to the review. The authors will compare their results with each other and in cases of disagreement, a third author (ZK) will be contacted to resolve the issue, with his/her opinion being decisive. The authors of the original articles may be contacted to clarify issues regarding eligibility. Full texts of the selected studies will be obtained and will be independently screened for inclusion or exclusion in the final review by two authors (SK and SR). The list of selected studies will then be compared and any disagreements will be resolved through a third author (ZK). Finally, the bibliographies of the selected studies will be screened for any relevant studies that might have been missed during the search process. The selection process for the studies will be presented using a flowchart.

Assessment of the quality of included studies

All selected studies will be assessed for their quality using the National Collaborating Centre for Methods and Tools, McMaster University's "Effective Public Health Practice Project Quality Assessment Tool for Quantitative Studies" [24]. The authors have previous experience with this tool [13], and in our opinion, it is well suited to the quality assessment of analytical study designs. Studies will be ranked as "strong," "moderate," or "weak" based on six parameters, i.e., selection bias, study design, confounding, blinding, data collection methods, and withdrawals and dropouts. In case there are eligible randomized studies, we will use the Cochrane Collaboration tool for assessing the risk of bias, to assess the quality of those studies. Quality assessment will be carried out by (SK and SR) independently. Any disagreements on the quality of the studies will be resolved in the presence of a third author (ZK).

Data extraction and management

Data regarding study type, publication year, authors name, place of study, sample size, type/s of exposure, exposure frequency and duration, and outcome/s and effect estimates (OR and RR) will be extracted independently by two authors (ZK and SK) and will be recorded onto a pre-designed data extraction spreadsheet (provided in Additional files 5 and 6).

Sample size of the studies where the only outcome is an OPMD will be extracted as such, i.e., the numbers reported in the study. In case there are multiple outcomes, e.g., oral cancer and OPMDs, then the oral cancer cases will be excluded from the sample size and only the cases with OPMD will be reported. Where applicable effect estimates for men and women will be recorded separately in addition to the overall effect estimate.

Based on original reporting in the included studies, the exposure/s (SLT) will be classified during data extraction as Gutkha, Betel quid with tobacco, areca nut with tobacco, chewing tobacco leaves or others. The term "any SLT" will be used for those exposures where the type of SLT is not mentioned in the included studies. Where applicable and depending on the availability of data, a single study might be treated as two or multiple studies if it reports separate risk of OPMD with the use two or more SLT types. Both adjusted (for smoking and alcohol) and crude effect estimates will be recorded.

The outcome for each study will be recorded as one of leukoplakia, erythroplakia, submucous fibrosis, multiple OPMDs or others depending on how they are reported in the included studies. In case the original study has not differentiated between the OPMD types than the term "All OPMD," will be used to record the outcome for that study. If a study reports separate effect estimates for different types of OPMDs than it will be treated as two or more separate studies corresponding to the related outcome. We will not address co-morbidities and/or secondary outcomes for this review.

Follow up for cohort and trials will be recorded as mean duration of follow up in years.

The individual data recording spreadsheet from each author will then be compared in the presence of a third author (OE), and if there are any differences, the opinion of the third author will be decisive. The third author (OE) can also decide to contact the authors of the included articles to resolve or further clarify issues regarding the data or for additional information. ORs and RRs will be calculated for studies which do not report an OR or RR but have enough data for their calculation. Efforts will be made to calculate an adjusted effect estimate; however, a crude estimate will be used if the available data is insufficient to calculate an adjusted one. The data will then be entered into RevMan 5.3. by ZK.

Meta, subgroup, and sensitivity analysis

Data synthesis

Each outcome will be combined and calculated using the statistical software RevMan 5.3, according to the statistical guidelines referenced in the current version of the “Cochrane Handbook for Systematic Reviews of Interventions” [25]. We will pool effect estimates from those included studies to calculate a meta-risk estimate for OPMDs with the use of SLT products using a random effects model. We will use the inverse variance method to account for the potential inconsistencies between the studies. Standard errors for the effect estimates will be calculated from the given confidence intervals after conversion to a log scale. We will carry out quantitative data synthesis if we have one representative study from at least three countries of South Asia. Previous systematic reviews from South Asia on SLT and oral cancer [13, 20, 21] have carried out meta-analysis despite the presence of very high inconsistency $I^2 > 75\%$ to provide a meta-risk estimate, because the magnitude of the pooled estimates are often so high that even if we consider the effects of heterogeneity, a causal link between the two can still be suspected. We expect a similar or an even higher magnitude of risk for OPMDs with the use of SLT products in South Asia and therefore will carry out a quantitative synthesis irrespective of the heterogeneity, publication bias and quality of the included studies. The readers though will be informed about the magnitude of the heterogeneity with every pooled effect estimate and the quality of studies, so they could interpret the results in the light of these findings.

The primary analysis will focus on “never versus ever” users of SLT products. We will also calculate individual meta-risk estimates for each subtype of OPMD and also perform a stratified analysis based on the type of SLT products. If feasible other subgroup analysis may include male versus female, stratification by country, as well as the relationship between the magnitude and intensity (dose-response) of the exposure and the outcome. Sensitivity analysis may include inclusion or exclusion of studies with different quality, studies reporting a very high or a very low effect estimate, studies with very large, or very small sample size etc. Reporting bias will be assessed using a visual funnel plot. The analysis will be carried out in RevMan 5.

Missing data

In case there are missing data, the original authors of the study will be contacted, to obtain the relevant missing data. If missing data cannot be obtained, an imputation method will be used. We will use sensitivity analysis to assess the impact of inclusion of studies which do not report an effect estimate and it has been calculated by the authors or with missing data.

Assessment of heterogeneity

Heterogeneity will be assessed by visually inspecting forest plots. The I^2 statistic will be calculated for the quantification of inconsistencies and assessment of the effects of heterogeneity in the pooled analysis. A meta-analysis will be performed irrespective of the presence or absence of heterogeneity or a high I^2 value. Causes of heterogeneity (if present) will be assessed through a subgroup and sensitivity analysis. The cause of heterogeneity may be a difference in sample size between the studies, retrospective versus prospective study designs, difference in background risk among the populations of different countries, representation of females (lower background risk) in the study sample, length of follow up, Inter and intra-country differences between the composition of SLT products, presence or absence of confounders and exposure quantification differences.

The subgroup analysis may involve stratified analysis by country, gender, length of follow-up, adjustment for smoking and alcohol, type of SLT, type of OPMD, and exposure-response categories. Sensitivity analysis may involve dropping of studies with a low quality and studies where crude effect estimates were reported or calculated by the authors. If applicable and depending on the availability of data, meta-regression will be performed to account for the effect of co-exposures and other confounders.

Assessment of meta-bias(es)

If available, protocols for randomized studies will be accessed and compared with the included study to assess selective reporting of outcomes. However, we expect that analytical studies will pre-dominantly form the core of our included studies and these usually do not have published protocols. We will run both the fixed effect model and the random effects model to assess the possible presence of small sample bias in the included studies. We will further assess publication bias through funnel plots (if the number of available studies is equal to or more than 10). This will be carried out by ZK and OE.

Confidence in cumulative evidence

The Grading of Recommendations Assessment, Development and Evaluation Working Group methodology will be used to assess the quality of the cumulative evidence. The quality of evidence will be assessed on the basis of risk of bias, consistency, directness, precision, and publication bias by two authors (ZK and FSZ).

Differences between protocol and review

If applicable, the differences between the published protocol and the review, and the circumstances and reasons, which necessitated these changes will be disclosed to the readers of the review.

Discussion

This review will identify and synthesize evidence regarding the use of SLT products and the risk of developing OPMDs in the context of South Asia. To the best of our knowledge, this is the first systematic review which will address the issue of SLT use and the risk of development of OPMDs. We therefore, hope that our findings will help inform tobacco control policies in the region as well as empowering health professionals and general public with evidence regarding the deleterious effects of SLT use and the risk of developing OPMDs associated with SLT.

The potential inclusion of pre-dominantly observational studies in the review may raise concerns over the quality of the evidence, as these designs are susceptible to bias(es). However, due to ethical reasons, it is not possible to carry out an experimental study to assess the risks associated with SLT use, and hence, we have to rely on observational data to estimate the risk estimates for OPMDs associated with SLT use. We are aware of this limitation and will aim to minimize the effects of both intra and extra-study factors which might influence the cumulative incidence.

Additional files

Additional file 1: PRISMA-P 2015 Checklist. (DOCX 29 kb)

Additional file 2: PubMed searches, CINAHL search, Science Citation Index search, Scopus search. (DOCX 18 kb)

Additional file 3: Search in Global Index Medicus. (DOCX 15 kb)

Additional file 4: Google Scholar Search. (DOCX 12 kb)

Additional file 5: Characteristics of included studies. (DOCX 14 kb)

Additional file 6: Data extraction spreadsheet. (XLSX 11 kb)

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N/A.

Availability of supporting data

Any data relevant to this protocol will be made available on written request.

Authors' contributions

ZK wrote the first draft of the protocol. FSZ and OE provided comments on the first and subsequent versions of the protocol. All authors were involved in developing the technical aspects of the review protocol. LC will conduct the electronic searches. LC, SK, and SR will conduct the quality assessment of the included studies. ZK, SK, and SR will extract and record data from the selected studies. ZK and OE will conduct the meta, sensitivity, and heterogeneity analysis. FSZ and OE will review the results, and all authors will contribute to the writing up of the manuscript. All authors read and approved this protocol.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors consent to publication of this protocol.

Ethics approval and consent to participate

N/A.

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ARTICLE IV

SMOKELESS TOBACCO AND ORAL POTENTIALLY MALIGNANT DISORDERS IN SOUTH ASIA: A SYSTEMATIC REVIEW AND META-ANALYSIS.

First author: Zohaib Khan

Order of authors: Zohaib Khan, Sheraz Khan, Lara Christianson, Sara Rehman, Obinna Ekwunife, Florence Samkange-Zeeb

Author's contributions: ZK and SD conceptualized the paper. ZK, SD, SK, SMHS and BR wrote the initial and subsequent drafts. ZU reviewed and contributed to final draft. All authors read and approved the final manuscript.

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Review

Smokeless Tobacco and Oral Potentially Malignant Disorders in South Asia: A Systematic Review and Meta-analysis

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Abstract

Oral Potentially Malignant Disorders (OPMDs) are chronic lesions or conditions characterized by a potential for malignant transformation. While recent meta-analyses show that smokeless tobacco (SLT) use is a risk factor for oral cancer in South Asia, there is a lack of pooled evidence regarding SLT use and the development of OPMDs. We searched Medline via PubMed, the Science Citation Index (SCI) via Web of Science, Scopus, CINAHL, Global Index Medicus and Google Scholar databases for relevant literature using a combination of keywords and MeSH terms. Eighteen case-control studies were included in the review, all of which reported significantly elevated risk estimates for OPMDs associated with SLT use. Overall and subgroup, Meta Odds Ratios (mOR) were calculated through a random effects analysis using “generic inverse variance” method in Rev Man 5.3. Heterogeneity was quantified by calculating the I^2 statistic. The mOR for any OPMD with the use of any SLT product was 15.5 [95% Confidence Interval (CI), 9.9–24.2]. Women had a higher risk, mOR = 22.2 (95% CI, 9.1–54.1) compared to men, mOR = 8.7 (95% CI, 2.1–34.8). Betel quid with tobacco carried the highest risk for OPMD, mOR = 16.1 (95% CI, 7.8–33.5). Although the cumulative evidence is informed by case-control studies only, the magnitude of the pooled estimates and the presence of exposure-response indicate a very strong association between OPMDs and SLT use. In addition to tobacco control, results of this review may help in informing oral cancer control policies in South Asia, since OPMDs lie on the causal pathway for oral cancer.

Implications: More than 250 million South and South East Asians use SLT in some form. As cigarettes prices climb up all over the world, more people could potentially take up SLT, particularly in the absence of epidemiological evidence regarding the harmful effects of these products, and SLT being advocated as a means of tobacco harm reduction. Our findings are thus relevant and timely in highlighting the harmful effects of SLT use, with a potential of influencing tobacco control policies in South Asia and beyond.

Introduction

Oral Potentially Malignant Disorders (OPMDs) are chronic lesions or conditions of the oral cavity characterized by a potential for

malignant transformation.¹ More specifically, “It is a group of disorders of varying etiologies, usually tobacco; characterized by mutagen associated, spontaneous or hereditary alterations or mutations in the genetic material of oral epithelial cells with or without clinical

and histomorphological alterations that may lead to oral squamous cell carcinoma transformation.”² Leukoplakia (LKP), Erythroplakia (EP), Oral Sub-Mucous Fibrosis (OSMF), Lichen Planus (LP), Actinic Keratosis (AK), Discoid Lupus Erythematosus (DLE) and Palatal lesions among reverse smokers, constitute OPMDs.³ The prevalence of OPMDs worldwide varies between 1% and 5%.⁴ The potential for malignant transformation among these conditions varies considerably,⁵ ranging from less than 1% to as high as 36%.⁶ Further, it is often influenced by the post-diagnosis cessation or continuity of the high-risk behaviors such as tobacco and alcohol use, as well as clinical intervention.⁷⁻⁹ LP, EP, and OSMF have a higher potential for malignant transformation compared to the other conditions.¹⁰ Apart from being possible precursors to oral cancer, OPMDs themselves, are potentially painful and debilitating conditions and influence the quality of life, both in terms of pain and social disability.^{11,12}

Smokeless Tobacco (SLT) that is, the forms of tobacco that are used without burning the product, is considered to be a risk factor for OPMDs,¹³ with SLT being estimated to contain more than 30 carcinogenic agents.¹⁴ SLT use is a culturally and socially acceptable habit in South Asia¹⁵ and according to a recent WHO report, 90% of the SLT burden of the whole world lies in South Asian countries.¹⁶ These countries include Pakistan, India, Sri Lanka, Bangladesh, Bhutan, Nepal, Afghanistan, and Maldives. According to the WHO,¹³ a variety of SLT products are used in South Asian countries, with the habits often being dictated by regional and cultural influences.¹⁷ The most widely used products include Betel quid or PAAN with tobacco, Gutkha, Naswar, Khaini, Misri, and chewable tobacco leaves. All these products have carcinogenic agents, usually the “Tobacco Specific Nitrosamines” that act by inducing changes at both genetic level and locally. Regarding the latter, they provide a conducive local environment for the hyperplastic transformation of the buccal cells.¹⁸ The SLT products used in South Asia usually have a high pH, aimed at a rapid and extensive absorption of Nicotine,¹⁹ resulting in stronger Nicotine urges and addiction. A strong Nicotine urge, in turn, induces more frequent and prolonged use of the SLT product,¹⁸ resulting in a prolonged exposure to the carcinogenic agents in SLT.

While recent evidence shows that SLT use is a major risk factor for the high incidence of oral cancer in South Asia,^{14,20} there is, however, a lack of pooled evidence with regards to SLT use and the development of OPMDs, the precursor lesions for oral cancer. The dearth of pooled evidence regarding the association between SLT use and OPMDs is apparent from a 2014 comprehensive report on SLT by the National Cancer Institute, United States²¹ and a 2015 study on the global burden of disease attributable to SLT,²² where summary risk estimates and SLT attributable burden for cancers and cardiovascular disease have been provided but estimates for OPMDs have not been included. Systematic reviews on the association of cancers with SLT use by Sinha and colleagues,²³ and Critchley and Unal,²⁴ have also pointed out the need for future studies on the assessment of the burden of precancerous conditions attributable to SLT use. In an effort to provide evidence that may inform both tobacco control policies and public opinion regarding the deleterious effects of SLT use in South Asian countries, we conducted a systematic review focusing on literature on the relationship between SLT and OPMDs. We aimed to assess the risk of developing OPMDs among SLT users and non-users by systematically reviewing, and if possible pooling effect estimates from, epidemiological studies carried out in South Asia.

Methods

Eligibility Criteria

Study Design, Participants/Population, and Setting

This review includes observational studies on the use of SLT and the risk of developing OPMDs carried out in the human population of the eight countries of South Asia. Studies carried out among both males and females irrespective of age, socio-economic, physical, or dental status, who were residents of South Asia were eligible for the review. Studies carried out among expatriate populations of South Asia were not eligible. Studies were included irrespective of the study setting, that is, hospital-based (private and public) or community-based, the only exception being laboratory based genetic epidemiology studies. Studies focusing on animals were also not eligible.

A pre-review scoping exercise had identified a lack of experimental studies on the topic, a fact further substantiated by existing systematic reviews of South Asian literature on the association of SLT with oral cancer, where all the included studies were case-control or cohort design.^{14,21,25} Therefore, we only focused on observational studies of case-control and/or cohort design, as these are the most appropriate observational study designs to establish epidemiological associations.²⁶ However, in order to avoid missing any potentially eligible study, we did not limit our search to specific study types.

Exposure

Studies in which exposure to any form of SLT had been ascertained through written records, for example, medical history, structured interviews or written self-reports and in which SLT was the main or one of the main exposures of the study were included in the review. For the purpose of this review, an “ever” exposed participant is defined as someone who might have used an SLT product at least once in life. Exposure was quantified in years and daily frequencies for the assessment of the exposure–response relationship between OPMD and SLT. Only the studies reporting daily frequencies and total duration of exposure or reporting data to calculate these were included for the exposure-response analysis. Studies in which exposure of interest was areca nut only were excluded.

Comparator(s)/Control

For case-control studies, the control group had to have subjects who had no history of OPMDs. Case-control studies where SLT was used as an inclusion criterion for both the case and control groups were hence excluded. In order to include maximum studies, matching for age, sex, and other potential confounders were not used as inclusion criteria. The source of controls, that is, hospital or community-based did not affect the inclusion/exclusion of a study from the review. For cohort studies, the use of any unexposed comparison group for example, internal comparison group, external comparison group, or population comparison group, was eligible as a comparator.

Outcome

Studies were eligible to be included in the review only when the cases had been ascertained to have an OPMD through histological confirmation. Studies reporting an OPMD as a primary or secondary outcome were eligible for this review. Studies with multiple outcomes (OPMDs), were included in the meta-analysis as separate studies with the corresponding data related to each specific OPMD. Articles reporting estimates of relative risk for example, Odds Ratio (OR) or Relative Risk (RR) with their 95% confidence intervals (CI) for SLT users versus non-users, or those reporting data from which these effect estimates could be computed were included in the review.

Language

No filters were used during the search process and hence studies published in any language were eligible for inclusion in this review.

Search Methods

A detailed search strategy is provided in [Supplementary Table 1](#). Briefly, we searched Medline via PubMed, the Science citation Index (SCI) via Web of Science, Scopus, and CINAHL databases from their inception till Feb 15, 2016, for relevant literature using a combination of keywords and MeSH terms. In order to include all relevant literature, no filters were used during the electronic search. Additionally, we also searched the Global Index Medicus and Google Scholar databases to identify relevant studies that might have been missed from our primary search. The electronic search was supplemented by a hand search of the bibliographies of selected articles. We also searched SLT related reports^{22,13,27} of the International Agency for Research on Cancer and the National Cancer Institute of the United States to identify studies that might be eligible for the review.

Selection of Studies

One author (LC) ran the search query in the electronic databases. After removal of duplicate articles, the remaining records were stored in an independent folder in a reference management software (Endnote). Two authors (SR, SK) separately went through the titles of all the records and selected the relevant studies. This was followed by the reading of the abstracts of the selected studies and exclusion of irrelevant ones, also carried out by SK and SR. Full texts of the selected studies were then obtained and screened independently by two authors (SK and ZK) for inclusion or exclusion in the final review. Any differences/discrepancies during the study selection process were resolved in consultation with a third author (OE). Finally, the bibliographies of the selected studies were screened by SR, SK, and ZK for any relevant studies that might have been missed by the search process.

Assessment of the Quality of Included Studies

Two authors (ZK, SR) assessed the quality of the included studies by applying the National Collaborating Centre for Methods and Tools, McMaster University's "Effective Public Health Practice Project Quality Assessment Tool for Quantitative Studies."²⁸ Studies were ranked as "strong," "moderate," or "weak" based on six parameters that is, selection bias, study design, confounding, blinding, data collection methods, and withdrawals and dropouts ([Supplementary Table 3](#)).

Data Extraction and Management

Two authors (ZK, OE) separately carried out data extraction using a pre-designed spreadsheet. Data regarding study type, publication year, authors, place of study, sample size, type/s of exposure, exposure intensity, outcome/s and overall as well as stratum-specific effect estimates (OR and RR) were extracted. ORs and RRs were calculated for studies that did not report an OR or RR but had sufficient data for their calculation. Wherever possible, efforts were made to calculate an adjusted or a Mantel-Haenszel odds ratio (OR_{MH}). However, crude estimates were used for the meta-analysis when the available data were insufficient to calculate an adjusted or MH estimate. Both authors compared their spreadsheets to rule out heterogeneity, which when present, were resolved in the presence of a third author. The extracted data were then entered into Cochrane Rev Man 5.3. software.²⁹

Statistical Analysis and Data Synthesis

Statistical analyses were carried out in Rev Man 5.0. Natural logs of individual ORs along with their corresponding standard errors were calculated. These log effect estimates were combined by the inverse variance method using a random effects model to get a summary meta- odds ratio (mOR). An mOR was calculated for the risk of developing any OPMD associated with the use of any SLT. Subgroup analyses included estimation of an mOR for: (1) Stratified risk for each subtype of OPMDs, associated with the use of any SLT; (2) risk of developing any OPMD with the use of different subtypes of SLT; (3) risk of developing OPMDs based on intensity and duration of SLT use; and (4) risk of developing OPMDs among male versus female SLT users. Country-specific estimates were also calculated. Heterogeneity among the studies regarding the stratification of duration and intensity of exposure were dealt with by collapsing strata and by calculating an OR_{MH} for the combined strata. Subgroup analyses were also carried out for studies reporting an estimate, which had been adjusted for smoking and alcohol use, and those reporting crude estimates or estimates calculated by the authors. Additional sensitivity analyses were carried out by excluding studies that had reported very high ORs (>20).

Where applicable we also calculated the country specific Population Attributable Fraction (PAF%) using the formula, signifying the proportion of OPMDs in a population that can be attributed to the use of SLT products. Following the "rare disease assumption,"³⁰ we substituted OR for RR and country-specific exposure prevalence (p) estimates for India and Pakistan were derived from the most recent Global Adult Tobacco Survey (age 15 and above, both sexes) estimates of the WHO,³¹ for Sri Lanka the estimates were drawn from the most recent estimates of the (STEPS) NCD risk Factor Survey (age 15–64, both sexes) provided in the Tobacco Atlas.³²

Heterogeneity among the studies was detected by visually inspecting forest plots. The I^2 statistic was calculated for the quantification of heterogeneity and assessment of the effects of heterogeneity on the pooled analysis. From our pre-review scoping exercise, we expected a high heterogeneity. However, we felt that the knowledge gap with regards to the risk of OPMDs associated with the use of SLT makes it pertinent and worthwhile to undertake meta-analyses and calculation of pooled effect estimates, with a view to point out the magnitude of the heterogeneity to the readers along with each pooled estimate. Causes of heterogeneity were explored through subgroup analysis based on geographical location, the type of SLT, gender, and exposure-response categories. To determine the effect of each study on the overall estimate, one study was dropped at a time. Reporting bias was assessed by visually inspecting the funnel plot.

This study is reported in accordance with the "Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines."

Results

Characteristics of Included Studies

The electronic search returned 712 articles, all in the English language. One additional study was identified through a search of the bibliographies of the included studies. Fifteen articles corresponding to 18 studies were included in the final review after the removal of duplicates and application of the inclusion/exclusion criteria. [Figure 1](#) describes the various steps of the study selection process.

The majority ($n = 12$) of the included articles were from India, two were from Sri Lanka and only one was from Pakistan. All

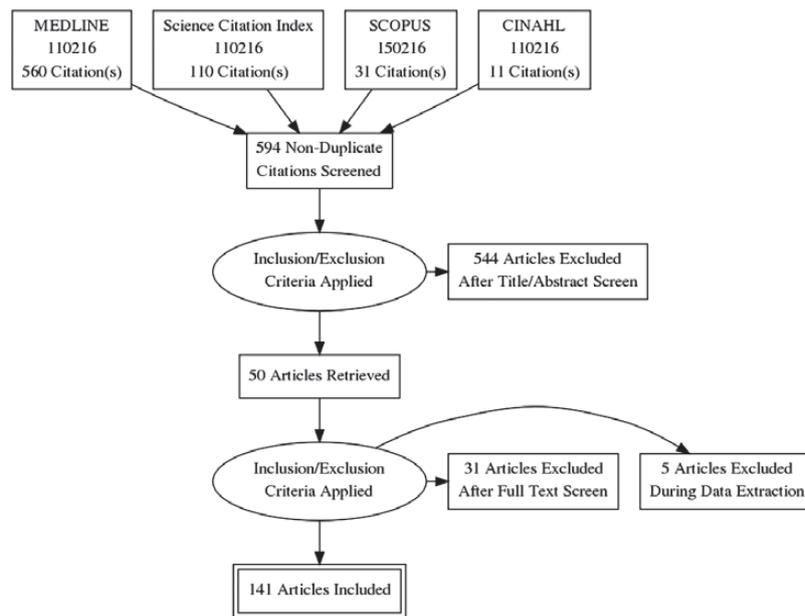


Figure 1. Flow chart of the study selection process.

of the included studies were of a case-control design. Important features of the included studies are given in Table 1. The majority of the studies ($n = 12$) were of “moderate” quality, while six studies were rated as “weak.” None of the studies were rated as “strong.” Two studies exclusively reported LP as an outcome, while OSMF was a primary outcome in 12 studies. A further two studies focused on any OPMD without specifying the subtype, one study had multiple OPMDs and another, EP, as an outcome. Betel quid was reported as the main exposure in five studies, Gutkha was the primary exposure in three studies while the remaining 10 studies reported SLT as the primary exposure without specifying the subtype. Seven studies reported or provided data on overall duration of exposure, while six studies reported or provided data on exposure frequency. Gender specific estimates or data to calculate them were reported by five studies. Twelve studies reported an effect estimate which was adjusted for smoking and alcohol, one study reported an effect estimate adjusted for smoking only, while a crude or an MH effect estimate was calculated for five studies from the data provided in the article. Six of the included studies, five from India and one from Sri Lanka, had population-based controls, while the remaining used hospital-based controls. All studies reported an OR with a magnitude higher than one.

Prior to carrying out the meta-analyses, we assessed publication bias by a visual inspection of the funnel plot. The funnel plot (Supplementary Figure 1) was skewed, indicating an underrepresentation of studies with smaller effect estimates. Exclusion of individual studies from the analysis did not have any effect on the funnel symmetry.

Risk of OPMDs with SLT Use

The mOR for any OPMDs with the use of any SLT products (All included studies, $n = 18$) was 15.5 (95% CI, 9.9–24.2), with a high heterogeneity of $I^2 = 89\%$ (Figure 2). The mOR for studies ($n = 15$) from India was 14.5 (95% CI, 8.9–23.5), $I^2 = 90\%$. The pooled OR for studies from Sri Lanka was 15.5 (95% CI, 7.2–33.6), $I^2 = 0\%$. An mOR for Pakistan could not be calculated

because only one study reported an adjusted OR of 64.0 (95% CI, 15–273.2). Sensitivity Analysis revealed that exclusion of studies (6/18) that had not reported an effect estimate adjusted for both alcohol and smoking decreased the magnitude of the effect estimate, mOR = 13.1 (95% CI, 8.3–20.7), $I^2 = 86\%$, these were the same studies which had a “weak” rating. Exclusion of studies (7/18), which had reported a very high effect estimate (OR > 20), resulted in an mOR of 8.0 (95% CI, 5.5–11.7), $I^2 = 84\%$. After excluding the India-based studies (6/15) in which the effect estimate was not adjusted for smoking and alcohol, the mOR for India decreased to 11.4 (95% CI, 6.9–18.8), $I^2 = 89\%$. The mOR for the six studies with population-based controls was 15.0 (95% CI, 9.1–24.8), $I^2 = 88\%$.

Risk of OPMDs Subtypes with SLT Use

The mOR for the development of OSMF with the use of any SLT product was 20.0 (95% CI, 12.3–32.5), $I^2 = 79\%$. After excluding the studies (5/12) with unadjusted risk estimates, the resultant mOR was 16.2 (95% CI, 8.7–30.0), $I^2 = 74\%$ (Figure 3). The risk for LKP was much lower, mOR = 4.33 (95% CI 1.4–13.2), $I^2 = 81\%$. Multiple OPMDs and EP were reported as primary outcomes in one study each. The OR for Multiple OPMDs was 37.8 (95% CI, 16.8–88.1), while that for EP was 19.8 (95% CI, 9.8–40.0). The pooled risk estimate from the studies ($n = 2$) that reported on OPMDs without specifying any subtype was mOR of 20.4 (95% CI, 7.6–54.3), $I^2 = 0\%$.

Risk of OPMDs with Different SLT Types

The pooled risk for the development of any OPMDs with the use of betel quid with tobacco was 16.1 (95% CI, 7.8–33.5), $I^2 = 47\%$. The mOR for OPMDs with the use of Gutkha was much lower at 4.9 (95% CI, 2.6–9.4), $I^2 = 33\%$. For studies ($n = 10$), which reported SLT as a primary exposure without specifying the subtype, the corresponding risk was 23.1 (95% CI, 10.6–50.2), $I^2 = 93\%$, after dropping studies (4/10) with unadjusted effect estimates, the mOR decreased to 13.8 (95% CI, 5.5–34.9), $I^2 = 93\%$.

Table 1. Characteristics of the Included Studies (18 Studies Corresponding to 15 Articles)

Title	Date	Author/s	Study location	Study type	No of cases (n1)	No of controls (n2)	Outcome	Exposure	Adjustment for confounders	Quality
A case-control study of oral submucous fibrosis with special reference to the etiology role of areca nut. ³³	1990	Sinor et al.	India	Case-control	60	60	Submucous fibrosis	SLT	Yes ^a	Weak
Role of areca nut in oral submucous fibrosis causation: a case-control study in Pakistan. ³⁴	1994	Maher et al.	Pakistan	Case-control	157	157	Submucous fibrosis	BQ + T	Yes ^b	Moderate
Role of chewing and smoking habits in the etiology of oral submucous fibrosis: a case-control study. ³⁵	1998	Shah and Sharma	India	Case-control	236	221	Submucous fibrosis	SLT	Yes ^a	Weak
Alcohol drinking, body mass index and the risk of oral leukoplakia in an Indian population. ³⁶	2000	Hashibe et al.	India	Case-control	927	47 773	Leukoplakia	SLT	Yes ^b	Moderate
Chewing tobacco, alcohol, and the risk of erythroplakia. ³⁷	2000	Hashibe et al.	India	Case-control	100	47 773	Erythroplakia	SLT	Yes ^b	Moderate
Body mass index, tobacco chewing, alcohol drinking and the risk of oral submucous fibrosis in Kerala, India. ³⁸	2002	Hashibe et al.	India	Case-control	170	47 773	Submucous fibrosis	SLT	Yes ^b	Moderate
Risk factors for multiple oral premalignant lesions. ³⁹	2003	Thomas et al.	India	Case-control	115	47 773	Multiple OPMD	SLT	Yes ^b	Moderate
Oral submucous fibrosis: a case-control study in Chennai, Southern India. ⁴⁰	2004	Ranganathan et al.(a)	India	Case-control	185	185	Submucous fibrosis	SLT	Yes ^b	Moderate
Oral submucous fibrosis: a case-control study in Chennai, Southern India. ⁴⁰	2004	Ranganathan et al.(b)	India	Case-control	185	185	Submucous fibrosis	BQ + T	Yes ^b	Moderate
Effect of betel chewing, tobacco smoking and alcohol consumption on oral submucous fibrosis: a case-control study in Sri Lanka. ⁴¹	2006	Aniyawardana et al.	Sri Lanka	Case-control	74	74	Submucous fibrosis	BQ + T	Yes ^b	Moderate
Epidemiological and etiological study of oral submucous fibrosis among gutkha chewers of Patna, Bihar, India. ⁴²	2006	Sami et al.	India	Case-control	157	135	Submucous fibrosis	SLT	Yes ^a	Weak
The role of gutka chewing in oral submucous fibrosis: a case-control study. ⁴³	2009	Bhati et al.	India	Case-control	220	220	Submucous fibrosis	SLT	Yes ^c	Weak
Betel quid chewing with or without tobacco is a major risk factor for oral potentially malignant disorders in Sri Lanka: a case-control study. ⁴⁴	2010	Amarasinghe et al.	Sri Lanka	Case-control	101	728	Any OPMD	BQ + T	Yes ^b	Moderate
Odds ratio of risk factors for oral submucous fibrosis in a case-control model. ⁴⁵	2013	Merhorra et al.(a)	India	Case-control	448	2 688	Submucous fibrosis	Gutkha	Yes ^b	Moderate

Table 1. Continued

Title	Date	Author/s	Study location	Study type	No of cases (n1)	No of controls (n2)	Outcome	Exposure	Adjustment for confounders	Quality
Odds ratio of risk factors for oral submucosis fibrosis in a case-control model. ⁴⁵	2013	Merhotra et al.(b)	India	Case-control	448	2688	Submucous fibrosis	BQ + T	Yes ^b	Moderate
Clinico epidemiological profile of potentially malignant and malignant oral disease. ⁴⁶	2013	Ray et al.(a)	India	Case-control	698	766	Leukoplakia	Gutkha + other SLT	No	Weak
Clinico epidemiological profile of potentially malignant and malignant oral disease. ⁴⁶	2013	Ray et al.(b)	India	Case-control	698	766	Submucous fibrosis	Gutkha + other SLT	No	Weak
Analysis of various risk factors affecting potentially malignant disorders and oral cancer patients of Central India. ⁴⁷	2015	Kadashetti et al.	India	Case-control	65	100	Any OPMD	SLT	Yes ^b	Moderate

OPMD = oral potentially malignant disorder; SLT = smokeless tobacco.

^aAdjusted for age and sex only.

^bAdjusted for age, sex, smoking, and alcohol use.

^cAdjusted for age, sex, and smoking only.

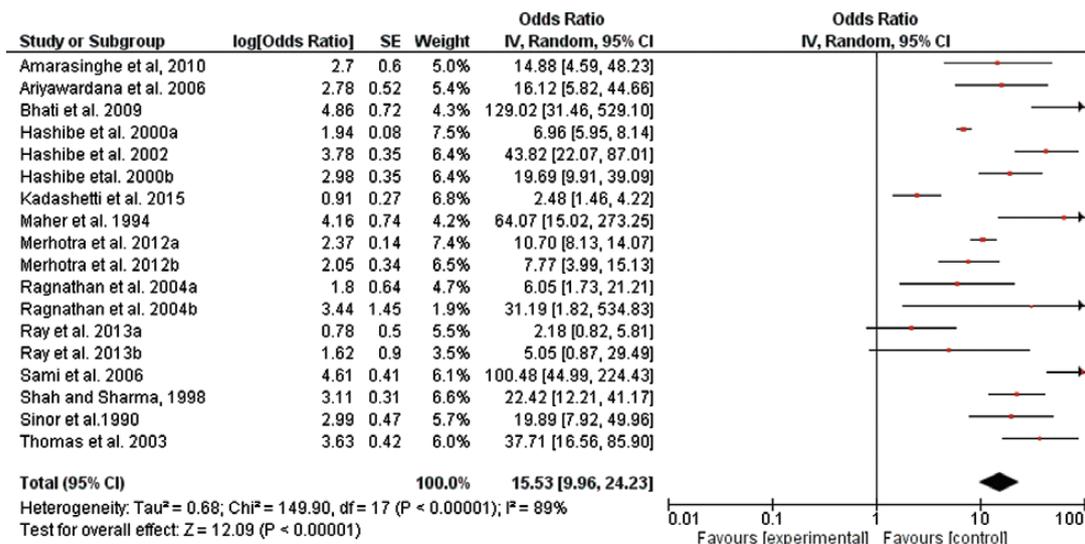


Figure 2. Risk of Oral Potentially Malignant Disorders (OPMDs) associated with the use of smokeless tobacco (SLT).

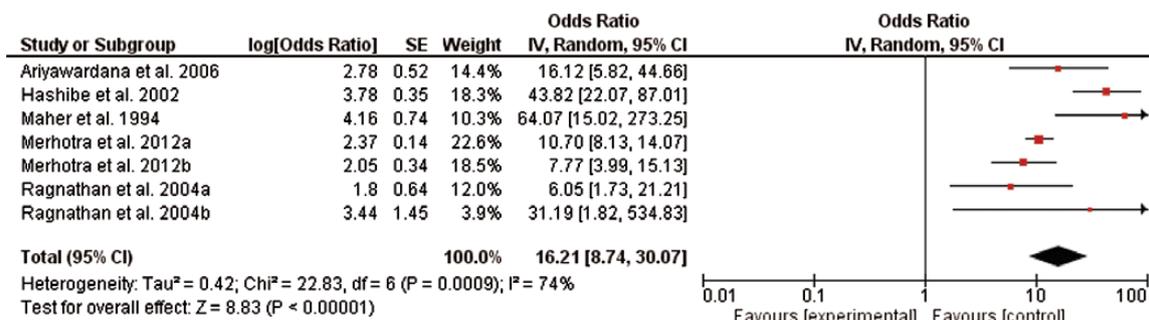


Figure 3. Risk of Oral Sub-Mucous Fibrosis (OSMF) associated with smokeless tobacco (SLT) use (Sensitivity analysis).

Risk in Males Versus Females (Five Studies)

Females had a considerably elevated risk of developing a OPMDs with the use of SLT, mOR = 22.2 (95% CI, 9.1–54.1), compared to men, mOR = 8.7 (95% CI, 2.1–34.8), I² = 79%.

Exposure–Response Relationship

The magnitude of risk was directly related to the total duration of exposure. Compared to non-users, the mOR for developing an OPMDs with the total duration of an SLT habit of up to 20 years was 67.5 (95% CI, 25.6–177.7), I² = 83%. After excluding studies (2/7) that did not adjust for alcohol or smoking, the mOR came down to 29.3 (95% CI, 20.3–42.1), I² = 0%. Compared to a non-user, the risk for persons having an SLT habit for up to 40 years increased to mOR = 95.2 (95% CI, 36.5–247.8), I² = 78% for smoking and alcohol adjusted and non-adjusted studies (n = 7) combined, and 41.9 (95% CI, 27.4–64.1), I² = 9%, when only adjusted estimates were pooled (5/7). Similarly, the frequency of SLT use was directly related to the magnitude of the risk estimates. Compared to non-users, the mOR for developing an OPMDs was 38.1 (95% CI, 24.6, 59.2), I² = 27%, for persons using SLT up to 10 times per day (six studies). The pooled estimate slightly decreased when alcohol and smoke adjusted ORs were pooled, mOR = 33.7(95% CI, 23.6–48), I² = 0% (five studies). The pooled risk increased

to 65.5 (95% CI, 39.2–109.5), I² = 13%, for persons using an SLT product up to 20 times a day, when combining both adjusted and non-adjusted studies (n = 6). The mOR after dropping the study (1/6), which had not adjusted for alcohol and smoking was 55.7(95% CI, 35.2–88.3), I² = 0%.

Population Attributable Fraction

Utilizing the country specific results from our meta-analysis, we calculated the PAF for India, Sri Lanka, and Pakistan. The PAF for India (SLT prevalence = 25.9%) was 74% while that for Sri Lanka (SLT prevalence = 15.8%) was 69%, the PAF for Pakistan (SLT prevalence = 11.4%) was 88%.

Discussion

The results of our review point towards a strong association between SLT use and OPMDs in South Asia. Although there is evidence of high heterogeneity among the studies included in this systematic review, the magnitude of risk of developing OPMDs with the use of SLT products was consistently high across all the included studies. The pooled risk was elevated for most types of the OPMDs, but was particularly high for OSMF (16-fold). In comparison, LKP had a 4-fold increased risk among SLT users compared to non-users. The high effect estimates and I² values in our study

are in line with those reported by previous systematic reviews on oral cancer risk with the use of SLT in the South Asian region.^{14,21,48} The magnitude of the pooled OR in our study was higher than the mORs reported by the meta-analysis of studies on oral cancer. This can be explained by the fact that OPMDs is an intermediate outcome, lying on the pathway of the development of oral cancer, and not all OPMDs progress to malignancy.⁴ Country specific analysis revealed high mORs for India and Sri Lanka, albeit a higher heterogeneity in the case of India. The latter might be due to the difference in the number of studies pooled together that is, two for Sri Lanka and 11 for India. Pooling of effect estimates which were adjusted for smoking and alcohol revealed a decrease in the magnitude of the pooled OR and also a slight decrease in the heterogeneity. These findings are similar to those of previous reviews on SLT and the risk of oral cancer and are suggestive of alcohol and smoking as factors in the etiology of OPMDs.^{21,49–52} The strong association between OPMDs and the use of SLT in South Asia that we are reporting is in accordance with results of independent studies carried out in other parts of the world. A study of 1569 men from Uzbekistan reported an OR of 5.1 (95% CI, 3.1–8.6) for oral and esophageal precancer among users of SLT compared to non-users.⁵³ An OR of 60 (95% CI, 40.5–88.8) for LKP among tobacco chewers compared to no-chewers, was reported by a study of 1109 baseball players in the United States.⁵⁴ A large study of 20 333 adults in Sweden revealed a 15.4% prevalence of Snuff-induced lesions in the study sample.⁵⁵

Our finding that there is a higher risk of developing OSMF compared to LKP, with the use of SLT, is supported by the fact that OSMF has a higher prevalence compared to any other OPMDs in South Asia,^{56–59} where SLT use is reaching an epidemic level.⁶⁰ Although the mOR decreased significantly after exclusion of studies which did not report an adjusted risk estimate, it was still significantly higher than the corresponding estimate for all OPMDs combined. Although the risk of OSMF progressing to malignancy is considered lower than LKP, OSMF is a very restrictive and debilitating condition with considerable implications on the quality of life.⁶¹ The lack of studies on EP and multiple OPMDs did not allow for the calculation of a pooled risk estimate for these conditions; however, the studies reporting EP and multiple OPMDs show highly elevated risks for these conditions with the use of SLT.

The stratified analysis by SLT products revealed a much lower heterogeneity among the studies. Among studies, which had adjusted for smoking and alcohol use, the combination of Betel quid with tobacco had the highest risk for developing OPMDs compared to other products. This finding is similar to the results reported by systematic reviews on oral cancer risk with the use of SLT products.^{14,21} All tobacco products induce carcinogenesis at the cellular level. The Tobacco Specific Nitrosamines in SLT help in the formation of DNA adducts, which if remain unrepaired, bring about abnormal cellular proliferation and the subsequent tumor formation.⁶² The higher risk associated with betel quid plus tobacco can be explained by the additional constituents of betel quid. Areca nut, which in itself is considered carcinogenic⁶³ is combined with slaked lime and tobacco in the preparation of betel quid. The slaked lime works 2-fold: (1) it helps in the release of Arecoline and its conversion into Arecaidine, which in turn trigger fibroblast proliferation and increase collagen synthesis in the oral mucosa, and (2) it also facilitates the production of Reactive Oxygen Species, which causes oxidative stress by increasing the pH of the oral microenvironment. This, combined

with the carcinogens in tobacco,¹³ may thus pose a higher risk of oral premalignancy and malignancy due to a synergistic effect, compared to other SLT products.^{64–67}

The results of our review suggest a considerably elevated risk of development of OPMDs among females compared to males associated with the use of SLT products. This finding is similar to previous findings on SLT use and the risk of oral cancer.¹⁴ This might be explained by a lower background risk of OPMDs among females as compared to males. The prevalence of other risk factors for OPMDs such as alcohol and smoking tobacco is considerably higher among men as compared to women in the countries of origin of the included studies.²¹ There might also be differences in the pattern of use of SLT products among males and females.^{68,69} Females in South Asia predominantly stay indoors, with very few going out for work, while most men go out for work etc. This may result in women chewing more and/or for a longer duration as compared to men, thus increasing their risk of development of OPMDs.^{70,71}

Our study reports a strong exposure–response relationship with regards to the frequency and duration of use of SLT products and development of OPMDs. The risk increases with increasing duration as well as the intensity of SLT use. This finding is in agreement with the results of a meta-analysis on the risk of oral cancer associated with SLT use^{14,54} and further suggests a strong association between OPMDs and SLT use. Future prospective studies are needed to assess the nature of this association for causal inference.

Strengths and Limitations

To the best of our knowledge, this is the first systematic review assessing the risk of OPMDs associated with the use of SLT products in South Asia and we believe it will help in informing tobacco control policies in South Asia, where control of SLT control has seldom been the main focus of tobacco control policies and actions.⁷² As pointed earlier a recent study on the global burden of disease attributable to SLT use has reported the burden of cancers and cardiovascular disease that are attributable to SLT use, however, that study did not report the OPMD burden attributable to SLT use.²³ As such this review adds to the body of knowledge on the burden of disease attributable to SLT use. The high heterogeneity among the included studies might negatively affect the pooled risk estimate reported by our study. Efforts were however made to minimize this through stratified analysis. Further, the magnitude of the heterogeneity was presented with every effect estimate that is reported in our review.

Another potential limitation of our study was the inherent bias related to observational study designs that might have been present in the included studies. This includes selection and recall bias, as well as bias linked to retrospective exposure assessment and under or over reporting of exposure status. There was a lack of a precise definition of what constituted “ever exposed,” in quantitative terms, in the included studies. Therefore, we had to use broad criteria for “ever exposed” that is, A person who had used SLT only once in their life and a person who had used it for 20 years were both considered as ever-exposed. This might have resulted in an overestimation of the true risk estimate.

Additionally, under-reporting of smaller effect estimates and publication bias may also have affected the results of our study. There was also a lack of studies addressing the less common OPMDs such as LP, DLE, and AK and as such, our findings are only applicable to the more common OPMDs conditions such as LKP, OSMF, and

EP. Moreover, we did not include studies carried out in expat South Asian populations which might have resulted in an under or over estimation of the true risk estimate. We excluded studies carried out in expat populations on the grounds that there may be differences in the composition of the SLT products used in South Asia and those used elsewhere. Another reason for excluding the expat populations was the potential difference in lifestyle related factors, such as diet and exercise, which are considered modifying factors for the risk of various disease. It may be worthwhile to conduct future reviews of studies carried out in expat populations.

It is also pertinent to mention that the biochemical composition, methods of use, and the duration of use vary among different SLT products. Additionally, the composition and the method of use of a similar product may vary among different geographical regions,¹⁹ which might have a bearing on the generalizability of our results. We have tried to address this issue by stratified analysis of the different SLT products to provide risk estimates associated with individual SLT products. Despite these limitations, we tried to address some of them through stratified analysis and provide the most robust risk estimates possible to quantify the relationship between OPMDs and SLT use.

Confidence in Cumulative Evidence

We used the “Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group methodology”⁷³ to assess the quality of the cumulative evidence (summary OR for the association of SLT use vs. no use, and OPMDs) reported in our study, via the GRADEpro GDT Software.⁷⁴ The reported evidence was downgraded on the basis of the high heterogeneity, risk of bias inherent to case-control studies, imprecision that is, increased the width of the confidence interval and presence of publication bias. The evidence was upgraded on the basis of the elevated magnitude of the risk estimate, plausible residual confounding lowering the demonstrated risk and the presence of a dose-response relationship. Application of the aforementioned parameters resulted in an overall quality of evidence that was rated as “Weak” (Supplementary Table 2).

There are plenty of reasons for the “weak” rating of the reported cumulative evidence, first and foremost is that GRADE methodology automatically assigns a “weak” rating to evidence from case-control studies, which, depending upon the methodological robustness and the validity of the reported effect estimates, of the pooled studies, may be upgraded to “Moderate” or very rarely to “High” strength.⁷⁵ There are indications of high heterogeneity and publication bias in addition to the usual methodological biases associated with case-control studies.⁷⁶ Although the quality of the cumulative evidence is “Weak,” it may be noted that the inappropriateness to conduct experimental studies for the assessment of the association between SLT and OPMDs on ethical grounds, renders the reported evidence as the “best evidence” available⁷⁷ and hence may be used to inform tobacco control policies in South Asia.

Conclusion and Implications

The findings of our study point towards a strong association between some forms of OPMDs and SLT use in South Asia. The risk estimates are high, irrespective of controlling for confounders such as smoking and alcohol or stratification by sex, country or source of controls. There is also an exposure–response relationship between OPMDs and SLT use. The high prevalence of both OPMDs and

SLT use in South Asia is a public health problem and this review provides the “best available” evidence on the association of OPMDs and SLT use, which can be used to educate the general public about the deleterious effects of SLT use and help inform both cancer and SLT control policies in South Asia as well as countries where SLT products of South Asian origin are used. Since OPMDs lie on the causal pathway to oral malignancy, preventing their development can potentially lead to a reduction in the incidence of oral cancer.

Differences Between Review and Protocol

We have tried our best to stay true to the published protocol for this review and hence report no significant differences between the review and the published protocol.⁷⁸

Supplementary Material

Supplementary Tables 1–3 and Figure 1 can be found online at <http://www.ntr.oxfordjournals.org>

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Declaration of Interest

None declared.

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We are thankful to Hajo Zeeb for his support and guidance. ZK and FSZ conceived the study. LC devised the search strategy and performed the electronic searches. SR and SK carried out study selection, quality assessment, and data abstraction. ZK and OE performed statistical analysis. ZK wrote the initial draft and all authors contributed to the subsequent drafts.

Authors Contribution

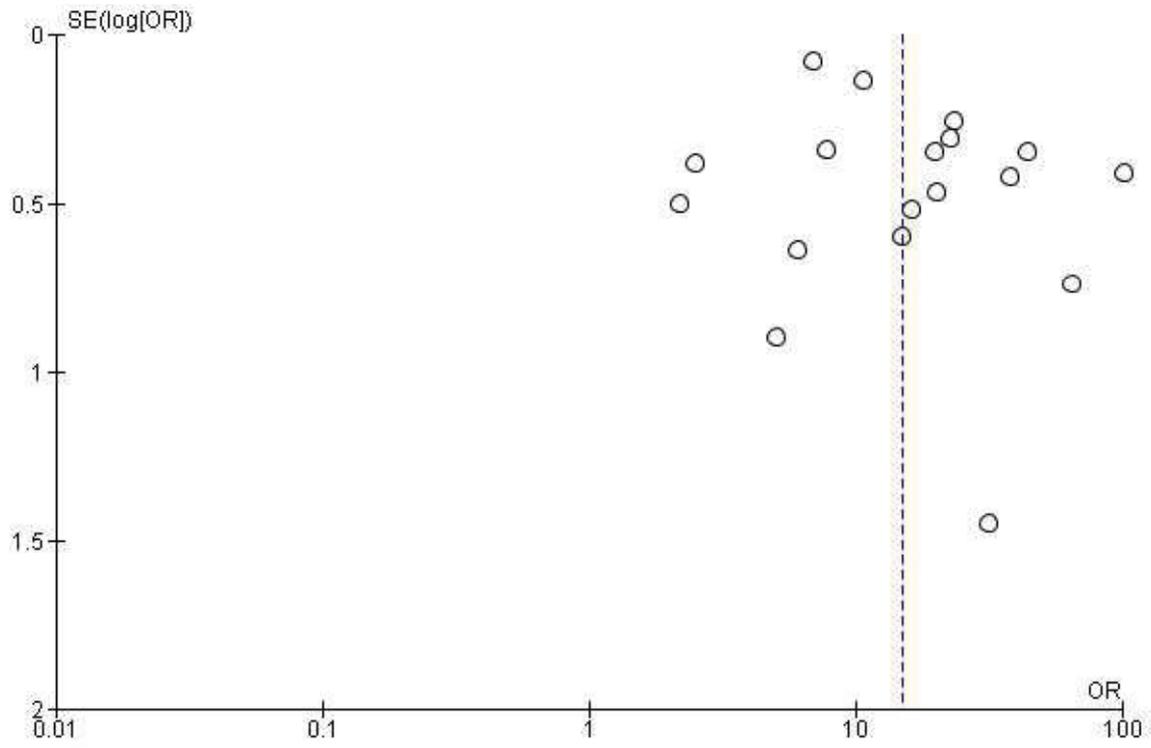
ZK and FSZ conceived the study. LC devised the search strategy and performed the electronic searches. SR and SK carried out study selection, quality assessment, and data abstraction. ZK and OE performed statistical analysis. ZK wrote the initial draft and all authors contributed to the subsequent drafts.

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SUPPLEMENTARY FIGURE 1. FOREST PLOT OF INCLUDED STUDIES

SUPPLEMENTARY TABLE 1: DETAILED SEARCH STRATEGY

PubMed searches

11.02.2016

Block 1: Smokeless tobacco

Search name	Search query	Type of search	Results
1A	("Betel quid" OR paan OR Pan OR "Pan Masala" OR "Creamy snuff" OR Gul OR gudhaku OR gutka OR khaini OR khawam OR "Dry snuff" OR mawa OR mishra OR naskar OR "Red tooth powder" OR tuibur OR zarda OR "smokeless tobacco")	keyword	98.001
1B	smokeless tobacco (*)	MeSH major & sub-terms	3.047
1	1A OR 1B		98.001

Block 2: South Asia

Search name	Search query	Type of search	Results
2A	(Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR "south* asia*")	keyword	525.381
2B	Asia, Western (*)	MeSH major & sub-terms	206.521
2	2A OR 2B		604.243

Block 3: Oral potentially malignant disease

Search name	Search query	Type of search	Results
3A	("Palatal lesions" OR "Lichen planus" OR "Discoid lupus erythematosus" OR "Oral Potential* Malignant" OR precancerous OR Leukoplakia OR "Submucous fibrosis" OR Erythroplakia OR "Actinic keratosis" OR OPMD)	keyword	49.434
3B	Mouth Diseases (*)	MeSH major & sub-terms	258.503
3	3A OR 3B		297.982

Summary & results

Search name (Saved in PubMed & EndNote)	Results
1 AND 2 AND 3	560

CINAHL search

11.02.2016

Search name	Search query	Type of search	Results
1	("Betel quid" OR paan OR Pan OR "Pan Masala" OR "Creamy snuff" OR Gul OR gudhaku OR gutka OR khaini OR khawam OR "Dry snuff" OR mawa OR mishra OR naskar OR "Red tooth powder" OR tuibur OR zarqa OR "smokeless tobacco")	keyword	1.912
2	("Palatal lesions" OR "Lichen planus" OR "Discoid lupus erythematosus" OR "Oral Potential* Malignant" OR precancerous OR Leukoplakia OR "Submucous fibrosis" OR Erythroplakia OR "Actinic keratosis" OR OPMD)	keyword	2.470
3	(Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR "south* asia*")	keyword	24.929
4	1 AND 2 AND 3		11

SCI search

11.02.2016

Search name	Search query	Type of search	Results
1	("Betel quid" OR paan OR Pan OR "Pan Masala" OR "Creamy snuff" OR Gul OR gudhaku OR gutka OR khaini OR khawam OR "Dry snuff" OR mawa OR mishra OR naskar OR "Red tooth powder" OR tuibur OR zarqa OR "smokeless tobacco")	keyword	44.509
2	("Palatal lesions" OR "Lichen planus" OR "Discoid lupus erythematosus" OR "Oral Potential* Malignant" OR precancerous OR Leukoplakia OR "Submucous fibrosis" OR Erythroplakia OR "Actinic keratosis" OR OPMD)	keyword	14.486

3	(Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR "south* asia*")	keyword	199.541
4	1 AND 2 AND 3		110

Scopus search

15.02.2016

Search name	Search query	Type of search	Results
1	("Betel quid" OR paan OR Pan OR "Pan Masala" OR "Creamy snuff" OR Gul OR gudhaku OR gutka OR khaini OR khawam OR "Dry snuff" OR mawa OR mishra OR naskar OR "Red tooth powder" OR tuibur OR zarqa OR "smokeless tobacco")	Title-abstract-keyword	80.139
2	("Palatal lesions" OR "Lichen planus" OR "Discoid lupus erythematosus" OR "Oral Potential* Malignant" OR precancerous OR Leukoplakia OR "Submucous fibrosis" OR Erythroplakia OR "Actinic keratosis" OR OPMD)	Title-abstract-keyword	56.376
3	(Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR "south* asia*")	Title-abstract-keyword	47.491
4	1 AND 2 AND 3		31

Google Scholar Search, run 10.03.2016

intitle:"smokeless tobacco" AND (Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR Asia) AND ("Oral Potential Malignant" OR "Oral Potentially Malignant" OR precancerous OR OPMD)

Discarded searches:

intitle:"smokeless tobacco" AND (Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR Asia) AND ("Oral Potential Malignant" OR precancerous OR OPMD)

109

intitle:"smokeless tobacco" AND (Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR Asia) AND ("Oral Potentially Malignant" OR precancerous OR OPMD)

65

intitle:"smokeless tobacco" AND (Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR Asia) AND intitle: ("Palatal lesions" OR "Lichen planus" OR "Discoid lupus erythematosus" OR "Oral Potential Malignant" OR precancerous OR Leukoplakia OR "Submucous fibrosis" OR Erythroplakia OR "Actinic keratosis" OR OPMD)

Too long doesn't work

"smokeless tobacco" AND (Afghanistan OR Bangladesh OR Bhutan OR India OR Maldives OR Nepal OR Pakistan OR Sri Lanka OR Iran OR Asia) AND ("Oral Potential Malignant" OR "Oral Potentially Malignant" OR precancerous OR Precancer OR OPMD)

1.100**Search in Global Index Medicus¹****Search conducted 10.03.2016**

This resource is a repository for scientific and technical literature which includes the following regional indexes: AIM (AFRO), LILACS (AMRO/PAHO), IMEMR (EMRO), IMSEAR (SEARO), WPRIM (WPRO), Global Index Regional Indexes, MEDLINE, SciELO as well as IRIS, the WHO institutional repository.

Search:

```
(tw:((afghanistan OR bangladesh OR bhutan OR india OR maldives OR nepal OR pakistan OR sri lanka OR iran OR "south* asia*"))) AND (tw:(("Betel quid" OR paan OR pan OR "Pan Masala" OR "Creamy snuff" OR gul OR gudhaku OR gutka OR khaini OR khawam OR "Dry snuff" OR mawa OR mishra OR naskar OR "Red tooth powder" OR tuibur OR zarqa OR "smokeless tobacco"))) AND (tw:(("Palatal lesions" OR "Lichen planus" OR "Discoid lupus erythematosus" OR "Oral Potential* Malignant" OR precancerous OR leukoplakia OR "Submucous fibrosis" OR erythroplakia OR "Actinic keratosis" OR opmd))) AND (instance:"ghl")
```

134 results

```
(tw:((afghanistan OR bangladesh OR bhutan OR india OR maldives OR nepal OR pakistan OR sri lanka OR iran OR "south* asia*"))) AND (tw:(("Betel quid" OR paan OR pan OR "Pan Masala" OR "Creamy snuff" OR gul OR gudhaku OR gutka OR khaini OR khawam OR "Dry snuff" OR mawa OR mishra OR naskar OR "Red tooth powder" OR tuibur OR zarqa OR "smokeless tobacco"))) AND (tw:(("Palatal lesions" OR
```

¹ About the Global Index Medicus, formerly known as the Global Health Library:

<http://www.globalhealthlibrary.net/php/level.php?lang=en&component=19&item=1>

"Lichen planus" OR "Discoid lupus erythematosus" OR "Oral Potential* Malignant" OR precancerous OR leukoplakia OR "Submucous fibrosis" OR erythroplakia OR "Actinic keratosis" OR opmd))) AND (instance:"ghl") AND (db:("IMSEAR" OR "LILACS"))

14 results, unique -> all hits from Medline excluded, these hits came from either IMSEAR or LILACS.

Quality assessment						
Nº of participants (studies) Follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence
18 observational studies of case control design	Serious ¹	Serious ²	Not serious ³	Serious ⁴	publication bias strongly suspected very strong association all plausible residual confounding would reduce the demonstrated effect dose response gradient ⁵	⊕⊕○○ LOW

SUPPLEMENTARY TABLE 2: QUALITY OF EVIDENCE

1. Case control design
2. High heterogeneity
3. The studies reported what they intended to find.
4. Confidence interval is moderately wide.
5. Indication of publication bias through visual assessment of funnel plot.

ARTICLE V

ORAL CANCER VIA THE BARGAIN BIN: THE RISK OF ORAL CANCER ASSOCIATED WITH A SMOKELESS TOBACCO PRODUCT (NASWAR).

First author: Zohaib Khan

Order of authors: Khan Z, Shah SMH, Rehman B, Ullah Z, Khan S, Dreger S, Pohlabein H, Zeeb H.

Author's contributions: ZK and HZ conceptualized the study. ZK and HP carried out data analyses. All authors contributed to the initial and final draft of the study.

Manuscript statistics: 4963 words (Abstract: 244), 5 tables and 2 supplementary figures.

Manuscript status: Under review in "*PLOS One*".

1 **Oral cancer via the bargain bin: the risk of oral cancer associated with**
2 **a smokeless tobacco product (*Naswar*).**

3

4 **Authors:** Zohaib Khan*^{1,2,3}¶, Syed Majid Hussain Shah⁴, Basheer Rehman⁵, Zakir Ullah⁶,
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16

17 **KEYWORDS**

18 Smokeless tobacco, *Naswar*, Case-control, Oral cancer, Pakistan

1 **ABSTRACT**

2 In the wake of smokeless tobacco (SLT) being advocated as a mean of tobacco harm
3 reduction, it is pertinent to establish individual health risks associated with each SLT
4 product. This case-control study was aimed at assessing the risk of oral cancer associated
5 with a smokeless tobacco product (*Naswar*). The study was conducted from September
6 2014 till May 2015 in Khyber Pakhtunkhwa, Pakistan. Exposure and covariate information
7 was collected through a structured questionnaire. Conditional logistic regression was used
8 to calculate odds ratios (OR) along with their 95% confidence intervals (CI). 84 oral cancer
9 cases (62% males) and 174 age- and sex-matched controls were recruited. Ever users of
10 *Naswar* had more than a 20-fold higher risk of oral cancer compared to never-users (OR
11 21.2, 95% CI 8.4-53.8). Females had a higher risk of oral cancer with the use of *Naswar* (OR
12 29.0, 95% CI 5.4-153.9) as compared to males (OR 21.0, 95% CI 6.1-72.1). Based on this
13 result, 68% (men) and 38% (women) of the oral cancer burden in Pakistan is attributable
14 to *Naswar*. The risk estimates observed in this study are comparable to risk estimates
15 reported by previous studies on other forms of SLT use and the risk of oral cancer in
16 Pakistan. The exposure-response relationship also supports a strong role of *Naswar* in the
17 etiology of oral cancer in Pakistan. Although still requiring further validation through
18 independent studies, these findings may be used for smokeless tobacco control in countries
19 where *Naswar* use is common.

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1 ABBREVIATIONS

2	CI	Confidence Interval
3	DAG	Directed Acyclical Graph
4	KPK	Khyber Pakhtunkhwa province
5	MAS	Minimum Adjustment Set
6	NPY	<i>Naswar</i> Pack-Year
7	OR	Odds Ratio
8	SLT	Smokeless Tobacco

9 INTRODUCTION

10 Oral cancer is one of the most common cancers in the world with approximately 300,000
11 incident cases each year [1]. Pakistan has one of the highest prevalence of oral cancer in the
12 world [2]. With an age-standardized incidence rate of 9.8/100,000, oral cancer has become
13 the most frequent cancer among males and the second most common cancer among both
14 sexes in Pakistan [1]. A variety of risk factors like diet, alcohol, tobacco use, infections,
15 genetic and environmental factors are associated with oral cancer. Among these, tobacco
16 smoking and alcohol use have been widely researched and are universally considered as
17 causal factors [3]. Smokeless tobacco (SLT) is labeled as carcinogenic by the World Health
18 Organization [4]. Studies from South Asia have established SLT as a risk factor for oral
19 cancer [5,6], but some investigations from industrialized countries, particularly Sweden,
20 where SLT use is common, do not show an increased risk of oral cancer linked to the use
21 of some SLT products [7,8]. These conflicting results become particularly important in the
22 light of SLT products being considered as an alternative to smoking [8], and as means of
23 harm reduction [9-11].

24 An estimated 250 million people use smokeless tobacco in South Asia [12]. Research on
25 SLT products and the risk of oral cancer in South Asia has traditionally focused on Betel-
26 quid and Gutkha [5,6]. This is understandable, as the majority of SLT research has been

1 carried out in India, where the most common forms of SLT are Gutkha and Betel-quin [13].
2 *Naswar* is a mixture of dried tobacco leaves, ash, lime and flavoring agents [14]. It is kept
3 in the buccal sulcus of the mouth and the active agents are absorbed through the oral
4 mucosa. *Naswar* use is often associated with the Pashtun tribes of Afghanistan and Pakistan
5 but is also used in Central Asia, India, Bangladesh and by expat communities of these
6 countries across the world [15].

7 *Naswar* is a much cheaper product compared to cigarettes. An average pack of *Naswar* costs
8 approximately a 10th of the price of a cigarette pack in Pakistan and as such is gaining
9 popularity as a cheap alternative to smoking [16]. It is also being advocated as a cheaper
10 nicotine replacement therapy for people trying to quit smoking [17]. The sale and
11 manufacture of *Naswar* in Pakistan are not regulated [18], and the sizing of the package
12 and the constituents vary from one manufacturer to the other. Thus, the amount of
13 carcinogenic agents also differs among the different brands available on the market [14].
14 Unlike cigarettes, the individual serving size also varies and is dependent upon personal
15 preferences. This renders the correct establishment of the magnitude of the risk of oral
16 cancer associated with a discernable *Naswar* “dose”, particularly challenging. A few studies
17 from the south of Pakistan, where other forms of SLT are more popular [19,20], have
18 reported risk estimates for oral cancer associated with *Naswar*, but there is scanty evidence
19 from the Khyber Pakhtunkhwa province (KPK), which has the highest number of *Naswar*
20 users in Pakistan [21]. The dearth of evidence needed to establish *Naswar* as carcinogenic
21 for humans has also been acknowledged by the International Agency for Research on
22 Cancer in its monograph on smokeless tobacco [22].

23 Given the conflicting research findings on the risks of SLT use and the scarcity of research
24 on assessment of *Naswar* as being carcinogenic to humans, we carried out a case-control
25 study in the KPK to assess the association between *Naswar* and the risk of oral cancer. We
26 particularly focused on exposure quantification by using a novel method of *Naswar* pack-
27 years (NPY), assessment of exposure-response relationships and gender stratified risks. We
28 also assessed the fraction of incident oral cancer among the study population that can be
29 attributed to *Naswar*.

1 METHODS

2 **Study design and setting**

3 A multi-center matched case-control study was carried out in two major cities of the
4 Khyber Pakhtunkhwa province of Pakistan between September 2014 and May 2015.
5 Peshawar is the capital city of the province; while Abbottabad is considered as the summer
6 capital. The province has an area of 74,521 sq km and a total population of 17.5 million. The
7 population of Peshawar is 3,575,000 while that of Abbottabad is 1,182,000 [21]. The
8 majority of the population lives in rural areas and agriculture and trade are the main
9 earning resources. Cases were recruited at three tertiary care centers (Maxillofacial Surgery
10 department of Khyber College of Dentistry, Peshawar, Ear, Nose, and Throat department
11 of the Khyber Teaching hospital, Peshawar and the Maxillofacial Surgery Department of
12 Rehmat Memorial Hospital, Abbottabad). Since primary and secondary healthcare facilities
13 in the province do not have adequate means to diagnose and/or manage oral cancer
14 patients, the included study centers are mainly responsible for the provision of both
15 diagnostic and curative services for oral cancer. The catchment area of the study centers
16 includes the whole province along with the Federally Administered Tribal Areas (FATA) of
17 Pakistan. Controls were recruited from the same centers as well as from two additional
18 health facilities in Peshawar (Pakistan Paraplegic Center, Peshawar and Institute for
19 Physical Medicine and Rehabilitation, Khyber Medical University, Peshawar). These
20 facilities also provide health services to the population of the whole province. All study
21 centers were selected based on expert opinions from local cancer physicians and dentists.
22 The recruitment was carried out for a nine-month period starting September 2014 and
23 ending in the first week of June 2015.

24 **Power calculation**

25 The study size for a case-control ratio of a 1:1 and 1:2, was calculated in Epi Info 7 by using
26 the Fliess method with continuity correction factor. The prevalence of *Naswar* (15%) among
27 the general population (controls) was derived from a nationally representative

1 survey[21,25]. To detect an OR of 3.0 with a two-sided 95% confidence level and a power
2 of 90%, we had to recruit 78 cases and 156 controls.

3 **Ethical approval**

4 Ethical approval for the study was granted by the ethical review board of Khyber Medical
5 University and also by the ethical review committee of Khyber College of Dentistry. Written
6 approvals to carry out the study were also obtained from the heads of the participating
7 centers. Written consent was taken from each study participant before the interview and
8 subsequent collection of biosamples. All study participants had the option to retract their
9 consent at any stage of the study if they did not want to be a part of the study. To ensure
10 maximum participation, laboratory charges related to the histopathological diagnosis and
11 confirmation of the presence of oral cancer were borne from the study fund. These charges
12 are normally paid out of pocket by the patients.

13 **Recruitment of cases and controls**

14 **Cases**

15 Potential cases were recruited based on a clinical differential diagnosis of oral cancer. For
16 the purpose of this study, “oral cancer” was defined as squamous cell carcinoma of the
17 buccal mucosa, lip, tongue and the oropharynx: The ICD-10 classification was used to
18 designate oral cancer sites to be included in the study. The eligible sites included lip, the
19 base of tongue, other and unspecified parts of the tongue, gum, floor of mouth, palate,
20 other and unspecified parts of the mouth, tonsil, and oropharynx (C00 - C06 and C09 -
21 C10). A potential case was confirmed as a “definitive case”, only after the histopathological
22 confirmation of the presence of squamous cell carcinoma at one of the above-mentioned
23 sites.

24 **Controls**

25 Subjects with any condition, except for cancer, pulmonary disease, cardiovascular disease,
26 gastrointestinal disease and periodontal disease, were eligible to be recruited as controls
27 because these diseases are known to be related to tobacco use. Two age (10-year bands)

1 and sex-matched controls were recruited per case from the out-patient and in-patient
2 departments of the study centers. Following are the inclusion and exclusion criteria for
3 recruitment:

4 **Inclusion criteria**

- 5 • Only incident cases who had not yet undergone any treatment for oral cancer were
6 included as cases;
- 7 • all included cases and controls were permanent residents and/or living in KPK or
8 FATA for at least twelve months prior to the interview;
- 9 • a case or control was only included if he/she could provide an informed consent and
10 was deemed physically fit to be interviewed by the resident doctor/s.

11 **Exclusion criteria**

- 12 • Subjects with tumors/malignancy of the hypopharynx, nasopharynx, and salivary
13 glands, or who had previous treatment for oral cancer before the interview;
- 14 • subjects who were not permanent residents and/or had not been living in the
15 Khyber Pakhtunkhwa province or the federally administered tribal areas for at least
16 12 months prior to the interview;
- 17 • unable to provide informed consent due to illness or deemed “physically not fit” for
18 interview by a resident doctor.

19 ***Matching***

20 Two controls per case, frequency-matched for age (10-year bands) and sex, were recruited
21 for the study.

22 **Exposure variables**

23 A Directed Acyclical Graph (DAG) analysis (Supplementary figures I and II) was carried out
24 to ascertain study variables for which data needed to be collected. Oral cancer was the main
25 outcome and *Naswar* was the primary exposure variable. Age, sex, socioeconomic status
26 (SES), tobacco smoking and alcohol use were determined as the Minimum Adjustment Set

1 (MAS) i.e. confounding exposures. Additionally, data were collected for Betel-quid
2 chewing, sunlight exposure, diet, oral hygiene habits and history of the systemic and oral
3 disease.

4 **Data Sources/ Measurement**

5 Data on the study variables was collected through a structured questionnaire adapted from
6 a large European case-control study on upper aero-digestive tract cancers [23]. Face to face,
7 interviews were conducted with both cases and controls. Apart from questions about the
8 “current illness”, the questionnaire used for both groups was the same.

9 ***Naswar use***

10 Data on ever use, daily frequency, total duration in years, duration of single use and type
11 of *Naswar* were recorded. To determine the cumulative exposure to *Naswar*, we developed
12 a novel measure of “*Naswar* pack-year (NPY)”

13 ***Tobacco smoking***

14 Data regarding ever smoking, past smoking, current smoking, frequency and total duration
15 of use in years for cigarettes and/or water pipe were recorded.

16 ***Alcohol drinking***

17 Although alcohol is an established risk factor for oral cancer and can modify or confound
18 the effects of other risk factors, the section of the questionnaire on alcohol use was
19 considerably shortened from the one in the ARCAGE study and had only six questions. This
20 was because alcohol use is forbidden in Islam, the main religion in this region, and is also
21 a culturally and socially unacceptable habit in Pakistan. This renders any talk about alcohol
22 as a taboo. However, we still collected data on ever and never use of alcohol and total
23 duration of alcohol use in order to account for the effects of alcohol use, if any, during
24 analysis.

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1 ***Socioeconomic status***

2 SES was assessed using a simple poverty scorecard developed for Pakistan[24]. The
3 scorecard is used to determine the probability of a household to be situated above the
4 national poverty line i.e. a higher score means a higher probability of being placed above
5 the national poverty line and vice versa. This method has been previously used in social
6 science research in Pakistan but never in health research. The scorecard consists of ten
7 close-ended questions pertaining to assets, education, job type, the number of children,
8 and source of drinking water. The responses are marked and scored according to pre-
9 determined scores. The overall score is then translated into the likelihood of a household
10 being below or above the national poverty line. The advantage of this approach is that it is
11 based on household-level data, which is cognizant of the Pakistani culture of joint families.

12 Dietary habits were assessed using a food frequency questionnaire, containing questions
13 about meat, vegetables, fruit and tea intake. The intake was recorded in terms of frequency
14 per month. The oral health section included questions regarding frequency and mode of
15 mouth and/or teeth cleaning along with the presence of oral disease and the use of
16 dentures. History of disease, such as candidiasis, herpes, warts and regurgitation, was
17 recorded in the systemic disease section. Pictures were used to aid the memory of
18 participants. Sun exposure was assessed by asking questions about the average time spent
19 in the sun during a day. Questions regarding any means of sun protection used by the
20 participants were also included.

21 **Exposure quantification**

22 ***Age***

23 Age was categorized into ten-year bands.

24 ***SES***

25 Based on the probability of lying above the national poverty line, we assigned our study
26 participants into three categories: high (probability > 66%), medium (34% - 66%
27 probability) and low (probability < 34%).

1 ***Habits***

2 An “Ever user” of *Naswar*, cigarette, betel-quid, water pipe, or alcohol was defined as a
3 person who had practiced the habit at least once per week for one year in his life. A “current
4 user” of *Naswar* was defined as someone who has been using *Naswar* at least once per week
5 in the 12 months preceding the interview, including those who had stopped the habit
6 within those 12 months. A “past user” was defined as a person who had used *Naswar* at least
7 once a week for a year but had quit the habit before the 12 months preceding the interview.

8 ***Naswar-pack-years***

9 *Naswar* production is not regulated in Pakistan and therefore the correct assessment of
10 exposure categories and dose-responses is very difficult. Usually, the packages come in
11 different sizes and the size of individual serving depends on users and varies to a great
12 extent based on personal preference. To address this issue, a selected sample of 50 case and
13 control participants, who were *Naswar* users, were asked to make a serving of *Naswar*,
14 similar in size, to what these participants had been or were currently using. These servings
15 were weighed and the average weight of a single serving was calculated. We also acquired
16 62 different *Naswar* packages from the 23 districts of the KPK, the capital city of Pakistan
17 and the five provincial capitals, and calculated the average weight of these *Naswar*
18 packages. The number of servings/package was computed by dividing the average weight
19 of a package by the average weight of a serving. From these data, NPY were calculated by
20 using the formula $(\text{Number of servings per day} \times$
21 $\text{Total duration of Naswar habit in years}) / (\text{Number of servings per Naswar packet})$

22 The average weight of a *Naswar* pack was 43.6 g (95% CI: 42.2-45.7 g).. The average weight
23 of a *Naswar* pellet was 2.1 g (95% CI: 2.0-2.3 g). The number of pellets per *Naswar* package
24 was 20.6. Conservatively, a *Naswar* pack-year was thus defined as 20 pellets of *Naswar* used
25 per day for one year. For the conditional model, NPY was categorized into 4 categories i.e.
26 None, 1-10, 11-20 and more than 20, the intensity of *Naswar* use (in minutes) was
27 categorized into None, 1-5, 6-10 and greater than 10.

28

1 **Bias reduction**

2 The study participants were blind to the main research hypothesis. Interviewers and cases
3 were partially blind to the case status of the participants, as interviews with the cases took
4 place when a definitive diagnosis had not been established. This approach helped us reduce
5 temporal ambiguity and of the interviewer and differential recall bias among cases and
6 controls. Recruitment of incident only cases was aimed at avoiding problems of recall of
7 pre-morbid history.

8 **Statistical methods**

9 Data were entered and stored in Epi Info 7 [26]. The analysis was carried out in SAS version
10 9.3 [27]. Crude odds ratios (OR¹) along with their 95% CI were calculated using conditional
11 logistic regression (conditioned for age and sex). Moreover, adjusted odds ratios were
12 derived (OR²), taking simultaneously into account the MAS of variables. We also calculated
13 the population attributable fraction (PAF) for KPK and Pakistan, using the OR from the
14 conditional logistic regression model and prevalence of *Naswar* (p) use from a nationally
15 representative tobacco prevalence survey from Pakistan [21] by using the formula: **PAF** =
16 $\frac{[p(OR-1)]}{[p(OR-1)+1]}$. The total number of attributable incident cases (AC) of oral cancer was
17 obtained by the formula $AC = PAF * TC$, TC is the total number of annual incident cases of
18 oral cancer. The estimated annual number of incident cases of oral cancer in Pakistan was
19 extracted from Globocan, 2012 [1].

20 **RESULTS**

21 **Participants profile**

22 Based on our initial sample size calculation, we had to recruit 107 cases and 107 controls
23 for a 1:1 case/control ratio, or 78 cases and 156 controls for a 1:2 case/control ratio. The study
24 initially started with a ratio of 1:1 among cases and controls. However, in December 2014,
25 Peshawar saw a deadly terrorist attack killing almost 150 children and resulting in a very
26 tight security situation in the whole province. The uncertain security situation led to a

1 decrease in patient in-flow at most hospitals in Peshawar city as both inter and intra-city
2 movement came to a halt. The security situation and the resulting decrease in patient in-
3 flow hampered recruitment of cases in Peshawar making it difficult to reach the desired
4 number of 106 cases for the study. Therefore, in February 2015, it was decided to recruit
5 two controls per case in order to be able to achieve the desired power for the study.

6 A total of 88 potential cases and 179 age and sex-matched controls were asked to participate
7 in the study. 86 cases and 174 controls agreed to participate, The participation rate was
8 98% for cases and 96 % for controls. The final sample included 84 cases and 174 age and
9 sex-matched controls (Table 1) as two cases were excluded from the analysis because they
10 had a cancer type other than squamous cell carcinoma. The majority of cases were males
11 (n= 52). The mean age of male cases and controls was 56.3 (\pm 13.0) and 57.4 (\pm 12.7) years,
12 respectively. Among females, the mean age of cases and controls was 51.4 (\pm 14.4) and 57. 3
13 (\pm 16.9) years, respectively. The male to female ratio was 1.7: 1 and about 34% (15 males, 14
14 females) of the cases were 50 years of age or younger 50.

15 The most common primary sites of oral carcinoma tumors were the gums (n=37) and the
16 buccal mucosa (n=24). Histologically, 75% of the tumors were “well-differentiated”, 17%
17 were “moderately differentiated”, and the remaining tumors either poorly differentiated or
18 “undifferentiated”. From a total of 23 districts in the Khyber Pakhtunkhwa province, only
19 two were not represented among the cases. Peshawar being the most populous city of the
20 province had the highest number of cases. Six cases originated from the federally
21 administered tribal areas. The distribution of MAS variables among the participants overall
22 and stratified by sex is provided in Table 2. *Naswar* was the most prevalent habit among
23 both cases (79.7%) and controls (27.5%). The majority of the participants (95 % cases, 92
24 % controls) belonged to the low or medium SES strata. Initial univariate analysis (chi-
25 square tests) revealed that *Naswar* use, smoking, and sex were significantly ($p < 0.05$)
26 associated with oral cancer.

Table 1: Distribution of cases and controls by study recruitment center

Study status	KCD	KTH	RMH	PPC	KMU	Total
Cases <i>n</i> (%)	57 (67.8)	9 (10.7)	18 (21.4)			84
Controls <i>n</i> (%)	63 (36.2)	36 (20.6)	20 (11.4)	28 (16)	26 (14.9)	174

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KCD: Khyber College of Dentistry, KTH: Khyber Teaching Hospital, RMH: Rehmat Memorial Hospital, PPC: Pakistan Paraplegic Center, KMU: Khyber Medical University

1 **Table 2:** Distribution of the lifestyle risk factors for oral cancer, by sex, among cases (n=84) and controls
 2 (n=174) in Khyber Pakhtunkhwa, Pakistan.
 3

Risk Factors	Males		Females		Total	
	Cases	Controls	Cases	Controls	Cases	Controls
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Naswar						
Never	3 (5.7)	64 (59.2)	14 (43.7)	62 (93.9)	17(20.2)	126 (72.4)
Ever	49 (94.2)	44 (40.7)	18 (56.2)	4 (6.1)	67 (79.7)	48 (27.5)
Current	34 (65.3)	28 (25.9)	12 (37.5)	0 (0.0)	46 (54.6)	28 (16.1)
Past	15 (28.8)	16 (14.8)	6 (18.7)	4 (6.1)	21 (25.1)	20 (11.4)
Cigarette Smoking						
Never	29 (55.7)	82 (75.9)	27 (84.3)	65 (98.4)	56 (66.6)	147 (84.4)
Ever	23 (44.3)	26 (24.1)	5 (15.6)	1 (1.6)	28 (33.3)	27 (15.5)
Betel-quid Chewing						
Never	50 (96.1)	108 (100.0)	30 (93.7)	66(100)	80 (95.2)	174 (100)
Ever	2 (3.8)	0 (0.0)	3 (6.2)	0 (0.0)	4 (4.7)	0 (0.0)
Water-pipe smoking						
Never	48 (90.3)	107 (99.1)	30 (93.7)	66 (100)	77 (91.6)	173 (99.4)
Ever	4 (9.69)	1 (0.9)	3 (6.2)	0 (0.0)	7 (8.3)	1 (0.6)
Alcohol						
Never	49 (94.2)	105 (97.2)	31(96.8)	66 (100.0)	80 (95.2)	171 (98.2)
Ever	3 (5.7)	3 (2.7)	1 (3.1)	0 (0.0)	4 (4.7)	3 (1.7)
Socio-economic status						
Low	26 (50.0)	50 (47.2)	13 (40.6)	16 (22.7)	39 (46.4)	66 (37.9)
Medium	24 (46.1)	51 (48.1)	17 (53.1)	43 (63.6)	41 (48.8)	94 (54.0)
High	2 (3.8)	7 (5.6)	2 (6.2)	7 (13.6)	4 (4.7)	14 (8.0)

1 **Main Results**

2 Table 3. shows the univariate as well as the simultaneously adjusted risk estimates for
3 different risk factors among the study participants. Ever and current *Naswar* users had a
4 more than 20-fold risk increase of oral cancer compared to non-users (ever: OR 21.2, 95%
5 CI 8.4-53.8), (current: OR 27.4, 95% CI 10.0-74.7). Ever smoking also doubled the risk of
6 oral cancer, compared to non-smokers (OR 2.2, 95% CI 1.4-4.5), while alcohol consumption
7 was not significantly related to the risk of oral cancer (p-value= 0.19). In general, a higher
8 SES was associated with a lower risk for oral cancer; however, this finding was also not
9 significant (p-value= 0.36). Tables 4 and 5 provide an overview of the risk of oral cancer
10 associated with *Naswar* stratified by males and females, respectively.

11 The overall PAF of *Naswar* for oral cancer in Pakistan was 59%. The sex-specific PAF of
12 *Naswar* for oral cancer in Pakistan was 68% and 38% for males and females, respectively.
13 The PAF was 75% for KPK. Sex-specific PAF for KPK was not calculated due to lack of data.
14 The total number incident cases of oral cancer in both sexes in Pakistan attributable to
15 *Naswar* (AC) was 9,094 (15,414 total incident oral cancer cases in Pakistan).

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Table 3: Risk of oral cancer associated with the lifestyle risk factors among both sexes (84 cases, 174 controls) in Khyber Pakhtunkhwa, Pakistan, derived from conditional logistic regression (conditioned on age and sex).

Risk Factors	Cases n (%)	Controls n (%)	OR ¹ (95 % CI)	OR ² (95 % CI)
Socio-Economic Status				
Low	39 (46.4)	66 (37.9)	1.0	1.0
Medium	41 (48.8)	94 (54.1)	0.7 (0.4-1.2)	0.7 (0.4-1.3)
High	4 (4.8)	14 (8.0)	0.5 (0.1-1.5)	0.5 (0.1-1.7)
Smoking				
Never	56 (66.6)	147 (84.4)	1.0	1.0
Ever	28 (33.3)	27 (15.5)	3.0 (1.5-5.8)	2.2 (1.4-4.9)
Alcohol				
Never	80 (95.2)	171 (98.2)	1.0	1.0
Ever	4 (4.7)	3 (1.8)	2.7 (0.6-12.1)	0.7 (0.1-4.1)
Naswar				
Never	17 (20.2)	126 (72.4)	1.0	1.0
Ever	67 (79.7)	48 (27.5)	22.9 (9.2-57.4)	21.2 (8.4-53.8)
Current	46 (54.7)	28 (16.1)	28.0 (10.5-74.0)	27.4 (10.0-74.7)
Past	21 (25.0)	20 (11.4)	16.4 (5.8-46.7)	14.3 (4.9-41.2)
Naswar Pack Years				
0	17 (20.2)	126 (72.4)	1.0	1.0
1-10	16 (19.0)	16 (9.1)	15.3 (5.2-44.9)	12.5 (4.1-38.0)
11-20	27 (32.1)	15 (8.6)	28.7 (9.9-82.8)	26.5 (9.0-78.2)
>20	24 (28.5)	17 (9.7)	28.3 (9.3-86.2)	28.9 (9.3-90.2)
Naswar dip duration (minutes)				
0	17 (20.2)	126 (72.4)	1.0	1.0
1-5	19 (22.6)	39 (22.2)	8.5 (3.1-23.3)	7.2 (2.5-20.4)
6-10	23 (27.3)	6 (3.4)	67.6 (18.6-245.6)	61.8 (16.6-229.5)
>10	25 (29.7)	3 (1.8)	142.2 (31.1-650.5)	136.2 (29.1-638.2)
Naswar Saliva *				
Swallow	20 (29.8)	8 (20.8)	1.0	1.0
Spit	47 (70.1)	40 (79.1)	0.4 (0.1-1.3)	0.4 (0.1-1.4)
Naswar Type				
Non-user	17 (20.2)	126 (72.4)	1.0	1.0
Black	50 (59.5)	37 (21.2)	22.2 (8.6-56.7)	21.3 (8.2-55.4)
Green	17 (20.2)	11 (6.3)	25.9 (8.0-83.0)	21.0 (6.4-68.9)

*Ever users only; OR¹: Basic model conditioned for age and sex; OR²: Basic model adjusted for other MAS variables.

Table 4: Naswar use and the risk of oral cancer among men (52 cases, 108 controls) in Khyber Pakhtunkhwa, Pakistan, crude and adjusted risk estimates from simple logistic regression.

Risk Factor	Cases		Controls		OR ¹ (95 % CI)	OR ² (95 % CI)
	n	%	n	%		
Naswar habit						
Never	3	5.8	64	59.3	1.0	1.0
Ever	49	94.2	44	40.7	23.7 (6.9-81.0)	21.0 (6.1-72.1)
Current	34	65.4	28	25.9	25.9 (7.3- 91.4)	23.4 (6.6-82.1)
Past	15	28.8	16	14.8	20.0 (5.1-77.5)	16.4 (4.1-65.4)
Naswar Pack Years						
0-10*	12	23.1	78	72.2	1.0	1.0
11-20	20	38.5	13	12.0	9.9 (3.9-25.2)	9.6 (3.6-25.5)
>20	20	38.5	17	15.7	7.6 (3.1-18.5)	8.7 (3.3-22.6)
Dip duration (minutes)						
0-5**	14	26.9	100	92.6	1.0	1.0
6-10	20	38.5	5	4.6	28.5 (9.2-88.3)	23.0 (7.4-71.5)
>10	18	34.6	3	2.8	42.8 (11.1-164.3)	39.7 (9.9-158.5)
Naswar type						
Green	11	21.2***	10	9.3	1.0	1.0
Black	38	73.1***	34	31.5	1.0 (0.3-2.6)	1.0 (0.3-2.8)

*, **Includes "Never users", *** Ever users only, OR¹: Crude Odds Ratio, OR²: Adjusted for age, SES, smoking, and alcohol, CI: Confidence Interval

Table 5. Crude and adjusted risk estimates for oral cancer associated with Naswar use among women (32 cases, 66 controls) in Khyber Pakhtunkhwa, Pakistan, derived from simple logistic regression

*Includes "Never users", **Ever users only, OR1: Crude Odds Ratio, OR2: Adjusted for age, SES, smoking and alcohol, CI: Confidence Interval

Variables	Cases		Controls		OR ¹ (95 % CI)	OR ² (95 % CI)
	N	%	n	%		
Naswar habit						
Never	14	44.1	62	93.9	1.0	1.0
Ever	18	55.9	4	6.1	19.9 (5.8-68.1)	29.0 (5.4-153.9)
Naswar Pack Years						
0-10*	21	65.6	64	97.0	1.0	1.0
>10	11	34.4	2	3.0	16.7 (3.4-81.8)	16.0 (2.7-93.7)
Dip duration (minutes)						
0	14	43.5	62	93.5	1.0	1.0
1-5	8	25.0	3	4.5	11.8 (2.7-50.2)	16.7 (2.2-124.1)
>5	10	31.3	1	1.6	44.2 (5.2-374.8)	50.2 (5.1-495.9)
Naswar type						
Green	6**	18.8	1	1.5	1.0	1.0
Black	12	37.5	3	4.5	0.6 (0.1-11.5)	0.8 (0.1-11.5)

DISCUSSION

Statement of main findings

Naswar contributes to about 70% of oral cancers in the study region. Ever users of *Naswar* were more than 20 times likely to develop oral cancer compared to non-users. Compared to non-users and participants with a comparatively low cumulative exposure (NPY) to *Naswar* (<11), both male and female ever-users with a higher NPY count had a significantly higher risk of oral cancer. A similar relationship was seen with the intensity of exposure between both sexes, with a significant increase in risk among *Naswar* users who kept

Naswar in the mouth for more than five minutes as compared to non-users and participants who kept *Naswar* in their mouth for a shorter duration.

Interpretation and generalizability

Smokeless tobacco is considered as a risk factor for oral cancer and its use is on a rise globally and in particular in South Asian countries [28,29]. *Naswar* has not been researched extensively, particularly in the context of cancer risk. A previous study from the KPK reported a high biochemical risk of cancer associated with the constituents of *Naswar* [14], the present study conducted in the same region provides epidemiological evidence to further strengthen that argument. A more than 80% prevalence of *Naswar* use among the oral cancer cases in our study is comparable to previous findings from the same region [30,31]. The prevalence of *Naswar* use among the controls at 27% is comparable to previous findings (31%) about *Naswar* use in Peshawar [32], yet substantially higher than the national figure of 7.3% [21]. The difference can be explained by the stark disparity in tobacco consumption practices among the different provinces of Pakistan. While the national figures are based on a representative sample of all the provinces of the country, our sample consists of subjects belonging to KPK only, where *Naswar* use is almost like a cultural practice [33].

We report a very high magnitude of risk for oral cancer associated with the use of *Naswar*. This finding is consistent with that of other studies from India and Pakistan on the risk of oral cancer associated with the use of other forms of SLT such as Gutkha and Betel-quid [5,6]. However, in our study, the observed risk estimates are even higher compared to those associated SLT products. A plausible explanation for this risk difference might be a comparatively higher amount of “Tobacco-Specific Nitrosamines” and nicotine, and a higher alkalinity (pH) of *Naswar* compared to Gutkha and Betel-quid [34]. Nicotine causes dependence and a higher nicotine level coupled with a high pH can cause stronger cravings and more frequent and/or prolonged use of the SLT products [35], leading to a stronger exposure to the carcinogenic agents. There are also suggestions that *Naswar* causes local

tissue trauma by erosion [36], and chronic tissue trauma is an independent risk factor for cancer [37]. The ash and lime used in the preparation of *Naswar* may also be contributing a high level of toxins and heavy metals to the composition, thus adding to its potential toxicity [14].

Some previous studies from Southern Pakistan have reported risk estimates for oral cancer and *Naswar* which are lower in magnitude than the risk estimates we report [19,20]. This difference may be attributed to the diverse SLT consumption practices in different parts of Pakistan. Betel--quid use is very common in the south, while *Naswar* is mostly used in the north of Pakistan, including our study region [33]. In our study, Betel-quid use was not significantly related to an elevated risk of oral cancer and the prevalence of Betel-quid use was much lower than previous reports [19,20]. Furthermore, a large case-control study from Pakistan carried out in the 1970s [19], reported a relative risk of 20 for oral cancer with the use of Nass (= *Naswar*), consistent with our findings. However, this study had some methodological limitations [38].

Our results show that current users of *Naswar* had a higher risk compared to past users. This finding is in line with those of a cohort study on SLT use and the risk of oral carcinoma from India [39]. The results of our exposure-response analysis are in accordance with those reported in independent studies as well as systematic reviews of literature from South Asia, where an increasing frequency, duration, and intensity of exposure were all related to a subsequent increase in the risk of oral cancer [5,6]. We have reported a higher adjusted OR for the risk of oral cancer with the use of *Naswar* among females as compared to males. Other studies of SLT and its effects on oral cancer reported similar findings that may be explained by lower background risk of oral cancer among females and a greater potential for oral mucosal damage among women as compared to men [6,40]. Our study reports population attributable risks of *Naswar* for oral cancer comparable to those reported for other forms of SLT from other South Asian countries [6]. Notably, the PAF for KPK is considerably higher than the national PAF due to a higher prevalence of *Naswar* use in the

province and signifies the importance of *Naswar* as a major risk factor for oral cancer in this population.

Strengths and limitations of this study

This study may suffer from drawbacks inherent to retrospective study designs. The study sample, particularly the hospital controls, may not be representative of the general population of KPK. However, we adopted wide eligibility criteria for the inclusion of controls with regard to their diagnosed disease to avoid recruitment of subjects who might be very similar to each other in terms of exposure and belonging to a narrow subset of the whole population. For recruitment of the participants, we chose the largest tertiary care facilities and in the case of oral cancer patients, the only public sector centers where diagnosis and treatment of oral cancer are carried out. We obtained a high response rate among potential study subjects, which may be attributed to the payment of laboratory charges on behalf of the case subjects as an incentive, and cooperation from the hospital staff at the study centers, who motivated control subjects to participate. We managed to exceed the number of cases and controls estimated during the sample size calculation. However, we still had to collapse a few exposure-response categories during the sex-stratified analysis, due to a small number of participants. This shortcoming warrants larger epidemiological studies to strengthen the evidence provided by this study.

Although we frequency-matched each case to at least two controls, there have been recent suggestions in the literature that an unconditional logistic regression analysis may yield equal or more robust and efficient results for matched studies [41]. We did not find any large differences between the effect estimates yielded by the conditional and the unconditional analysis, both being highly elevated and suggestive of a causal link between *Naswar* and oral cancer. This is the first adequately powered case-control study to be carried out on the risk factors for oral cancer in the Khyber Pakhtunkhwa province and the use of a “simple poverty card”, utilization of causal diagrams and “*Naswar* pack-years” gives it novelty among other similarly designed studies on use of smokeless tobacco and the risk

of oral cancer. Another important feature of the study was the partial blinding of the study cases, as they were only differentially diagnosed at the time of interview and hence not fully aware of their condition. This may have diminished selective recall bias among the cases.

Policy and practice Implications

These findings are highly relevant for South and Central Asia, where *Naswar* use is common. As prices of cigarettes soar, more people might take up products like naswar, because of their lower prices [42]. The lack of published evidence on health risks associated with SLT, such as *Naswar*, may also contribute to this. It is, therefore, pertinent to produce further local evidence to inform public policy, as findings from developed countries may not be applicable in the local context because of a difference in composition of SLT products, which may be responsible for the observed differences in risk of oral cancer and other diseases between industrialized and developing countries [43]. To the best of our knowledge, this study is one of a handful of case-control studies focusing on *Naswar* and the associated risk for oral cancer. Until larger cohort studies are carried out to further assess this risk, the evidence from this study may be used to inform SLT control policies in countries where *Naswar* is used. The use of *Naswar* pack-years could also be incorporated into research and clinical practice to assess future risks for oral cancer with the use of *Naswar*.

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Declaration

The authors declare no conflict of interest.

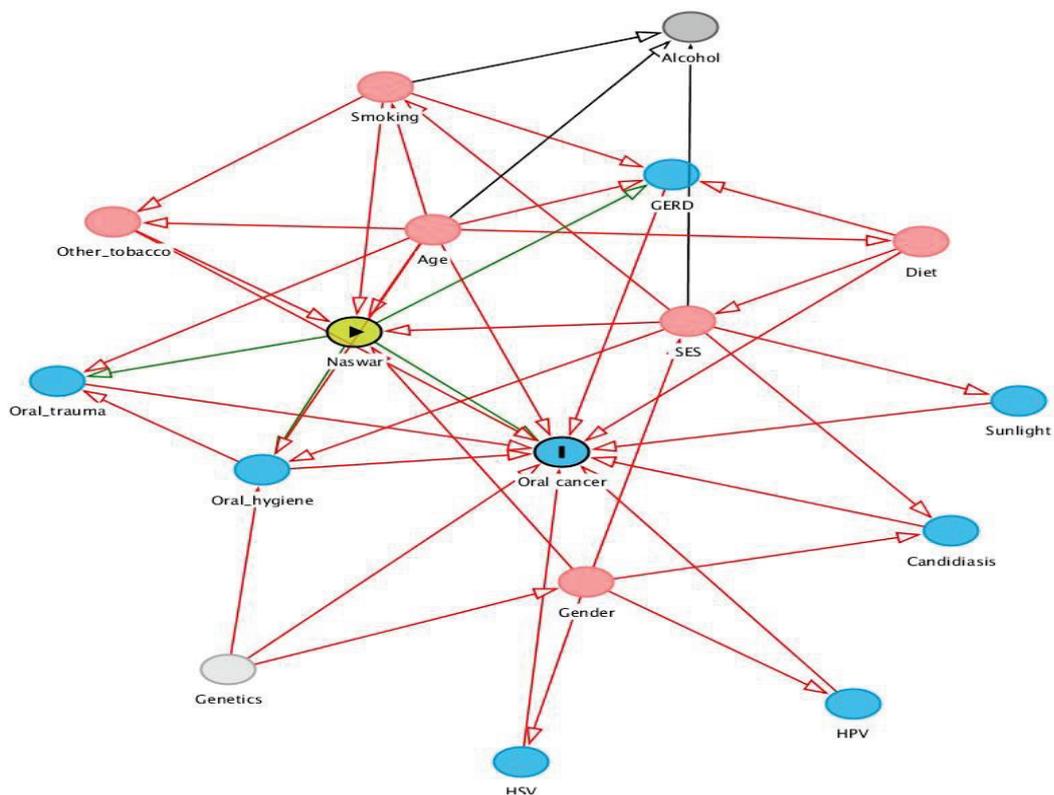
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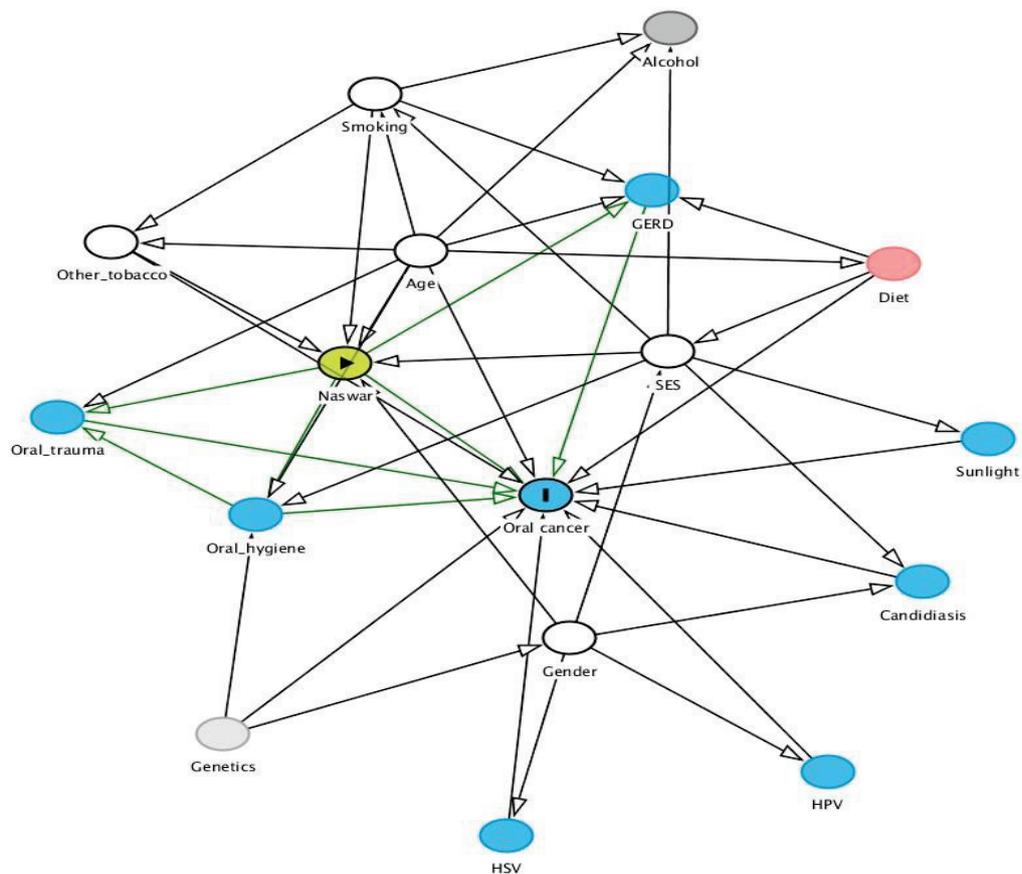
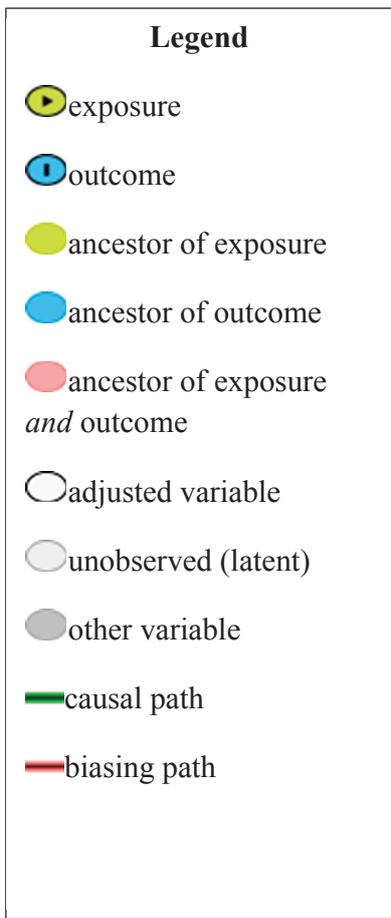
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Supplemental Figure 1 a. Relationship between the study variables before adjustment for the minimal adjustment set.



Supplemental Figure 1 b. Relationship between the study variables after adjustment for the minimal adjustment set.

ARTICLE VI

A GAPING GAP (SMOKELESS TOBACCO CONTROL IN PAKISTAN)

First author: Zohaib Khan

Order of authors: Zohaib Khan

Author's contributions: ZK developed the concept for the study, conducted the literature search, assessed studies for inclusion in the review and extracted data, also prepared drafts and undertook edits.

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REVIEW

Open Access



A gaping gap (smokeless tobacco control in Pakistan)

Zohaib Khan^{1,2}

Abstract

Oral cancer is second most common cancer in Pakistan and one of the major contributing factors to its high incidence is smokeless tobacco (SLT) use. 5.3% of Pakistan's youth are current SLT users. The World Health Organization requires the signatories of its "Framework Convention on Tobacco Control" to officially ban the sale of tobacco products to minors. We reviewed the Government of Pakistan's tobacco control, and related supporting policies, to assess how these address the issue of sale of SLT products to minors and found evident gaps in this regard. Legislations need to be in place to ban the sale of SLT products to minors and avoid an SLT epidemic in the future.

Keywords: Smokeless tobacco, Youth, Oral cancer, Tobacco control, FCTC, Sales ban

Background

Smokeless tobacco is associated with a variety of oral and systemic disease [1, 2], in particular with oral and pharyngeal cancers [3]. Pakistan has one of the highest incidence rates of oral cancer in the world [4]. It is the most common cancer among men and the second most common cancer among women in the country [5]. Tobacco use and alcohol consumption are considered as the leading modifiable risk factors for oral cancer and account for over 70% of the population attributable fraction for oral cancer [6]. A World Health Organization (WHO) report from 2001 suggests that Pakistan has one of the lowest per capita consumption of alcohol in the world [7], which might be due to a public ban on consumption and sale of alcohol. Tobacco, therefore, seems to be a major reason for the high incidence of oral cancer in Pakistan, this is substantiated by recent evidence from systematic reviews of literature pertaining to South Asia which implies that SLT is one of the main factors responsible for a high incidence of oral cancer in the region [8, 9]. Results from the Global Youth Tobacco Survey (GYTS), carried out recently in Pakistan, show that 5.3% (approx. 4.2 million) of the country's youth currently use smokeless tobacco (SLT) products and another 4.7% were past users [10]. The results from

GYTS are particularly alarming because the survey sample consisted of school going children aged 13–15 years. Evidence shows that a social disparity exist with regards to both oral cancer incidence and SLT use i.e., People from low socioeconomic status, and lower or no education level, are at a higher of oral cancer and having an SLT habit [11]. This could imply, that potentially the actual prevalence of SLT use might even be higher among the youth of Pakistan, given that the national literacy rate is just 46% [12].

Pakistan is a signatory of the WHO's Framework Convention for Tobacco Control (FCTC) since 2005, and has taken significant steps to curb smoking in the country [13]. Article 16 of the FCTC states, "Each Party shall adopt and implement effective legislative, executive, administrative or other measures at the appropriate government level to prohibit the sales of tobacco products to persons under the age set by domestic law, national law or eighteen", an intervention aimed at curbing the use of tobacco among minors. However, the results of the GYTS and evidence from more recent studies [14, 15], suggest that tobacco control in Pakistan may be lagging in its effectiveness to reduce the prevalence of tobacco products use among minors.

A 2014 research article explored public policy gaps with regards to SLT control in four Asian countries including Pakistan, by conducting a review of policy documents and interviews with key informants [16], one of the findings of this study was that, "the sale of

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smokeless tobacco to and by minors is prohibited in Pakistan". The dichotomy between the alarming prevalence of SLT use among minors in Pakistan and the reported findings of the aforementioned policy review study [16], warranted a review of the Government of Pakistan policies to identify, how the issue of sale to and by, and consumption of SLT by minors has been addressed in these policy documents.

Methods

We downloaded the official documents related to tobacco control from the official website of the Government of Pakistan's Tobacco Control Cell, a body responsible for research, advocacy and legislation pertaining to tobacco control in Pakistan. Documents pertaining to child labor in Pakistan were downloaded from the United Nations' International Labor Organization's "database of national labor, social security and related human rights legislation (NATLEX)" database. Provincial policy documents, if applicable, were downloaded from the official websites of the respective provinces of Pakistan. In order to supplement the information from the official documents, we performed an electronic search in Medline via PubMed using various combinations of MeSH terms and keywords, to identify tobacco control policy related literature from Pakistan. The final search query used in PubMed was ((("pakistan"[MeSH Terms] OR "pakistan"[All Fields]) AND ("tobacco"[MeSH Terms] OR "tobacco"[All Fields] OR "tobacco products"[MeSH Terms] OR ("tobacco"[All Fields] AND "products"[All Fields]) OR "tobacco products"[All Fields])) AND ("policy"[MeSH Terms] OR "policy"[All Fields])). From the retrieved articles those which reviewed, or were specifically aimed at informing public tobacco control policy in Pakistan, were included in this review. Additionally, an email request was sent to the authors of the 2014, SLT control policy review article, to explain the basis of their findings regarding the prohibition of sale of SLT to and by minors in Pakistan.

Results

Table 1 refers to key findings from the relative official documents of the Government of Pakistan. The PubMed search returned 27 articles out of which two were eligible to be included in this review. The first article was the afore-cited review of the SLT control policies in South Asia which had concluded that SLT sale to and by minors is prohibited in Pakistan [16]. The second article was based on a core Non-communicable disease prevention policy document from Pakistan and only addressed the prohibition of sale and consumption of cigarettes to minors, without any mention of SLT use among minors [17]. In response to the authors' query, the authors of the article on SLT control policies in South Asia [16] cited

"section 9" of the "Prohibition of Smoking and Protection of Non-Smokers Health Ordinance 2002", and information from a key personnel that the child labor act prohibits children from working (Including selling tobacco products), as the basis of their finding, that sale of SLT products to and by minors in Pakistan is prohibited.

Discussion

The findings from table 1 clearly suggest gaps in public policy with regards to SLT sale, to and by minors. The "section 8" of the 2002 Ordinance only focuses on "smoking tobacco" products sales to and by minors without mentioning smokeless tobacco. On the contrary, the monitoring tool designed to assess the implementation of the same ordinance assesses the sale of both smoking and other forms of tobacco, to and by minors, which implies a disconnect between the two documents. The 2002 ordinance also puts a selective ban on the sale of any tobacco products to any age group i.e., only inside and in the near vicinity (50 m) of educational and public sector institutions, and renders these public and educational buildings "smoke free". This would technically imply that a person, irrespective of age, can consume smokeless tobacco within these institutions. Additionally, given that 54% of Pakistan's population is not literate [12], the potential applicability of the section 9 of the ordinance may only be limited to half of the country's population. In Sindh province there is a selective ban on manufacture and sale of Gutka but other forms of SLT are still manufactured and sold. In Khyber Pakhtunkhwa both children and adolescents are not allowed to work in the manufacture or processing of Naswar but there are no provisions for prohibition of sale of Naswar to and by minors.

With regards to the difference between our finding and those reported by Khan et al. [16]. We believe that the basis on which they concluded that "In Pakistan, the sale of SLT to and by minors is prohibited", are too vague and open to interpretation, to draw a solid conclusion from. Firstly, "section 9" of the 2002 ordinance puts only a "geographically limited" ban/prohibition on the sale of tobacco products in, or in the near vicinity of selected public/private buildings. Given that section 8 of the ordinance only prohibits sale of cigarettes and other smoking substances (not SLT) to and by minors, thus, technically a minor could sell or buy an SLT product at a 51 m distance from the institutions/buildings mentioned in the ordinance. Secondly, the national act related to child labor in Pakistan explicitly bans children from selected "occupations and processes", none of which are related to SLT sale. It is also pertinent to note that apart from the Khyber Pakhtunkhwa province, where adolescents are also covered by its own child labor law, the national act is applicable only to children under the age of 14, while "minor" has been defined as a person under the age of 18 in the

Table 1 Smokeless tobacco control and minors in Pakistan

No	Document name	Available at	Relevant sections/articles/paragraphs	"SLT and minors" related findings
	Prohibition of Smoking and Protection of Non-Smokers Health Ordinance 2002.	http://www.tcc.gov.pk/downloads.php	<ul style="list-style-type: none"> Section 8, titled <i>Prohibition of sale of cigarettes, etc. to minors</i>- "No person shall sell cigarettes or any other smoking substance to any person who is below the age of eighteen years". Section 9, "<i>Prohibition of storage, sale and distribution of cigarettes, etc., in the immediate vicinity of educational institutions</i>" – No person shall himself or by any person on his behalf, store, sell or distribute cigarettes or any other smoking substance or any other tobacco product within (fifty) meters from any college, school or educational institution. Explanation of Educational institutions: "All schools in the country, Primary, Secondary, Higher Secondary, all Colleges, Intermediate degree, Medical Colleges, Engineering Colleges, Agriculture and all other institutions and all the Universities, private or Government have declared 100% smoke free. No student, teacher, persons who are working in the above mentioned institutions and all heads of institutions cannot smoke in the premises". "No tobacco products can be sold within the 50 meters area of the premises of all above mentioned" 	<ul style="list-style-type: none"> Sale of cigarettes and other smoking substances to minors is banned, no mention of SLT. Selective prohibition i.e., Ban on sale of all kinds of tobacco in and in the immediate vicinity of various institutions. All institutions mentioned are declared "smoke free" and not "tobacco free".
	SRO on "The Prohibition of Sale of Cigarettes to Minors Rules, 2010	http://www.tcc.gov.pk/downloads.php	<ul style="list-style-type: none"> No mention of smokeless tobacco. 	<ul style="list-style-type: none"> None
	Monitoring Checklist for Implementation Committee, 2002	http://www.tcc.gov.pk/downloads.php	<ul style="list-style-type: none"> Section 8, Sale of cigarettes and other tobacco products to minors Yes/No Section 9, Presence or absence of cigarette sales outlet/s within 50 m of the institution, and the presence or absence of cigarette sale in the institutions canteine. 	<ul style="list-style-type: none"> Monitoring of tobacco products sale to minors. Monitoring of sale of tobacco products in, or in the vicinity, of institutions mentioned in section 9 of the 2002 ordinance.
	Media Resource Kit on Tobacco Control	http://www.tcc.gov.pk/downloads.php	The media tool kit has a section on SLT, however, there are no particular references to minors.	<ul style="list-style-type: none"> None.
	Employment of children act, 1991 ^a	http://www.na.gov.pk/uploads/documents/1335242011_887.pdf	<ul style="list-style-type: none"> Section 3. <i>Prohibition of Employment</i>. "No child shall be employed or permitted to work in any of the occupations set forth in Part I of the Schedule or in any workshop wherein any of the processes set forth in Part II of that Schedule is carried on: Tobacco processing and manufacturing including niswar^c and bidi^b making." Is mentioned as one of the processes (part II) where child employment is illegal. 	<ul style="list-style-type: none"> None Children under 14 years of age are not allowed to be involved in processing or manufacturing of tobacco.
	The Khyber Pakhtunkhwa Prohibition of Employment of Children Act, 2015 (Act No. XIX of 2015).	http://www.pakp.gov.pk/2013/acts/the-khyber-pakhtunkhwa-prohibition-of-employment-of-children-act-2015/	<ul style="list-style-type: none"> Section 3. <i>Prohibition of employment</i>. "(1) No child shall be employed or permitted to work in any establishment: Provided that a child not below the age of 12 years may be engaged in the light work, alongside his family member, for a maximum of two hours per day mainly for the purpose of acquiring skills, in a private undertaking, or in any school established, assisted or recognized by Government for such purpose. (2) No adolescent shall be employed or permitted to work in any hazardous work included in the Schedule". The above mentioned "Schedule" includes "Tobacco processing and manufacturing including Niswar and bidi making". 	<ul style="list-style-type: none"> No children or adolescent is allowed to work in tobacco processing and manufacturing.

Table 1 Smokeless tobacco control and minors in Pakistan (Continued)

Shops and Establishments Ordinance 1969	http://www.ilo.org/dyn/travail/docs/1008/West%20Pakistan%20Shops%20and%20Establishments%20Ordinance%201969.pdf	Section 20. <i>Prohibition of employment of children.</i> "No child shall be required or allowed to work in any establishment" Section 7. <i>Opening and closing hours of establishments</i> "Except with the permission of Government, no woman or young person shall be employed in any establishment otherwise than between the hour of 9–00 a.m. to 7:00 p.m".	Children under 14 years are not allowed to work in any establishment, which may also include tobacco sale shops.
Government of Sindh Resolution, PAS/Legis-R-202/2016/734-A	http://www.pas.gov.pk/index.php/mediacenter/ntf/en/31/1404	Resolution- "This Assembly resolves that Provincial Government impose ban on import, sale and purchase of beetal nut & Gutka in the entire Province of Sindh. According to medical statistics chewing Gutka is injurious to health and is pre-cancerous".	• Selective ban on SLT i.e., Gutka ^c only.

^aAmended version (via SRO 387)^bHand rolled cigarettes comprising of tobacco wrapped in a plant leaf^cDifferent forms of SLT

official documents of the Tobacco Control Cell of Pakistan. The “shops and establishments act” is also aimed at children under the age of 14, prohibiting them to work in any establishment. Again the word “establishment” is not clearly defined in the ordinance and may or may not be applicable to shops where tobacco is sold.

From these findings it is evident that there are gaps regarding children and particularly “adolescents”, in the SLT control policies of Pakistan. Adolescence is considered one of the most vulnerable age groups for tobacco uptake and therefore shall be one of the primary targets of a tobacco control policies and interventions [18]. From the review of the official documents of the Government of Pakistan, we can also infer that most of the focus is on the supply side i.e., manufacture and sale of tobacco products, with no legislations regarding the demand side i.e., Possession, use, and purchase (PUP) of tobacco products by minors. Sufficient evidence exists that PUP laws aimed at reducing access to tobacco products are an effective adjuvant to any tobacco control measures [19].

Conclusions and policy implications

From the results and discussion we can conclude that a differential focus with regards to smoking and smokeless tobacco products exists in the current tobacco control policies in Pakistan. There is also some evidence that the tobacco industry as well as some part of the scientific community suggest SLT use as means of harm reduction [20], which might lead to an increase in the uptake of SLT products in Pakistan. As such, this warrants a non-differential focus on both smoking as well as SLT products from the Government of Pakistan and calls for fashioning of legislative measures, aimed to curb both the sale and consumption of SLT products among minors. Some of the gaps identified above are technical and others need more explicit explanation or clarification. The sale and consumption of SLT by minors has not been adequately and explicitly addressed in the relevant legislation. The supporting laws in tobacco control e.g., “Employment of children act”, may need revisions and must also address adolescents.

Abbreviations

FCTC: Framework Convention on Tobacco Control; GYTS: Global Youth Tobacco Survey; SLT: Smokeless tobacco; SRO: Statutory rules and orders; TCC: Tobacco Control Cell (Ministry of National Health Services, Regulations and Coordination Government of Pakistan); WHO: World Health Organization

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Authors' contributions

ZK wrote the preliminary and revised draft of the manuscript.

Competing interests

The author declares that he has no competing interests.

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ARTICLE VII

NASWAR, ORAL CANCER, AND TOBACCO CONTROL IN KHYBER PAKHTUNKHWA

First author: Zohaib Khan

Order of authors: Zohaib Khan

Author's contributions: ZK was involved in the study conceptualization, literature search, and draft preparation.

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NASWAR (SMOKELESS TOBACCO PRODUCT), ORAL CANCER AND TOBACCO CONTROL IN KHYBER PAKHTUNKHWA, PAKISTAN

Zohaib Khan¹✉

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The Oxford Medical Companion is a very useful resource to have in the arsenal of any health professional dealing with illness and disease. But perhaps one of its greatest contributions to public health has been its description of tobacco. A medicinal herb to some and a recreational drug for others, the Oxford Medical Companion describes tobacco as "...tobacco is the only legally available consumer product which kills people when it is used entirely as intended".¹ Tobacco kills one person every six seconds.² Tobacco killed more people in the 20th century than the two world wars combined and if the current trends of tobacco consumption continue, it will be responsible for more than a billion deaths by the end of the 21st century.³

Recent estimates suggest that more than 250 million people in South Asia use smokeless tobacco (SLT) products⁴ and Pakistan is no stranger to these products with 13.3% of the population using either Naswar, Paan, Gutkha and other SLT products.³ Naswar use is a major public health challenge in Khyber Pakhtunkhwa, where an estimated 15% of the province's population is addicted to this mixture of tobacco, ash and lime.⁵ Naswar use is associated with a variety of conditions including upper aerodigestive tract cancers, cardiovascular and gastrointestinal disease.⁶ Oral cancer has become the most common cancer among men and the second most com-

mon cancer among women in Pakistan. An estimated 6000 Pakistanis lose their lives to oral cancer every year.⁷ Studies from Karachi show that Naswar is a major player in the etiology of oral cancer in Pakistan.^{8,9}

As part of a doctoral research, the author has recently concluded a multi-center case control investigation into the risk of oral cancer associated with Naswar use in Khyber Pakhtunkhwa. It was the first adequately powered epidemiological study to assess this causal association in the context of the province. The results of our study, which have been submitted for publication elsewhere, suggest a strong causal association between Naswar use and oral cancer in Khyber Pakhtunkhwa. Users of Naswar were 20 times more likely to develop oral cancer compared to non-users. We also observed a dose-response relationship between Naswar and oral cancer i.e. the risk of oral cancer increased with increasing frequency, duration of each use and the total duration of the habit. One of the most striking findings of our study was that if Naswar use was stopped in the Khyber Pakhtunkhwa, there will be a 70% decline in oral cancer incidence in the province. The results of our study are supported by the findings of a biochemical analysis of 30 brands of Naswar use in Khyber Pakhtunkhwa reported by Zakiullah et al.⁶ They concluded that Naswar available in the Pakistani market

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had a high concentration of biochemical agents that are labelled as carcinogenic to humans by the International Agency for Research on Cancer.

In the light of these findings, we reviewed the various tobacco control policy documents in Pakistan to identify areas which could be strengthened to curb SLT use in the country. The analysis of the three tobacco control ordinances i.e. "Cigarettes (Printing of Warning) Ordinance"-1979, "Prohibition of Smoking and Protection of Non-smokers Health Ordinance No. LXXIV"-2002, "The Cigarette (Printing of Warning) (Amendment) Ordinance No. LXXV"-2002, and the related statutory regulation led us to the following conclusions:

- Smoking tobacco is the main emphasis of tobacco control in Pakistan.
- Any references to SLT are at best, vague.
- There are no provisions to regulate the manufacture of SLT products.
- Health warnings on the packaging do not apply to SLT products.
- Only cigarettes and smoking tobacco products, are prohibited to be sold to and by minors.

The biggest point of concern among these conclusions is the sale of SLT, to and by minors. Although it can be argued that the article 5 of the 2002 ordinance on prohibition of smoking which prohibits tobacco use inside and in the vicinity of Public buildings as well as educational institutions, includes minors, a counter argument can be made that "use" does not entail "sale". The only official document from the current day Pakistan which forbids the consumption of SLT by a minor can be dated back to 1959, when the Princely State of Swat (Not a part of Pakistan at the time), by the decree of

the Wali-e-Swat, levied a punishment and a monetary fine on the use and sale of Naswar, to and by minors. This decree was absolved when Swat acceded to Pakistan in 1969. There are other federal legislations e.g. "Employment of children act, 1991" and the "Shops and Establishments Ordinance, 1969", which prohibit children under 14 years of age, from working in certain occupations, which could potentially include manufacture and/or sale of smokeless tobacco products, but the only tobacco product mentioned explicitly in these documents is "Bidi" (بیدی), a form of local cigarette. Another argument can be put forward that "Minors" are defined as "a person who is below the age of 14" in Pakistani law and hence adolescents between the age of 14 and 18 are exempted from all the articles pertaining to minors. To its credit though, the 2015 provincial act of Khyber Pakhtunkhwa, "The Khyber Pakhtunkhwa Prohibition of Employment of Children Act, 2015 (Act No. XIX of 2015)", does explicitly prohibits children to work in tobacco manufacturing.

The Khyber Pakhtunkhwa Government is currently in the process of devising a new tobacco control legislation for the province. Based on our study findings and the review of policy documents, following recommendations are proposed to be addressed in the new legislation.

- i. First and foremost, "Smokeless Tobacco" is a recognized term by the World Health Organization and hence should be used as such, rather than vague terminologies like "other tobacco", which can be misleading and may be misinterpreted.
- ii. The use of smokeless tobacco, explicitly citing "Naswar", shall be prohibited in public places, offices

and educational institutions, akin to the prohibition of tobacco smoking.

- iii. Sale of all forms of tobacco (smoking and smokeless), to and by a person who is younger than 18 years of age shall be prohibited.
- iv. Health warnings should be made mandatory on Naswar packaging.
- v. As opposed to an absolute ban on Naswar, the Government should try to introduce regulatory legislation regarding the composition of Naswar. The content of carcinogenic agents in Naswar could be reduced using the "Swedish Snus" model.
- vi. These policy provisions should be supplemented with media and school campaigns on the deleterious effects of Naswar.

These changes in policy are necessary, particularly in the wake of soaring cigarette prices and the absence of evidence based advocacy regarding SLT use. The easy availability and considerably lower prices of SLT like Naswar may lead to more people taking up these products as alternatives to smoking. A differential focus on smoking and SLT could potentially prove counterproductive in the long run, a drop in smoking may be accompanied by an increase in SLT products use, as has been seen in Sweden.¹⁰ A comprehensive approach, including targeting both smoking and smokeless tobacco use, is therefore needed to avoid this scenario. At the same time legislation regarding the contents of Naswar and the regulation of its production need to be fashioned. In the authors view, the policy considerations that have been recommended, would ensure a gradual decline in the prevalence of SLT habits in Khyber Pakhtunkhwa, as well as reducing its potential toxicity. This could ultimately

lead to a lower tobacco related morbidity and mortality, which should be the primary goal of any tobacco control program and policy.

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CONFLICT OF INTEREST

Author declared no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE

NIL

ARTICLE VIII

SMOKELESS, NOT HARMLESS: FINDINGS OF A SYSTEMATIC REVIEW OF CASE-CONTROL STUDIES ON ORAL CANCER ASSOCIATED WITH *NASWAR* USE IN PAKISTAN.

First author: Zohaib Khan

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Author's contributions: ZK carried out the online search, data abstraction, and data synthesis. RAS was responsible for data abstraction, analysis, and synthesis. SM and TH performed study selection and quality assessment of the included studies. ZK and RAS wrote the initial draft. All authors contributed to the subsequent and the final draft.

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SMOKELESS, NOT HARMLESS: FINDINGS OF A SYSTEMATIC
REVIEW OF CASE-CONTROL STUDIES ON ORAL CANCER
ASSOCIATED WITH *NASWAR* USE IN PAKISTAN.

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INTRODUCTION

Oral cancer is the most common cancer among men and the second most common cancer among women in Pakistan [1]. Given an age-adjusted rate of 12.0/100,000, Pakistan is one of the global frontrunners with regards to oral cancer incidence. Approximately 16,000 new cases of oral cancer are diagnosed each year in Pakistan and around 5,500 Pakistanis lose their lives to oral cancer every year [2]. Oral cancer has a multifactorial etiology but tobacco use and alcohol are considered as the universal modifiable risk factors for oral cancer [3]. Smokeless tobacco (SLT) used in the Indian sub-continent is associated with an elevated risk for oral cancer [4-6], however, evidence from Sweden and other developed countries show a negligible risk for oral cancer with the use of SLT products [7, 8]. This difference may be attributed to the varied composition of smokeless tobacco products used around the world [9]. The variance in the composition of the products necessitates the establishment of individual risks related to each SLT product [10].

It is estimated that in South and South East Asia more than 250 million people use smokeless tobacco (SLT) in some form [11]. According to the World Health Organization around 10 million (7.7%), adults in Pakistan are SLT users [12]. A multitude of SLT products are consumed in Pakistan including Betel quid with tobacco, Gutkha and *Naswar* [13]. *Naswar* is a form of smokeless tobacco which has historically been used in the northern part of Pakistan, Afghanistan and in Central Asia. Its use, however, is now becoming more common in other parts of the world [14]. *Naswar* is a mixture of slaked lime and tobacco with different flavorings added to it. It is usually kept in the buccal sulcus of the mouth or put under the tongue. *Naswar* has one of the highest Tobacco-Specific Nitrosamines content and alkalinity among the SLT products used in South Asia [15]. Additionally, it also contains other Group 1 and 2 carcinogens, as classified by the International Association for Research on Cancer, which renders it potentially more toxic and carcinogenic compared to the other forms of SLT used in Pakistan [16].

Epidemiological research on oral cancer and tobacco in Pakistan has been scarce [1] and as such the public policies regarding tobacco control in Pakistan have to depend on anecdotal evidence. Furthermore, the SLT products used in the country often evade the taxation net, hence, the very low prices of these products [17]. The easy availability and cheap prices of SLT products coupled with the lack of scientific evidence on the harms of SLT use in Pakistan make SLT an attractive substitute to smoking. There is also an assertion in some parts of the scientific community that SLT products can and should be used as a tobacco harm reduction strategy [18, 19] and some even suggest it as a cheaper substitute for nicotine replacement therapies in smoking cessation [20]. It is, therefore, pertinent to provide evidence regarding the carcinogenicity of *Naswar* to avoid more people from taking up the habit or using *Naswar* as a smoking cessation means.

The high rates of oral cancer in Pakistan coupled with the high prevalence of SLT use and the relative dearth of cumulative evidence on the carcinogenicity of *Naswar* serve as a rationale for this review. We aimed to identify epidemiological evidence produced from Pakistan and synthesize cumulative evidence regarding the role of *Naswar* in the etiology of oral cancer in Pakistan. The specific objectives of the review included the calculation of a pooled estimate for the risk of oral cancer associated with *Naswar* use, computing estimates stratified by region and sex, and the calculation of the population attributable fraction of *Naswar* for oral cancer.

METHODS

Eligibility criteria

Study design

Epidemiological studies of case-control or cohort design were eligible for this review. A previous review of oral cancer research in Pakistan had suggested a lack of any experimental or cohort design study on oral cancer in Pakistan [1], and some preliminary searches confirmed that finding. Therefore, we focused on case-control studies, which are considered as a valid design to assess epidemiological associations. Cross-sectional and descriptive studies were not eligible. Laboratory-based case-control studies and studies carried out on animals were also not eligible.

Participants/population

This review included studies carried out on the resident human population of Pakistan. Studies carried out among Pakistanis residing outside Pakistan were not eligible. The inclusion criteria were independent of the source of cases and controls i.e. both hospital and population-based studies were eligible. Studies were included in the review only when the case ascertainment had been carried out through histological/medical records.

Exposure

Studies in which exposure to *Naswar* had been ascertained through written records, e.g. medical history, structured interviews or written self-report were eligible. Studies in which *Naswar* was a primary or secondary exposure were included in the review. Case-control studies where exposure was used as an inclusion criterion for both the case and control groups were not eligible for inclusion. Articles reporting estimates of risk e.g. Odds Ratio (OR) or Relative Risk (RR) with their 95 % confidence intervals (CI) for SLT users versus non-users, or those reporting data from which these effect estimates could be computed were included in the review.

Comparator(s)/control

Studies were included when the control group included subjects who had no history of oral cancer. Only those studies were eligible in which controls were chosen from the same target population as the cases.

Outcome

Studies reporting oral cancer as a primary or secondary outcome were included in this review. Oral cancer was defined as “Squamous cell carcinoma arising in the oral cavity or the oropharynx”, We used the ICD-10 classification to identify the following anatomical sites which were included in our definition of oral cancer, C00-C06, C8-C10, and C12-14.

Search methods

A detailed search strategy is provided in the supplementary table I. Briefly, we searched Medline via PubMed, the Science citation Index (SCI) via Web of Science, the WHO Index Medicus for Eastern Mediterranean region, and “PakMedinet” for relevant literature using a combination of keywords and MeSH terms. Google searches were also run to identify studies, which might not have been indexed in these databases. In order to include all relevant literature, no filters were used during the electronic search. The electronic search was supplemented by a hand search of the bibliographies of selected articles.

Selection of studies

One author (ZK) ran the search query in the electronic databases. After removal of duplicate articles, the remaining records were stored in an independent folder in a reference management software. Two authors (TH, ZK) separately went through the titles and abstracts of all the records and selected the relevant studies. Full texts of the selected studies were then obtained and screened independently by the two authors (TH and ZK) for inclusion in the final review. Finally, the bibliographies of the selected studies were screened by SM, TH, and ZK for any relevant studies that might have been missed by the search process. Figure I. Provides the details of the study selection process.

Assessment of the quality of included studies

Two authors (SM and TH) assessed the quality of the included studies through the National Collaborating Centre for Methods and Tools, McMaster University’s “Effective Public Health Practice Project Quality Assessment Tool for Quantitative Studies”. Studies can be ranked as “strong”, “moderate”, or “weak” based on six criteria i.e. selection bias, study design, confounding, blinding, data collection methods, and withdrawals and dropouts.

Data extraction and management

Two authors (ZK, RAS) separately carried out data extraction on a pre-designed spreadsheet. Data about the following study characteristics was recorded; study type, year of publication, author name/s, study location, number of cases and controls, source of cases and controls, type/s of exposure, exposure frequency and intensity, reported outcome/s and risk estimates [overall as well as stratum-specific effect estimates Odds Ratio (OR) and/or relative risk (RR)]. ORs were calculated for studies that did not report an OR, but data were available to calculate them. Wherever possible, efforts were made to calculate an adjusted or a Mantel-Haenszel odds ratio (OR_{MH}). Crude estimates were used for the meta-analysis when the available data was insufficient to calculate an adjusted or OR_{MH} . Spreadsheets from both the authors were compared for consistency in the presence of a third author, whose decision was considered final in case there were disagreements between the two sets of abstracted data. The extracted data were then entered into the Cochrane Rev Man 5.3. software [21].

Data synthesis

RAS carried out the data synthesis. All the meta-analyses were carried out on the logit scale due to their desirable statistical properties and back-transformed for logical interpretation. We used the fixed effects model by assuming that the component studies represented a similar underlying population. However, we also ran the random effects model to confirm our assumptions. The effect size to be meta-analyzed was the Odds Ratios from the different studies that represented the association between consumption of *Naswar* and development of oral cancer. Gender wise subgroup analysis was also carried out using data from studies which reported gender wise estimates. Sensitivity analyses were carried out by the exclusion of studies which had not adjusted for smoking/alcohol exposures and which of poor evidence quality. Also, one study was dropped at a time to see its effect on the overall estimate. A narrative synthesis was carried out for individual studies for exposure-response relationships as well as inter-regional differences in the estimates due to lack of adequate data for quantitative synthesis. Heterogeneity among the studies was assessed by visual inspection of the forest plot and estimation the I^2 statistics (a value of >50% was considered as evidence of heterogeneity). Publication bias was assessed by visual inspection of the funnel plots and by means of Begg–Mazumdar’s test and Egger’s test. Asymmetry in the funnel plots and significant p values in the two statistical tests were considered to be evidence of publication bias. Statistical significance was set at a p-value <0.05. All analyses were carried out in Stata 12 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP) [22].

We calculated the overall Population Attributable Fraction (PAF%) of *Naswar* by using the formula, $PAF = [p(RR - 1)] / [p(RR - 1) + 1]$ where “p” is the % of the population exposed to *Naswar* and RR is the relative risk of oral cancer among those exposed to *Naswar* compared to non-exposed. Following the “rare disease assumption,” we substituted the RR for the meta-OR. The prevalence of *Naswar* use among adults aged ≥ 35 years in Pakistan was derived from a nationally representative tobacco prevalence survey carried out in 2012.

The total number of attributable incident cases (AC) of oral cancer was obtained by the formula $AC = PAF * TC$, where PAF is the population attributable fraction and TC is the total number of annual incident cases of oral cancer. The estimated annual number of incident cases of oral cancer in Pakistan was extracted from Globocan, 2012 [2].

This review was carried out in accordance with the MOOSE checklist [23].

RESULTS

Characteristics of included studies

The electronic searches returned 53 articles (after removal of duplicates), all in the English language. Additionally, two articles were identified through a google search. Five studies [24-27] were included in the final review after the application of the inclusion /exclusion criteria. Figure 1 describes the various steps of the study selection process.

Two studies were carried out in Karachi [24, 25] and one each in Peshawar, Lahore [27], and Abbottabad [26]. Important features of the included studies along with the results of their quality assessment are given in table 1. Three studies exclusively focused on *Naswar* as the exposure of interest [26, 27], while in the other studies *Naswar* was a co-

exposure. All the studies had hospital-based controls as the comparator. Four studies reported an effect estimate, which was adjusted for smoking and/or alcohol [24-26]. Two studies provided an exposure-response analysis between *Naswar* and the odds of oral cancer [27]. Gender-specific estimates were provided by only two studies [25].

Prior to carrying out the meta-analyses, we assessed publication bias by a visual inspection of the funnel plot. The funnel plot (Supplementary figure 1) was skewed, indicating an underrepresentation of studies with smaller effect estimates.

Key findings of the meta-analysis

The mOR for oral cancer associated with the “ever use” of *Naswar* compared to “never use” was 13.5 (95% C.I, 9.5-19.2), with a moderate heterogeneity, $I^2 = 56\%$ (Fig 2). Exclusion of one study with a small sample size and one study reporting an unadjusted estimate resulted in an mOR of 17.0 (95% C.I, 11.4-25.3), $I^2 = 0\%$, in both random and fixed effect models (Figure 3). The pooled estimate for oral cancer among female “Ever-users” of *Naswar* compared to “Never-users was 18.8 (95% CI, 12.5-28.2), $I^2 = 0\%$ and among men it was 16.4 (95% CI, 10.7-25.1), $I^2 = 0\%$. The pooled estimate for oral cancer in “Ever-users” of *Naswar* compared to “Never-users”, in the Khyber Pakhtunkhwa province was 18.3 (95% CI, 8.7-38.5), $I^2 = 0\%$, while in Sindh it was 16.2 (95% CI, 10.5-25.2), $I^2 = 0\%$. Only one study, OR= 3.9(95% CI, 1.5- 9.7), was included from Lahore so a pooled estimate could not be calculated.

Key results from the narrative synthesis

Riaz et al. reported a four-fold increase in the odds of oral cancer associated with the use of *Naswar* for up to five years compared with “Never-users”, OR= 4.2 (95% CI, 1.0- 17.7) and it increased to 6.0 (95% CI, 1.8-19.7) for a duration of more than five years. Khan Z et al. reported a 12-fold (95% CI, 4.1-38.0), a 26-fold (95% CI, 9.0-78.2), and a 28-fold(95% CI, 9.3-90.2) increase in the odds of oral cancer among subjects who had a cumulative exposure of 1-10, 10-20, and more than 20 *Naswar* pack-years respectively, compared to “Never-users”. *Naswar* pack-years were derived from the daily frequency and total duration of the *Naswar* habit. Khan Z et al., also reported the odds of oral cancer associated with the intensity of *Naswar* use i.e. duration of a single use. Subjects, who used *Naswar* for up to five minutes had an OR of 7.2 (95% CI, 2.5-20.4) compared to “Never-users” and those who used *Naswar* for 6-10 minutes had an odds ratio of 61.8 (95% CI, 16.6-229.5). They also reported a lower odds of oral cancer associated with the spitting of saliva, during and after *Naswar* use, compared to those who swallowed saliva, OR= 0.4 (95% CI, 0.1-1.4).

Population Attributable Fraction

Utilizing the overall and region-specific estimates from the meta-analysis, we calculated the overall and the region-specific population attributable fraction (PAF) for oral cancer attributed to *Naswar*, respectively. The overall PAF for oral cancer associated with the use of *Naswar* in Pakistan, with the national prevalence for *Naswar* being 7.3 % [28], was 48% (95% CI, 38%-57%). In males (*Naswar* prevalence= 13.7%) [28], the PAF of *Naswar* for oral cancer was 68% (95% CI, 57%-77%), while in females (*Naswar* prevalence= 3.8%) [28], the PAF was 40% (95% CI, 30%-50%). In Khyber Pakhtunkhwa (*Naswar*

prevalence= 15.3%) [28], the PAF of *Naswar* for oral cancer was 73% (95% CI, 54%-85%). In Sindh (*Naswar* prevalence= 5%) [28], *Naswar* was responsible for 43% (95% CI, 32%-55%) of the oral cancer cases, while in Punjab (*Naswar* prevalence= 1%) [28], the PAF for oral cancer with the use of *Naswar* was 3% (95% CI, 0.5%-8%). The total number of incident oral cancer cases in Pakistan attributable to *Naswar* was 7,400.

DISCUSSION

Statement of main findings

The findings of this review suggest a strong association between oral cancer and *Naswar* use in Pakistan. There are also indications of an exposure-response relationship between *Naswar* use and oral cancer. The odds ratio of oral cancer is more pronounced in the Khyber Pakhtunkhwa province of Pakistan, where *Naswar* use is very common. More than 60% of the oral cancer cases in Khyber Pakhtunkhwa can be attributed to *Naswar*.

Interpretation and Generalizability

Our findings are comparable to the findings of systematic reviews of literature from the Indian-subcontinent, which report elevated risks of oral cancer associated with the use of smokeless tobacco products. Guha et al., [5] reported an 8-fold increase in the risk of oral cancer associated with betel quid+tobacco use, meta-relative risk (mRR)= 7.7 (95%CI, 5.3-11.1). Sinha and colleagues [4] reported an mOR of 5.6 (95% CI, 3.8-8.4) for oral cancer associated with the use of smokeless tobacco products in India. Gupta and Johnson [29] reported a 7-fold increases in the risk of oral cancer associated with the use of SLT products in South Asia and the pacific, mRR of 7.5 (95% CI, 5.8-9.5). However, the pooled estimate that we are reporting is of a higher magnitude than the above-cited reviews. This can be explained by the differences between the composition of *Naswar* and the other SLT products used in South Asia [15].

According to the National Cancer Institute of the U.S, *Naswar* contains a significantly higher amount of tobacco-specific Nitrosamines and free form Nicotine, compared to the other SLT products available in the Indian subcontinent [15]. Additionally, *Naswar* has one of the highest alkalinity (pH) of any smokeless tobacco products found across the globe [30]. Tobacco-specific Nitrosamines are the primary carcinogenic agents in tobacco [31-33], they act by inducing changes at the molecular level leading to the formation of DNA adducts and mutations, and subsequent carcinogenesis [32, 34]. Nicotine is the main psychoactive agent found in tobacco [31, 35]. A higher Nicotine levels mean a greater dependence [36], which leads to more frequent and prolonged use of *Naswar* to curb the need for the nicotine craving [37-39]. This implies a greater exposure to the cancer-causing agents and hence the high potential carcinogenicity of *Naswar*. The high alkalinity of *Naswar* facilitates a rapid and efficient absorption of nicotine, thus aiding in *Naswar* dependence, and contributing to the *Naswar* related carcinogenesis. Moreover, the slaked lime used in the preparation of *Naswar* to increase its alkalinity also induces the formation of a free radical, reactive oxygen species, which causes cellular damage and promotes carcinogenesis [40-42]. The biochemical plausibility for our results comes from the study of Zakiullah et al., who studied the composition of 30 *Naswar* samples found on the Pakistani market and reported high levels of both group I and II carcinogens and

other toxic agents [43]. Animal studies also suggest a high incidence of different tumors associated with the administration of *Naswar* [44].

Our main findings are in contrast with the reported risks of oral cancer associated with the use of Swedish snuff. Systematic reviews of the association of oral cancer with the snuff used in Sweden have reported minimal increases in the risk of oral cancer with the use of Swedish snuff [8, 45-49]. This difference can be also be contributed to the variability in the composition of *Naswar* and the Swedish snuff. The Snuff found in Sweden has a much lower level of TSNAs as well as free form Nicotine [7, 19, 50], compared to *Naswar*. The contents of the snuff found in Sweden are regulated by law and kept under a safe limit, which is not harmful to the human beings. In contrast, the production of *Naswar* is unregulated in Pakistan and the composition varies from one manufacturer to the next and hence, the level of carcinogens.

We report slightly higher risks of *Naswar* associated oral cancer among females as compared to men. These results are in line with the findings of the systematic reviews on the association of oral cancer with the use of SLT in South Asian countries [4, 6]. A low background risk for oral cancer among females as compared to males can be a plausible explanation for this difference. There is a low prevalence of smoking and alcohol use among women in Pakistan as compared to men, implying a lower background risk of oral cancer among the women. Another possible explanation can be the social structure in Pakistan, women usually stay at homes, while men work outside, and this can possibly lead to more frequent and longer uses of *Naswar* by females [51, 52]. An increased susceptibility of the female buccal mucosa to oral cancer, explained by hormonal differences between men and women, has also been touted as the reason for the higher risk of oral cancer among females with the use of SLT [53, 54].

We report a 44% PAF of *Naswar* for oral cancer, which is slightly less than that reported by Sinha et al., for India, PAF= 60% [4]. This is understandable because the PAF calculated by Sinha and colleagues was related to all types of SLT used in India, rather than a specific one like *Naswar* as in the case of our review. The reported PAF for Pakistan is however much closer to the one reported by Guha and colleagues for betel quid in India, PAF= 49.5% [5], confirming our earlier assertion about the differences in PAF attributable to multiple as opposed to single SLT forms.

Strengths and limitations

To the best of our knowledge, this is the first systematic review on the association between oral cancer and *Naswar* use. We have also for the first time reported *Naswar* attributable burden of oral cancer in Pakistan. We have reported pooled estimates for all included studies, as well only for studies that had adjusted for confounding by smoking and alcohol drinking.

The cumulative evidence synthesized in this review is based on case-control studies, which is a recognized research design in cancer etiological research [55]. However, the case-control study design is also associated with some inherent limitations owing to its retrospective nature [56-58]. As such, this review may also be suffering from the same limitations that underline case-control studies. However, given the ethical considerations involved in conducting experimental studies on human beings and the practical limitations with regards to carrying out longer prospective studies in a developing country like Pakistan [59-62], this evidence constitutes the “best available evidence” [63] on the carcinogenicity of *Naswar*.

There were no high-quality studies included in the review which further downgrades the quality of evidence reported in this review. The generally low heterogeneity, however, favors the pooled estimates and increase confidence in our findings. The small number of studies included in the review may also be a potential limitation, especially in the estimation of the exposure-response relationship, which is a necessary requirement to establish causality in epidemiology. However, oral cancer research in general and research on *Naswar* in particular, are scant in Pakistan [1]. This has also been recognized by the IARC, which in 2007 deemed the then available evidence on the carcinogenicity of *Naswar*, as inadequate [64]. Although efforts were made to include all eligible studies available on the topic, it has previously been noted that a considerable amount of oral cancer research output from Pakistan is published in journals that are not indexed in well-recognized databases [1] and have a limited presence in the electronic databases. This may potentially have resulted in the non-inclusion of some studies. However, we contacted experts from Pakistan with an interest in oral cancer research about any other potential study that could be included in this review but were unsuccessful in identifying further studies.

The PAF method of calculating attributable burden was limited in the sense that it does not represent the purest effect of exposure and therefore risks due to all possible factors may add up to >100%. In the case of oral cancer, such factors include smoking, alcohol drinking, genetic factors, diet, and infections with HPV. A possibility of residual confounding due to these factors can also not be ruled out. Additionally, the *Naswar* attributable fractions and incidence of oral cancer are based on estimates from a single survey [28] and only one cancer registry [65] respectively, and hence, may not be reflective of the true fractions in the whole population of Pakistan. However, the estimates regarding the incidence case of oral cancer and the prevalence of *Naswar* use are the most recent and perhaps the only available estimates in the context of Pakistan and hence our findings can be considered valid for Pakistan.

Finally, we have only focused on oral cancer in this review, but the harmful health effects of the use of *Naswar* may not be limited to oral cancer only. Evidence from India show associations between SLT use and other head and neck cancers e.g. Pharyngeal and laryngeal cancers [4]. However, a pre-review scoping exercise to identify literature on head and neck cancers from Pakistan identified just one case-control study on esophageal cancer that could have been eligible for this review. The authors decided not to include that study due to the considerations for specificity and heterogeneity in the review.

CONCLUSION

This review confirms a strong relationship between oral cancer and the use of *Naswar* in Pakistan. It adds to the evidence base on the carcinogenicity of SLT products in humans and indicates a need for further research on *Naswar* related carcinogenicity. Although the synthesized evidence may not be of a high quality, it represents the “best available evidence” which can be used to inform policy and generate further hypothesis for future research.

POLICY IMPLICATIONS AND RECOMMENDATIONS

Tobacco control in Pakistan has largely focused on smoking tobacco with little if any emphasis on smokeless tobacco control in Pakistan. Due to a lack of taxation smokeless

tobacco products are much cheaper than cigarettes e.g. a *Naswar* packet costs 1/10th of the price of a cigarette pack in Pakistan. The soaring prices of cigarettes coupled with the lack of reported evidence on the deleterious effects of *Naswar* and other SLT use in Pakistan may potentially induce more people to take up SLT products. To avoid such a scenario, findings from this review could be used to inform tobacco control policies in Pakistan. We recommend the following changes in the light of the findings of this review.

- *Naswar* shall be brought under the tobacco tax-net.
- *Naswar* packets shall bear health warnings, similar to cigarette packets in Pakistan.
- The use of *Naswar* shall be banned in the premises of all public sector building including hospitals and educational institutions.
- Sale and use of *Naswar* by people under the age of 18 shall be prohibited.
- Regulations shall be put in place to reduce the concentration of carcinogenic agents in *Naswar* on the lines of the Swedish snuff.

AUTHORS CONTRIBUTIONS

ZK carried out the online search, data abstraction, and data synthesis. RAS was responsible for data abstraction, analysis, and synthesis. SM and TH performed study selection and quality assessment of the included studies. ZK and RAS wrote the initial draft. All authors contributed to the subsequent and the final draft.

CONFLICT OF INTEREST

None declared

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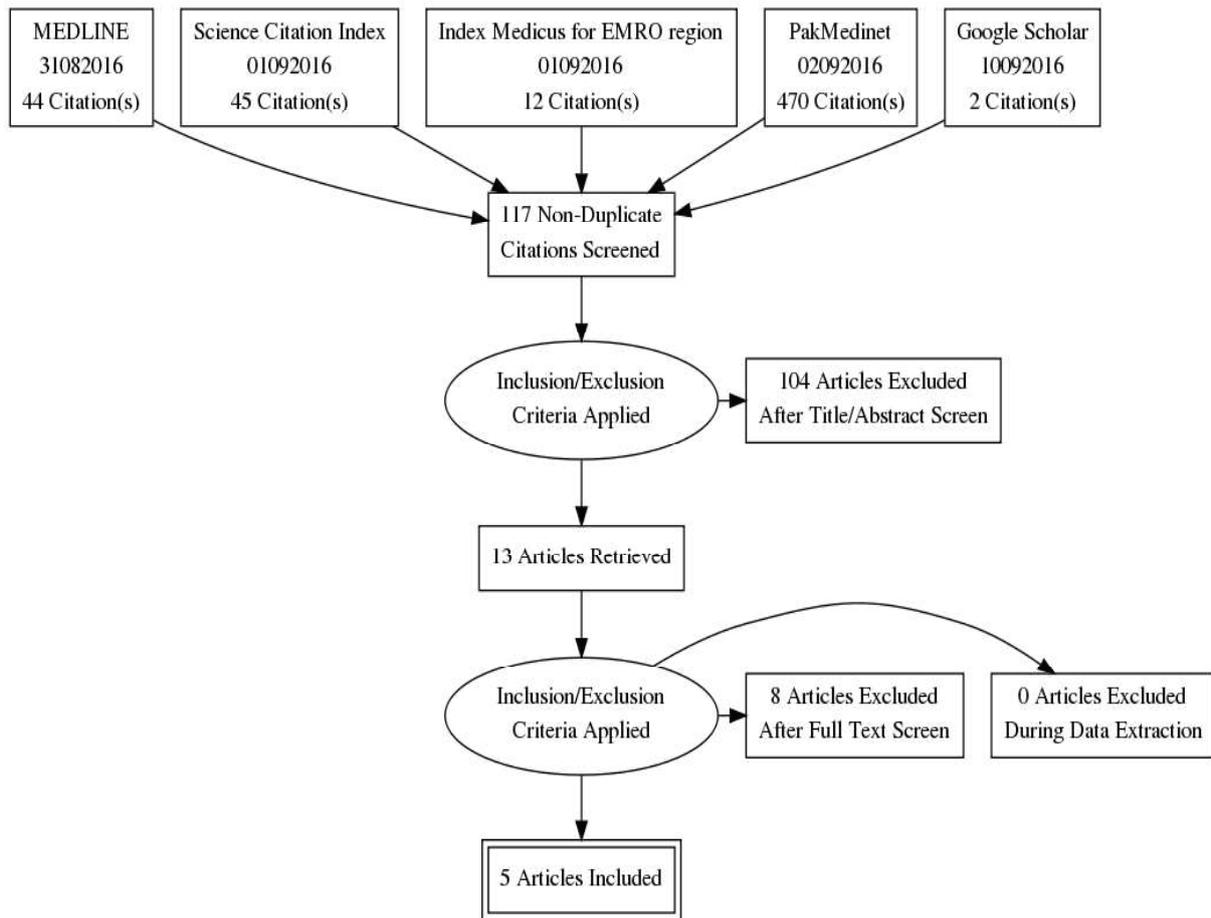


Figure 1. Flow diagram of the study selection process.

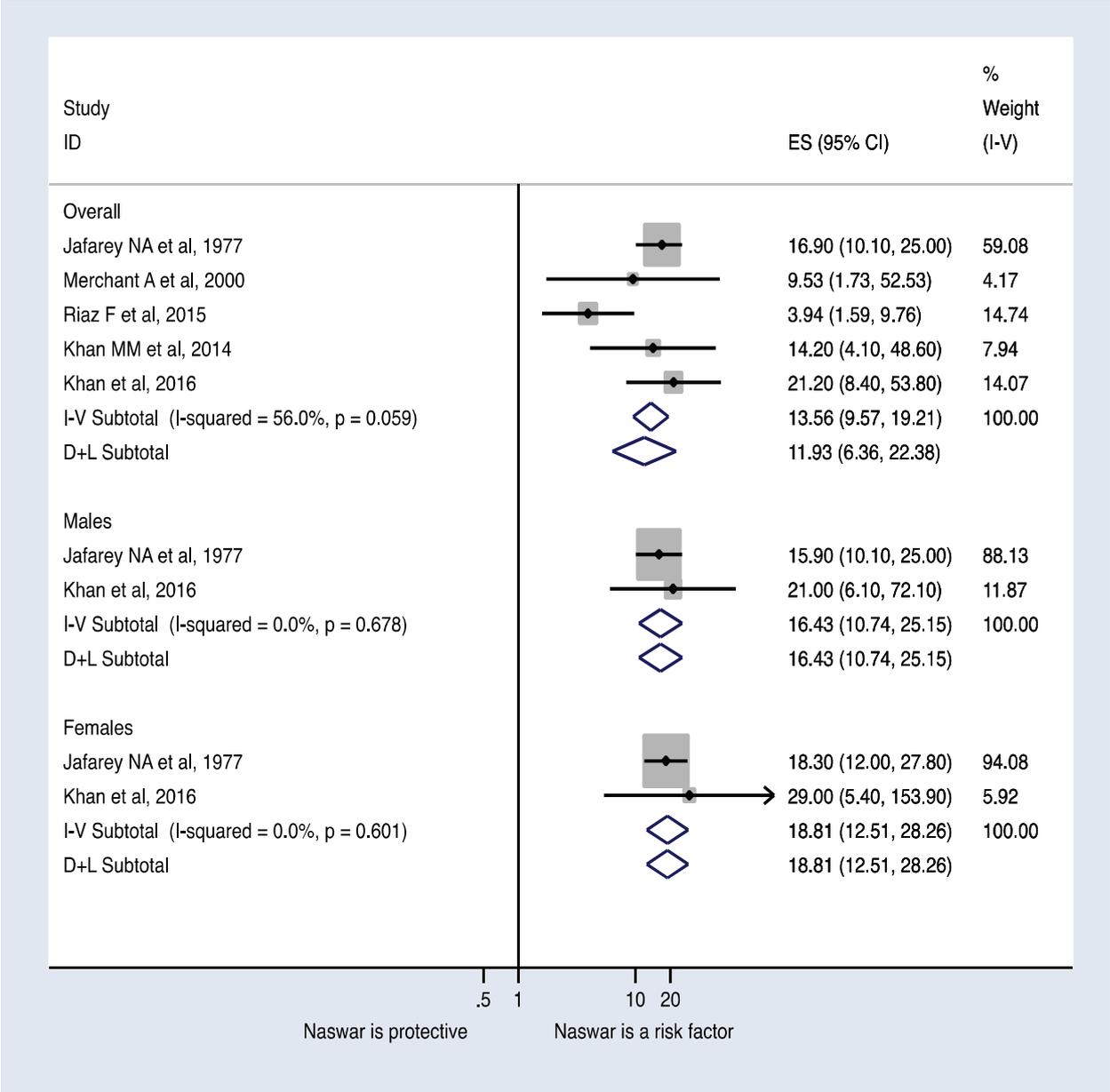


Figure 2. Overall and sex-stratified pooled estimates of oral cancer associated with the use of *Naswar* in Pakistan.

Note: *Year of publication, ** Adjusted for smoking a
Haenszel odd ratio.

Table 2. Pooled estimates and PAF of ora
naswar use in Pakistan

Study location	Cases (n)	Controls (n)	Adjustment**	Overall OR (95%CI)	Males OR (95% CI)	Females OR (95% CI)	Exposure response	Quality
Karachi	1192	3562	Yes	16.9 (10.1-25.0)***	15.9 (10.1 - 25.0)***	18.3 (12.0- 27.8)**	NA	Moderate
Karachi	79	149	Yes	9.5 (1.7-52.5)	NA	NA	NA	Moderate
Lahore	118	352	No.	3.7 (1.5- 9.7)	NA	NA	Yes	weak
Abbottabad	31	31	Yes	14.2 (4.1-48.6)	NA	NA	NA	weak
Peshawar	84	156	Yes	21.2 (8.4-53.8)	21.0 (6.1-72.1)	29.0 (5.4 -153.9)	Yes	Moderate
	Number of studies	Fixed effects pooled OR (95% CI)	P value	Test of heterogeneity, p value	I squared	Prevalence of Naswar use %	PAF % (95% CI)	
5	13.55 (9.56, 19.20)	<0.001	0.06	56%	7.3	48 (38, 57)		
Males 2	16.43 (10.73, 25.14)	<0.001	0.67	0%	13.7	68 (57, 77)		
males 2	18.80 (12.51, 28.26)	<0.001	0.6	0%	3.8	40 (30, 51)		
Sindh 2	16.27 (10.5, 25.2)	<0.001	0.52	0%	5	43 (32, 55)		
khwa 2	18.34 (8.73, 38.54)	<0.001	0.61	0%	15.3	73 (54, 85)		
unjab 1	3.7 (1.5- 9.7)	-	-	-	1	3 (0.5, 8)		
3	17.07 (11.49, 25.37)	<0.001	0.71	0%	7.3	54 (43, 64)		

SUPPLEMENTARY MATERIAL

1. Detailed search strategy

PubMed searches

31/8/2016

Block 1: Naswar and Smokeless tobacco

Search name	Search query	Type of search	Results
IA	((((nass[All Fields] OR naswar[All Fields]) OR niswar[All Fields]) OR ("tobacco, smokeless"[MeSH Terms] OR ("tobacco"[All Fields] AND "smokeless"[All Fields]) OR "smokeless tobacco"[All Fields] OR "snuff"[All Fields])) OR ("tobacco, smokeless"[MeSH Terms] OR ("tobacco"[All Fields] AND "smokeless"[All Fields]) OR "smokeless tobacco"[All Fields] OR ("smokeless"[All Fields] AND "tobacco"[All Fields])))	Keyword & MeSH	5,667
IB	smokeless tobacco (*)	MeSH major & sub-terms	3,985
1	IA OR IB		5867

Block 2: Pakistan

Search name	Search query	Type of search	Results
2A	((((((((((("pakistan"[MeSH Terms] OR "pakistan"[All Fields]) OR Punjab[All Fields]) OR Sindh[All Fields]) OR Balochistan[All Fields]) OR Baluchistan[All Fields]) OR NWFP[All Fields]) OR (Khyber[All Fields] AND Pakhtunkhwa[All Fields])) OR Kashmir[All Fields]) OR Islamabad[All Fields]) OR Peshawar[All Fields]) OR Karachi[All Fields]) OR Lahore[All Fields]) OR Quetta[All Fields])	Keyword + MeSH major & sub-terms	44,173

Block 3: Cancer

Search name	Search query	Type of search	Results
3A	"neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]	Keyword + MeSH	3,347,826
3B	Mouth Diseases (*)	MeSH major & sub-terms	271,867

3	3A OR 3B		3,541,299
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Summary & results

Search name (Saved in PubMed & EndNote)	Results
1 AND 2 AND 3	44

(((((("pakistan"[MeSH Terms] OR "pakistan"[All Fields]) OR Punjab[All Fields]) OR Sindh[All Fields]) OR Balochistan[All Fields]) OR Baluchistan[All Fields]) OR NWFP[All Fields]) OR (Khyber[All Fields] AND Pakhtunkhwa[All Fields])) OR Kashmir[All Fields]) OR Islamabad[All Fields]) OR Peshawar[All Fields]) OR Karachi[All Fields]) OR Lahore[All Fields]) OR Quetta[All Fields]) AND (((nass[All Fields] OR naswar[All Fields]) OR niswar[All Fields]) OR ("tobacco, smokeless"[MeSH Terms] OR ("tobacco"[All Fields] AND "smokeless"[All Fields]) OR "smokeless tobacco"[All Fields] OR "snuff"[All Fields]))) AND (((("head and neck neoplasms"[MeSH Terms] OR ("head"[All Fields] AND "neck"[All Fields] AND "neoplasms"[All Fields]) OR "head and neck neoplasms"[All Fields] OR ("head"[All Fields] AND "neck"[All Fields] AND "cancer"[All Fields]) OR "head and neck cancer"[All Fields]) OR ("mouth neoplasms"[MeSH Terms] OR ("mouth"[All Fields] AND "neoplasms"[All Fields]) OR "mouth neoplasms"[All Fields] OR ("oral"[All Fields] AND "cancer"[All Fields]) OR "oral cancer"[All Fields])) OR ("oesophageal cancer"[All Fields] OR "esophageal neoplasms"[MeSH Terms] OR ("esophageal"[All Fields] AND "neoplasms"[All Fields]) OR "esophageal neoplasms"[All Fields] OR ("esophageal"[All Fields] AND "cancer"[All Fields]) OR "esophageal cancer"[All Fields])) OR ("laryngeal neoplasms"[MeSH Terms] OR ("laryngeal"[All Fields] AND "neoplasms"[All Fields]) OR "laryngeal neoplasms"[All Fields] OR ("laryngeal"[All Fields] AND "cancer"[All Fields]) OR "laryngeal cancer"[All Fields])) OR ("pharyngeal neoplasms"[MeSH Terms] OR ("pharyngeal"[All Fields] AND "neoplasms"[All Fields]) OR "pharyngeal neoplasms"[All Fields] OR ("pharyngeal"[All Fields] AND "cancer"[All Fields]) OR "pharyngeal cancer"[All Fields])) OR ("trachea"[MeSH Terms] OR "trachea"[All Fields] OR "tracheal"[All Fields]) AND ("neoplasms"[MeSH Terms] OR "neoplasms"[All Fields] OR "cancer"[All Fields]))))

SCI search

11.02.2016

Search name	Search query	Type of search	Results
1	TS= (cancer OR disease*)	keyword	4,583,649
2	TS=(naswar OR nass OR snuff OR smokeless tobacco)	keyword	3,929
3	CU=Pakistan	keyword	80,457
4	1 AND 2 AND 3		45

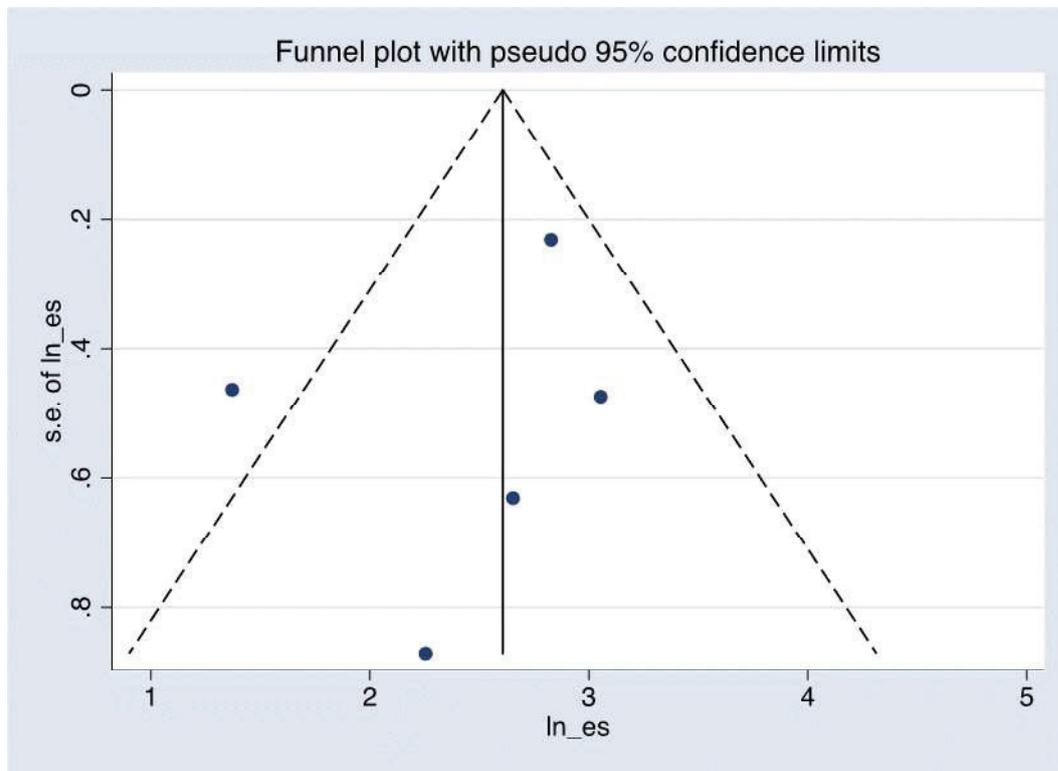
IMEMR

01.09.2016

(((NASWAR) or "NISWAR") or "SNUFF") or "NASS" [KeyWords] and Pakistan [KeyWords] and ((cancer) or "CARCINOMA") or "NEOPLASM") or "MALIGNANCY" [KeyWords]

12

PakMedinet



Naswar =19

Niswar =13

Nass=0

Snuff=14

Oral cancer=94

Oral squamous cell carcinoma=68

Mouth cancer=37

Lip cancer=65

Pharyngeal cancer=11

Carcinoma tongue=48

Mouth cancer=37

Head and neck cancer=54

2. Funnel plot of included studies

3. Quality Assessment of the included studies

	Selection Bias	Design	Confounders	Blinding	Data Collection Methods	Withdrawals and Drop-outs	Global Rating
Jafarey et al. (1977)	+	o	o	o	-	n/a	+
Khan et al. (2014)	-	o	o	o	-	n/a	-
Khan et al. (unpublished)	+	o	+	o	-	n/a	+
Merchant et al. (2000)	o	o	+	o	-	n/a	+
Riaz et al. (2015)	o	o	-	o	-	n/a	-

Component ratings: + strong; o moderate; - weak

Global ratings: + strong (no weak ratings); o moderate (one weak rating); - weak (two or more weak ratings)

4. MOOSE Checklist

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	3
2	Hypothesis statement	4
3	Description of study outcome(s)	6
4	Type of exposure or intervention used	5
5	Type of study designs used	5
6	Study population	5
Reporting of search strategy should include		
7	Qualifications of searchers (eg, librarians and investigators)	Supplementary file 1.
8	Search strategy, including time period, included in the synthesis and keywords	-do-

9	Effort to include all available studies, including contact with authors	6
10	Databases and registries searched	6
11	Search software used, name and version, including special features used (eg, explosion)	N/A
12	Use of hand searching (eg, reference lists of obtained articles)	6
13	List of citations located and those excluded, including justification	Figure 1
14	Method of addressing articles published in languages other than English	N/A
15	Method of handling abstracts and unpublished studies	N/A
16	Description of any contact with authors	N/A
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	5
18	Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	7, 8
19	Documentation of how data were classified and coded (eg, multiple raters, blinding and interrater reliability)	7
20	Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	Supplementary file 3
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	7
22	Assessment of heterogeneity	9
23	Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	9
24	Provision of appropriate tables and graphics	Table 2 and figure 2
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	Figure 2
26	Table giving descriptive information for each study included	Table 1
27	Results of sensitivity testing (eg, subgroup analysis)	Table 2
28	Indication of statistical uncertainty of findings	Table 2



Oral Cancer Epidemiology in Khyber Pakhtunkhwa, Pakistan

Lifestyle questionnaire “Cases”

1. Identification number
 Centre Person number

The identification number is composed of the values for centre and person number.

Person numbers are consecutive numbers within each centre and should not include the identifier of case or control status.

2. General guidelines

- The columns should be filled in justified to the right e.g., valid |__|_1|_2| , not valid |_1|_2|
- Leave blank if question is not asked or not applicable.
- Avoid missing or unknown codes; insist to get an answer even if it is only estimation.
- If you do not succeed in getting an answer or estimation, the columns should be filled in with 9.
- When “specify” is written, note your answer on the uninterrupted line.

Good morning.

My name is and first of all I would like to thank you for agreeing to participate in this study. We are conducting a study in order to clarify if certain characteristics and habits of men and women are related to certain diseases. For this purpose, we will interview many patients attending this and other hospitals.

If you agree, I will ask you several questions and the answers will be recorded on this form. I would like to reassure you that all that is said during the interview will be strictly confidential and that the information collected from several hundreds of people will only be used in scientific reports without any personal name or identifiers being mentioned.

Any likely benefits of the study for the well-being of the population rely on the accuracy of your answers. Therefore, if you do not understand the meaning of any of the questions, please don't be afraid to ask. At any time you may refuse to continue or to answer specific questions.

Before starting, I invite you to carefully read the enclosed acceptance form and to sign it. I will be happy to explain to you any detail regarding the study before you decide to sign the form. By signing the form, you accept to participate in this research: the acceptance as well as the refusal to participate, however, will have no consequence on the medical acts related to your current disease.

Can we start now?

A. Personal Data

A.1. Age _____ (In Years) , D.O.B d d / m m / y e a r (Optional)

A.2. Sex

(1) Male

(2) Female

(3) Other

A.3. Address

House # _____

Street / Mohalla / Village _____

District _____

A.4. For how long have you been living at this address?

(1) \geq 1 year

(2) $<$ 1 year

A.5. Which country were you born in?

(1) Pakistan

(2) Afghanistan

(3) If other specify _____

A.6. What is your ethnic origin?

(1) Pashtun

(2) Hindkowi

(3) Persian

(4) Muhajir

(5) Chitrali

(6) Other (Specify) _____

A.7. What is your religion?

(1) Muslim

(2) Christian

(3) Hindu

(4) Sikh

(5) Parsi

(6) Other (Specify)

B. Oral cancer history (To be crosschecked / filled with the help of clinical report, The questions requiring checking of clinical records should be dealt with at the beginning or the end of the interview).

B.1. Date of definitive diagnosis (Or date of return of biopsy) d d / m m / y e a r

B.2. Site of tumor according to definitive diagnosis

1. Upper Lip
2. Lower Lip
3. Gums
4. Tongue
5. Floor of the mouth
6. Soft Plate
7. Hard Palate
8. Oro pharynx
9. Buccal mucosa
10. Others (Specify)

B.3. Tumor Stage at time of diagnosis (If available in records)

- (1) Stage 0
- (2) Stage I
- (3) Stage II
- (4) Stage III
- (5) Stage IVA
- (6) Stage IVB
- (7) Stage IVC

B.4. Can you recall when did you became aware of your condition d d / m m / y e a r

1. Can you recall when did you first consult a health provider for this condition (date)
 d d / m m / y e a r

B.5. Which healthcare provider did you consult first for this condition?

(1) Dentist

(2) Medical Practitioner

(3) Others (Specify)_____

B.6. What course of action did the health provider take?

(1) Referral

(2) Biosy

(3) Treatment and follow up

(4) Other (Specify)_____

1. When did you first report to a Maxillofacial/ENT deptt (Date, verbal and from record)
 d d / m m / y e a r

B.7. When was the Biopsy taken (Date, verbal and from record) d d / m m / y e a r

B.8. What treatment is planned (Ascertain from clinical records and mark as many as apply)

(1) Surgery

(2) Chemo therapy

(3) Radio Therapy

(4) Other (Specify)_____

B.9. When will this Treatment start (Date, verbal and from record) d d / m m / y e a r

C. Socioeconomic status

C.1. How many household members are 13 years old or younger?

- (1) Five or more
- (2) Four
- (3) Three
- (4) Two
- (5) One
- (6) None

C.2. How many children ages 5 to 13 attend school?

- (1) Not all
- (2) All, or no children ages 5 to 13

C.3. How many household members work in elementary occupations (not senior officials, managers, professionals, technicians or associated professionals, clerks, salespeople, service or shop workers, skilled workers in agriculture or fishery, craft or trade workers, or plant/machinery operators)?

- (1) Two or more
- (2) One
- (3) None

C.4. What is the highest educational level completed by the female head/spouse?

- (1) Less than Class 1 or no data
- (2) No female head/spouse
- (3) Class 1 or higher

- C.5. What is the main source of drinking water for the household?
- (1) Others
 - (2) Hand pump, covered/closed well, motorized pump/tube well, or piped water
- C.6. What type of toilet is used by your household?
- (1) None or other
 - (2) Flush connected to pit/septic tank or open drain
 - (3) Flush connected to public sewerage
- C.7. Does the household own a refrigerator or freezer?
- (1) No
 - (2) Yes
- C.8. Does the household own a television?
- (1) No
 - (2) Yes
- C.9. Does the household own a motorcycle, scooter, car, or other vehicle?
- (1) No
 - (2) Yes

D. Oral Hygiene and Oral Health

D.1. How often do you clean your teeth (Think about one year ago)?

(0) Never

(1) Less than once a week

(5) 2 times a day

(2) 1 to 2 times a week

(6) 3 times a day

(3) Every other day

(7) more 3 times a day

(4) Once a day

D.2. Which of the following do you use to clean your teeth? (These may not be mutually exclusive)

(1) Tooth brush

(2) Dental floss

(3) Toothpaste

(4) Other (specify) _____

D.3. Do you wear a denture?

(1) Yes ► D.4.

(2) No ► D.7.

D.4. If yes, in the upper jaw do you wear?

- (1) Full denture
- (2) Partial denture
- (3) No denture

D.5. In the lower jaw do you wear?

- (1) Full denture,
- (2) Partial denture,
- (3) No denture

D.6. At which age did you start wearing dentures?

D.7. Did your gums bleed when you clean your teeth?

- (1) No
- (2) Sometimes;
- (3) Always or almost always

D.8. Have you ever felt that your teeth are shaky?

- (1)Yes
- (2)No

D.7. How often do you visit a dentist?

- (1) Once an year
- (2) Twice an year
- (3) Only when there is a need
- (4) Never

D.8. Have you ever been diagnosed with an oral disease?

(1) Yes

(2) No

D.9. If yes, can you specify what it was? _____

D.10. Have you ever experienced trauma inside your mouth?

(1) Yes

(2) No

D.11. If yes, do you remember when (year)?

D.12. Did the condition resolved by itself or did you seek any medical help for it?

(1) Resolve

(2) Help

D.13. Did the condition resolve after you sought medical help?

(1) Yes

(2) No

E. Diet and lifestyle

1. How often did you consume the following foods and beverages one year ago?

	Unit	Food item	How many times per day, week, month, year? (mark one column only)				
			day	week	month	year	Never
E1	1 portion	Beef	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E2	1 portion	Mutton	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E3	1 portion	Poultry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E4	1 portion	Other meat	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E5	1 portion	Fish	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E6	1 portion	Liver, Kidney, Paaye	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E7	1 portion	Raw green vegetables and salads	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E8	1 portion	Cooked green vegetables	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E9	1 portion	Carrots	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E10	1 portion	Fresh tomatoes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E11	1 portion	Pulses (peas, beans, etc.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E12		As a summary, how often would you say that you ate any kind of vegetables (potatoes excluded)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E13	1 portion	Fresh fruit juices	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E14	1 portion	Apples or pears	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E15	1 portion	Citrus fruit (oranges, Kino, lemons)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E16	1 portion	Bananas	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E17	1 portion	Melons (Water Mellon, Mellon etc)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E18	1 portion	Plums, peaches, apricots (in season)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E19	1 portion	Grapes	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E20	1 portion	As a summary, how often would you say that you ate any kind of fresh fruit (including fruit salads)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

E21 1 cup Green Tea

E22 1 cup Black Tea

E23 At what temperature did you usually drink your tea?
 1 very hot 2 hot 3 warm

E24 1 portion How often do you use olive oil:
For salads?
For cooking

E25 Do you use vegetable oil or ghee for cooking?

(1) Ghee

(2) Vegetable oil

F. Substance UsageF.1. Do you or did you ever smoke cigarettes, at least once a week for a year?

(1) Yes, still

(2) Only in the past (stopped at least 12 months ago)

(3) Never ► F2

<i>First period:</i> a) At what age or in what year did you first start smoking cigarettes?	c) Which type of cigarettes did you mostly smoke?	d) How many cigarettes did you smoke? (per day or per week)	e) Did you continue to smoke in this way or did you stop or change your smoking habits substantially anytime?	f) When was that? (Probing: At which age or in which year did you stop smoking or change your smoking habits?)	g) (If stopped) Did you ever start smoking cigarettes again subsequently?
(age) or (year)	(1)Manufactured with filter (2)Manufactured without filter (3)Hand-rolled	per day or week	(1) no change ► F2 (2) stopped ► f (3) changed ► f	(age) or (year)	yes ► a next period no ► F2
____ or _____	____ ____	____ or ____	____	____ or _____	

<p><i>Subsequent periods:</i> a) When did you begin smoking again or change to smoking a different amount or different product? <i>(Probing: ...at which age or in which year was that?)</i></p>	<p>c) Which type of cigarettes did you mostly smoke?</p>	<p>d) How many cigarettes did you smoke? <i>(per day or per week)</i></p>	<p>e) Did you continue to smoke in this way or did you stop or change your smoking habits substantially anytime?</p>	<p>f) When was that? <i>(Probing: At which age or in which year did you stop smoking or change your smoking habits?)</i></p>	<p>g) (If stopped) Did you ever start smoking cigarettes again subsequently?</p>
<p>____ or _____</p>	<p>(1)filter (2)non-filter (3)hand-rolled <input type="checkbox"/></p>	<p>____ or ____ ____</p>	<p>(1) no change ▶ F.2. (2) stopped ▶ f (3)changed▶ f <input type="checkbox"/></p>	<p>____ or _____ _____</p>	<p>yes▶ a next period no▶ F.2.</p>

F.2. Do you or did you ever smoke Cheelum/Hukkah at least once a week for a year?

(1) still

(2) Only in the past (stopped at least 12 months ago)

(3) Never ►F.3.

<i>First period:</i> (a) At what age or in what year did you first start smoking Cheelum?	d) How many cheelums did you smoke? (per day or per week)	e) Did you continue to smoke in this way or did you change your smoking habits substantially at anytime?	f) When was that? (Probing: At which age or in which year did you stop smoking or change your smoking habits ?)	g) (If stopped) Did you ever start smoking Cheelum again subsequently ?
(age) or (year)	per day or week	(1) no change ►F.3. (2) stopped ►f (3)changed►f <input type="checkbox"/>	(age) or (year)	yes► a next period no►F.3.
<input type="text"/> or <input type="text"/>	<input type="text"/> or <input type="text"/>		<input type="text"/> or <input type="text"/>	
<i>Subsequent periods:</i> a) When did you begin smoking again or change to smoking a different amount or different product? (Probing: ...at which age or in which year was that?)	d) How many Cheelum did you smoke? (per day or per week)	e) Did you continue to smoke in this way or did you change your smoking habits substantially anytime ?	f) When was that? (Probing: At which age or in which year did you stop smoking or change your smoking habits ?)	g) (If stopped) Did you ever start smoking Cheelum again subsequently ?
<input type="text"/> or <input type="text"/>	<input type="text"/> or <input type="text"/>	(1) no change ►F.3. (2) stopped ►f (3)changed►f <input type="checkbox"/>	<input type="text"/> or <input type="text"/>	yes► a next period no►F.3.

F.3. Do you or did you ever used Paan at least once a week for a year?

- (1) Yes, still
 (2) Only in the past (stopped at least 12 months ago)
 (3) Never ► F.4.

<i>First period:</i> a) At what age or in what year did first start paan?	b) Which type of paan did you mainly take?	d) How many paans? (per day or per week)	e) Did you continue to use in this way or did you stop or change your eating habits substantially anytime?	f) When was that? (Probing: At which age or in which year did you stop using paan or change your paan habits?)	g) (If stopped) Did you ever start paan again subsequently?
(age) or (year)	(1) With tobacco (2) Without tobacco	per day or week	(1) no change ► F.4. (2) stopped ► f (3) changed ► f	(age) or (year)	yes ► a next period no ► F.4.
____ or _____	<input type="checkbox"/>	____ or _____	<input type="checkbox"/>	____ or _____	
<i>Subsequent periods:</i> a) When did you begin paan again or change to different amount or different product? (Probing at which age or in which year was that?)	b) Which type of paan did you mainly use? (1) With tobacco (2) Without tobacco	d) How many paans did you take? (per day or per week)	e) Did you continue to take paan in this way or did you stop or change your paan eating habit substantially anytime?	f) When was that? (Probing: At which age or in which year did you stop paan?)	g) (If stopped) Did you ever start paan again subsequently?
			(1) no change ► F.4. (2) stopped ► f (3) changed ► f		yes ► a next period no ► F.4.
____ or _____	<input type="checkbox"/>	____ or _____	<input type="checkbox"/>	____ or _____	

F.4. Do / did you you ever take Naswar at least once a week for a year?

- (1) Yes, still
- (2) Only in the past (stopped at least 12 months ago)
- (3) Never ► G

<i>First period:</i> a) At what age or in what year did first start using Naswar ?	b) Which site of the Mouth you generally used to put Naswar in? <i>Which Quadrant</i>	c) What is/was the average duration of each dip?	d) Which type of Naswar did you mostly use?	e) How many units did you dip? (per day or per week)	f) What did/ do you do with saliva coming into mouth?	g) Did you continue to use in this way or did you stop or change your use habits substantially anytime?	h) When was that? <i>(Probing: At which age or in which year did you stop using or change your usage habits?)</i>	i) (If stopped) Did you ever start naswar use again subsequently?
(age) or (year)	(1) Upper Right (2) Upper Left (3) Lower Right (4) Lower Left	Time in mins	(1)Black Moist (2) Black Dry (3) Green Moist (4) Green Dry (5) Other	per day or week	(1)Spit (2)Swallow (3)Both	(1) no change ► G (2) stopped ► h (3) changed ► h	(age) or (year)	yes ► a next period no ► G
<input type="text"/> or <input type="text"/> <input type="text"/>	<input type="checkbox"/> if more than one <input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/> or <input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/> or <input type="text"/>	

<i>Subsequent period:</i> a) At what age or in what year did first start using Naswar ?	b) Which site of the Mouth you generally used to put Naswar in? <i>Which Quadrant</i>	c) What is/was the average duration of each dip ?	d) Which type of Naswar did you mostly use?	e) How many units did you dip? (per day or per week)	f) What did/ do you do with saliva coming into mouth?	g) Did you continue to use in this way or did you stop or change your use habits substantially anytime?	h) When was that? <i>(Probing: At which age or in which year did you stop using or change your usage habits?)</i>	i) <i>(If stopped)</i> Did you ever start naswar again subsequently?
(age) or (year)	(1) Upper Right (2) Upper Left (3) Lower Right (4) Lower Left	<i>Time in mins</i>	(1)Black Moist (2) Black Dry (3) Green Moist	<i>per day or week</i>	(1)Spit (2)Swallow (3)Both	(1) no change ► G (2) stopped ► h (3) changed ► h	(age) or (year)	yes ► a next period no ► G
____ or _____	____ if more than one _____	____	(4) Green Dry (5) Other ____	____ or ____	____	____	____ or _____	

G. DRINKING HABITS

G.1. Have you ever drunk alcoholic beverages?

(1) Yes ► G.2.

(2) No ► H

G.2. If yes how frequently did you drink alcoholic beverages one year ago?

(1) Every day

(2) Most days but not every day

(3) 1 to 3 times per week

(4) More than once per month & less than once per week

(5) Less than once per month

G.3. Regarding your normal drinking habits one year ago, when did you normally drink?

(1) With meals

(2) Between meals

(3) Both

G.4. Have you ever in your lifetime drunk large amounts of alcohol in a short period of time, (eg more than 10 drinks in a couple of hours)?

(1) Yes

(2) No

G.5. If yes how often did you do this?

(1) Every day

(2) 4 to 6 times per week

(3) 1 to 3 times per week

(4) More than once per month & less than once per week

(5) Less than once per month

G.6. From what age did you do this? |_|_| to what age ? |_|_|

H. Systemic Health

H.1. Throughout your life, have you ever had skin warts/ veruccae (Picture) ?

- (1) Yes ► Next part of the question
- (2) No;
- (3) Don't know [if 'No' or "don't know" go to H.2.]

If 'Yes', where?

- (1) Hands
- (2) Feet
- (3) Head & Neck
- (4) Other (specify) _____

H.2. Throughout your adult life, have you ever had Candida Albicans/ thrush? (Explanation with pictures)

- (1) Yes ► Next part of the question
- (2) No
- (3) Don't know [if 'No' or "don't know" go to H.3.]

If 'Yes', where?

- (1) Genitals
- (2) Mouth
- (3) Other (specify) _____

H.3. Have you ever had herpetic lesions (cold sore)? (Picture explanation)

- (1) Yes ► Next part of the question
- (2) No
- (3) Don't know [if 'No' or "don't know" go to H.4.]

If 'Yes', where?

- (1) Lip
- (2) Genitals
- (3) Other (specify) _____

H.4. Have you ever had heartburn?

- (1) Yes ► Next part of the question
- (2) No

[If 'No' go to H7]

If yes, how frequently?

- (1) At least once a day
- (2) 2 to 6 times per week
- (3) Once per week
- (4) Less than once per week

H.5. At what age did you first begin suffering from heartburn?

H.6. Do you take or have you taken medication for heartburn?

- (1) Yes;
- (2) No

If yes which (name of medicine) _____

H.7. Do you ever suffer from regurgitation?

(1) Yes ► H.8.

(2) No ► H.10.

If yes, how frequently?

(1) At least once a day

(2) 2 to 6 times per week

(3) Once per week

(4) Less than once per week

H.8. At what age did you first begin suffering from regurgitation?

H.9. Do you take or have you taken medication for regurgitation?

(1) Yes

(2) No

If yes which _____

H.10. Have you ever taken aspirin regularly (at least once a week for a year)?

(1) Yes

(2) No

If yes, from age to age

I. Sun Exposure

Think of your exposure to direct sunlight during the past 1 year, and respond regarding a typical week

I.1. Usually, how many days in a week did you go out in the sunlight during the daytime? |_|

- (1) One
- (2) Two
- (3) Three
- (4) Four
- (5) Five
- (6) Six
- (7) seven

I.2. Usually, between 9 am-4 pm, what was the average amount of time (hours) spent outdoor in the sunlight in a typical day? |_|

- (1) One
- (2) Two
- (3) Three
- (4) Four
- (5) Five
- (6) Six
- (7) seven

I.3. How much of your head and neck was generally covered during outdoor hours (between 9 am - 4 pm)? |_|

- (1) Head
- (2) Face

(3) Neck

I.4. Were any of these a part of your attire during outdoor hours (between 9 am- 4 pm)?

(1) Jelbaab/abaya/Burka_____

(2) Niqab_____

(3) Pagree_____

(4) Other (specify)_____

Dear Mr / Ms _____, Thankyou very much for your cooperation, we are done now with the interview. We hope the valuable information that we got from your interview will be very useful for us in identifying potential causes for oral cancer in this population and help in its prevention.

Signature Interviewer _____

Date and place_____

Signature Field Coordinator (After checking for completeness)_____

Date and place_____

Signature Data entry Operator_____

Date and place_____



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Dated: 11-09-2014

TO WHOM IT MAY CONCERN

Certified that after desk review Ethical Approval has been granted to the project title "Epidemiology of oral cancer in Khyber Pakhtunkhwa Province of Pakistan" submitted by Dr. Zohaib Khan, PhD Scholar Epidemiology, Leibniz Institute for Prevention Research and Epidemiology (BIPS) Bremen, Germany.

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Study I.D __/__/---

“Epidemiology of Oral Cancer in the Khyber Pakhtunkhwa Province”

Consent Form

Zohaib Khan (Investigator),
Prof. Dr. Hajo Zeeb (Study Supervisor),
Prof. Dr. Wolfgang Ahrens (Co- Supervisor),
Leibniz Institute for Prevention Research and Epidemiology (BIPS)
Achterstrasse 30, 28359
Bremen, Germany.

Dear Sir/Madam,

Thank you for taking your time to read this. BIPS is a public sector research oriented institute affiliated with the University of Bremen, Germany. The principal investigator is a PhD student from Pakistan working at BIPS. We want to assess the role of different risk factors in the development of oral cancer in the Khyber Pakhtunkhwa province. For this purpose we are conducting a case control research study in different hospitals of the province to get information from people who have been diagnosed with oral cancer and also people who are not suffering from oral cancer, we tend to compare different habits among these two groups to come up with evidence regarding risk factors for oral cancer in the Pakistan in general and Khyber Pakhtunkhwa in specific. We hope that our study will provide evidence to inform policy for prevention against oral cancer. We hereby request you to spare some time for a verbal interview and access to your clinical record, the interview will last from 30-60 mins. Your consent is also needed for access to your biopsy sample, collection of 3cc of your blood and a sample of your oral mucosal cells through exfoliation by a tooth brush.

- If you have any questions regarding the study please do not hesitate to ask the interviewer or contact the Principal Investigator at the given address.
- You may refuse to answer any question
- You may choose to stop the interview at any time
- Your name will not be disclosed at any time during or after the study.
- The information gained by this study will be kept confidential and used entirely for the purpose of improving the prevention strategies against oral cancer.

Do I have your consent to proceed Yes No

Name of **Respondent**: _____ Thumb Impression

Name of **Witness**: _____ CNIC #: _____

Name of Person obtaining consent: _____

Signature: _____

Date: _____

Versicherung der eigenständigen Verfassung

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig verfasst und keine weiteren als die angegebenen Quellen und Hilfsmittel verwendet habe. Alle Stellen, die ich wörtlich oder sinngemäß aus anderen Werken entnommen habe, sind unter Angabe der Quellen als solche kenntlich gemacht.

Diese Arbeit hat in gleicher oder ähnlicher Form noch keiner anderen Prüfungsbehörde vorgelegen.

Ort und Datum

Unterschrift