

The impact of smoking sheesha on gene expression in salivary cells

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Lists of Acronyms

Sheesha Smoking (SS)

University of Ottawa (uOttawa)

Systematic Review (SR)

Abstract

Background: The health effects of sheesha smoking are not well addressed.

Objective: To assess the association between sheesha tobacco smoking and gene expression pertinent to cancer.

Methodology: Three linked studies were carried out: (1) investigation of gene expression in salivary cells before and after exposure to sheesha tobacco smoke in 15 participants; (2) a systematic review of the association between sheesha and cancer; and (3) a pilot survey to collect data on factors potentially relevant to the uptake and cessation of sheesha tobacco smoking.

Results: In the short-term, sheesha smoking significantly reduced the expression of both xenobiotic metabolism genes and other genes known to have altered expression in tobacco related cancers in a range between 1.7 times and 55 times. The systematic review showed that sheesha may increase the risk of lung and esophageal cancers. The pilot survey identified misperceptions about safety, in line with other studies, an approach that could be used to investigate the characteristics of sheesha smokers on a larger scale, and specific issues to probe.

Conclusion: High quality epidemiological evidence on long-term effects of sheesha smoking on cancer is lacking. However, sheesha smoking has short-term effects on the expression of genes known to be involved in tobacco-related cancers. This is of major concern given widespread misperceptions about the likely safety of sheesha tobacco smoking.

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In the name of Allah, Most Gracious, Most Merciful.

All Praise and thanks be to Allah! We praise and thank Him, ask Him for His Help and Forgiveness, and we seek refuge in Allah from the evils of our souls and the mischiefs of our deeds. He whom Allah guides will not be misled, and he whom Allah misleads will never have a guide.

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Chapter 1. Background

Origin

Sheesha originated in the North Western provinces of India near the border of Pakistan in the states of Rajasthan and Gujarat hundreds of years ago. From India sheesha travelled to the Persian Kingdom, then towards Pakistan and Afghanistan, and then to the Arab countries. The name and shape evolved over many years, reflecting its long journey. In India traces of primitive sheesha have been found, with its jar made from coconut shell and without a hose. In Persia, where “shisha” means glass, the coconut was replaced by a glass jar; that is how sheesha acquired its name. In Turkey the design of sheesha was reshaped with the addition of a hose and introduced to the Middle Eastern people by the Ottomans. Sheesha has been largely unchanged over the last few hundred years. (1)

Apparatus

Sheesha consists of a smoking apparatus which includes a head, body, bowl and hose (Figure 1). These parts are assembled together and sealed by pieces of rubber. The burned charcoal used to heat the tobacco is placed on the head. The perforated tin foil that separates the tobacco from charcoal adjusts the temperature and prevents the tobacco from being combusted. The smoker uses the hose to inhale a sufficient amount of air that pulls down the smoke from the head, where the tobacco is heated by charcoal, through the body tube into the water jar, where it bubbles, and then is deposited in the top part of the jar where the hose is attached, to be ready to consume during the next inhalation (4).

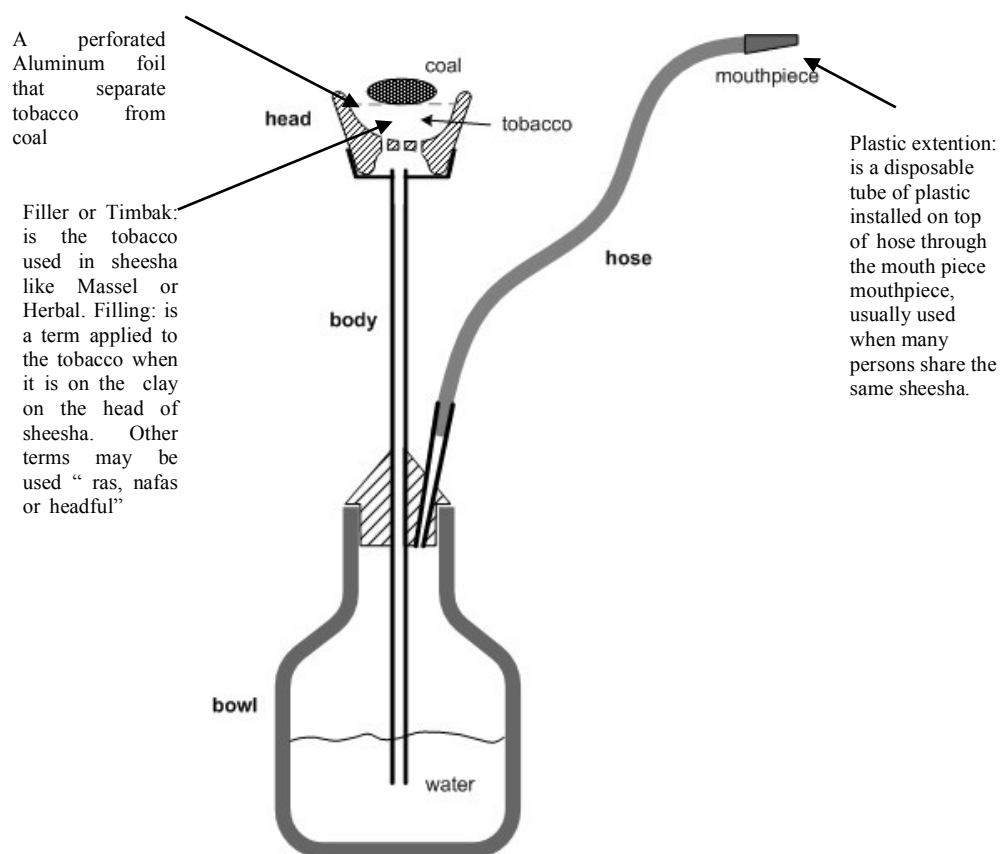


Figure 1 A diagram representing sheesha.

(diagram extracted from: WHO Study Group on Tobacco Product Regulation (TobReg). (2005) Advisory note. waterpipe tobacco smoking: Health effects, research needs and recommended actions by regulators. (11).

Nomenclature

Different names are given to sheesha depending on locality for example “Nargyleh” or “Arghile” is a frequently-used term in Lebanon, Palestine, Jordan and Syria. “Sheesha”, “Jajeer” (5) “boory” or “goza” are the most widely used names for the waterpipe in Egypt and Saudi Arabia. In Africa and India, the waterpipe is known as the “Hookah”, and in the United Kingdom sheesha is known as “Hubble bubble”. Sheesha has different spellings, varying between translations, for example, “Argyleh”, “Argyle”, “Arguileh”, “Argileh” are pronounced the same in Arabic. However sheesha is the most commonly used term in Canada, as it was introduced by the Middle Eastern immigrants (6). Different kinds of tobacco, also called “timbak”, can be used to fill the head of sheesha, such as Massal, Ajami (7) and Herbal. The head, when filled, is called “nafas or ras” (Table 1). In this thesis “sheesha” will be used as a generic term to refer to diverse types of filled “waterpipes”.

Table 1 Different kinds of timbac "filling" used in sheesha based on personal observation.

Tobacco (timbac)	Consumed by	Availability	Produces fumes	Contains nicotine	Excluded from tobacco tax	Cost
Tombac Ajami	Older people	Not available in Canada	Less	Yes	NA	NA
Massal	Younger people	Yes can be sold only to people aged 18 years or older	More	Yes	No	Expensive >10 dollars per nafas
Herbal	Younger people	Yes and can be sold to younger people	More	No	Yes	Cheap <3 dollars per nafas

Putting sheesha into context

The World Health Organization (WHO) recognizes Canada's leadership in regulating tobacco (4). Regulations for tobacco smoking encompass policy development, legislation, taxation, health warning and public education, restricting youth access, marketing, media and antismoking campaigns (8). Despite these regulations, Canada has witnessed an unexplained sharp rise in sheesha use especially among young adults and adolescents (6, 9).

The Smoke-Free Ontario Act bans tobacco smoking in all enclosed places. However, it does not give a clear definition of smoke (10). Sheesha bars are exempt from the legislation as the law doesn't specify the aromatic smoke emitted from sheesha as being included in the decomposition of tobacco smoke (11, 12).

Indeed, the Public Health Agency Canada (PHAC), when accessed in 2011, under "Terry's case: A Youth at Risk" stated that sheesha may be:

"Considered the healthiest way to smoke pot (and tobacco). The water acts as a filter, removing more of the toxins than a cigarette filter, giving the smoker THC-rich smoke" (13).

However, recently PHAC has deleted this statement from their website and as of May 2013 the website provides no information about sheesha smoking (SS).

According to the Non-Smokers' Rights Association in February 2012, more than 20 establishments, known as "sheesha bars", had opened in Ottawa. A WHO Study Group on Tobacco Product Regulation noted that the increase in popularity of the sheesha among young people is, in part, thought to be attributable to the belief that SS is the healthiest way to smoke tobacco (4, 14). This perception may have been reinforced by the introduction of new flavored and aromatic Massal tobacco that masks the noxious fumes (7, 15), as well as the paucity of data in the literature concerning the adverse health

effects of SS. Moreover, the quality of the small body of evidence on potential outcomes associated with SS has been deemed to be “very low” to “low” (16).

The chemical profile and characteristics of sheesha’s smoke

In contrast to the perception that SS may be less harmful than other forms of tobacco use, many studies have highlighted the potential deleterious effects of SS by studying the composition of mainstream sheesha smoke (3, 17). Sheesha tobacco smoke contains high levels of the same chemicals found in cigarettes such as polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO) and tar; these chemicals have been classified to be causal factors for cancer and other chronic disease (3, 18).

Table 2 Principal constituents of sheesha and cigarette mainstream smoke

Substance	Sheesha in 4.7 g of tobacco used	In a single cigarette
Nicotine (mg)	2.96	0.5-3.32
Tar (mg)	802	6.1-48.7
Carbon monoxide (mg)	143	11-40.7
<i>Polycyclic aromatic hydrocarbons (PAH)</i>		
Phenanthrene, (µg)	0.784	0.2-0.4
Fluoranthene, (µg)	0.221	0.009-0.099
Chrysene, (µg)	0.112	0.004-0.041

Data extracted from (2,3)

It has been estimated that during a one-hour sheesha use session, a sheesha smoker generates carcinogenic PAH, volatile aldehydes, carbon monoxide and ultrafine particles equivalent to 2-10 times those emitted from cigarette smoking (Table 2) (17, 19). Indeed, the WHO has stated that a session of SS is equivalent to 100 cigarettes (4). The report has been criticised by Chaouachi et al., in 2006 (20), who were of the opinion that the conclusions of the WHO report are not valid because they drew conclusions from a review of the literature that was not comprehensive or systematic. A study conducted in Virginia showed that in comparison to smoking a single cigarette, a single sheesha tobacco smoking session emits similar peak nicotine exposure, almost 4-fold greater carboxyhemoglobin and 56-fold greater smoke volume (21). Hadidi et al., 2004 concluded that smoking one filling of flavoured tobacco increases the plasma nicotine level by 20% (22). Neergaard et al., 2007 found that both sheesha and cigarettes produce the same amount of nicotine in a day (23). In addition to the toxicants derived from the tobacco, sheesha smokers are also susceptible to be exposed to large quantities of toxicants that came from the lit charcoal placed on top of the aluminum foil that separates it from the tobacco (18, 24).

Undoubtedly, what we estimated from the content of mainstream smoke is not in line with what has been found from many epidemiological studies, in which SS has not been found to be significantly associated with the adverse health outcomes, although the quality of these studies has been classified as “low” to “very low” (16). For example, Ben Saad has argued that:

“Indeed, we are witnessing a growing confusion in biomedical studies, including the relationship between the use of the narghile and certain diseases such as lung cancer and bacterial or parasitic infections.” (25).

The water in the tank -“filter” - as asserted by some commentators -acts as an absorbent but the retained gaseous components depend on the absorbent coefficient of each gas (26) and the degree of liquid saturation. In practice, the water that is in sheesha jar remains unchanged during a typical sitting of SS (27). Therefore, during a session of SS, the water will become saturated and lose its “filter” characteristic. One study (26) showed that the

water retains only a limited amount of nicotine. This suggests that the water has limited, if any, ability to filter the noxious fumes.

The temperature attained by a gas affects its physical characteristics and its ability to react within the body (28, 29). The range of temperature in a burning cigarette is between 700° C and 900° C. However the temperatures recorded in sheeshas are much lower (26). The temperature depends on the tobacco content, the method of preparation and the method of application (26). The Ajami filling typically used by older people (Table 1) needs to be moisturised and firmly packed on the head of sheesha with charcoal placed on the top. However, the Massal and Herbal fillings are already wet when taken out of the packet for use and just need to be shredded or finely separated and lightly adjusted on the head with perforated tin foil on top that separates it from the burning coal (30). The coarse physical state of the filling, the extent to which it is moisturised, as well as the way in which the tin foil on top of the filling that separates it from the charcoal is applied, determine the amount of air that pass through the filling and therefore the temperature. This mechanism of heating and passing the smoke through water, instead of burning and cooling, gives the gas coming from the filling a colloidal characteristic and not a combustible one (26). This results in inhalation of a larger quantity of fumes while smoking sheesha than while smoking cigarettes (31). The large amount of smoke that sheesha produces when Massal or the Herbal mix is heated, makes this practice appealing for its taste and smell (32, 33).

Characteristics of sheesha smokers and the method of smoking sheesha

Smoking sheesha is seen as a recreational activity (34). In practice, sheesha does not appear to be a tool used to reduce stress, in contrast to cigarette smoking (34, 35). It is enjoyed in a social context. Smoking sheesha is usually done in a group setting among friends or family members (36).

The types of people who smoke sheesha have changed over the years. In the 1990s, the time of introduction of Massal tobacco smoking, there has been a significant upsurge of

SS among female and youth in Asia and the Middle Eastern region (37, 41). However, in the last 5 years or so, this trend of tobacco smoking has crossed the borders to reach the Western Countries (6, 38, 39). Sheesha use is depicted in cartoon movies (Figure 2) and in advertisements that are directed towards young women and tourists (40, 41). Sheesha smokers, during a typical sheesha gathering, chat, eat, drink and share the same sheesha hose, which might increase the potential risk of spreading infectious diseases, such as tuberculosis, hepatitis and herpes (42, 43). A sheesha gathering may take place in someone's home or in lounges, called "sheesha bars". Sheesha bars, crowded with young adults, are the most popular public venue for smoking sheesha. They are decorated in a way to present a very relaxing and enjoyable ambiance. Sheesha bars are considered to be a prime destination for young adults, even for non-smokers, to spend time with friends (10). In contrast to cigarette smoking, sheesha use is more common among students who have been reported to believe that it enhances concentration (36).

Akl et al., (55) have conducted a comprehensive SR to assess the prevalence of sheesha smoking across countries. The SR comprises 38 cross-sectional studies, with 4 national surveys and 34 studies among specific populations. The main concern in the included studies was the method of reporting sheesha smoking. They noted that the highest prevalence of current sheesha smoking was among school students across countries: in the United States, especially among Arab Americans (12%-15%); the Arabian Gulf region (9%-16%); Estonia (21%); and Lebanon (25%). In addition, they observed a high prevalence of current sheesha smoking among university students in the Arabian Gulf region (6%), the United Kingdom (8%), the United States (10%), Syria (15%), Lebanon (28%), and Pakistan (33%). The prevalence of current sheesha smoking among adults was not as high as that reported among young population: Pakistan (6%), Arabian Gulf region (4%-12%), Australia (11% in Arab speaking adults), Syria (9%-12%), and Lebanon (15%).

Chan et al 2011 (44), using data from the 2006 Canadian Youth Smoking Survey, evaluated the prevalence of sheesha smoking among Canadian youth. They found that the prevalence of ever sheesha smoker among all students was 6.8 %, and that the highest

prevalence (12.1 %) was recorded among 12th grade students. Males were more prone to smoke sheesha than females, with reported prevalences of 8.1 % and 5.5 % respectively. Comparing provinces, they found that Quebec has the highest prevalence (10.5%), while Ontario had a lower prevalence of ever sheesha smoking (5.6%). In a survey conducted in Montreal (Canada) in 2007 using mailed self-reported questionnaire, Dugas et al (9) found that 23 % out of 871 adults included in the study, age between 18 and 24 years, had smoked sheesha in the past year.

Sheesha is a new trend of smoking in Canada. It started among young immigrants “primarily from Middle Eastern region” and is gaining popularity among young “white” Canadians (6). Little work has been done on the prevalence and determinants of sheesha use in Canada and other countries outside the Middle East (6). Such work as has been done indicates that sheesha use is particularly common in adolescents and young adults (9, 44).

In health risk behavioral studies, surveillance is a crucial component of evaluation and assists in developing recommendations. The engagement of a range of stakeholders helps to refine these recommendations, and facilitates implementation. However, any assessment needs baseline data on who is smoking sheesha, why, how, for how long and what they use as filling. As yet, it is not well understood what drives young people to adopt this behavior in a country that has enacted rigorous multi-faceted tobacco control practices such as Canada.



Figure 2 The Caterpillar from Alice in wonderland movie smokes sheesha. The cartoon is one illustration of the perception of smoking sheesha.

Investigations on the potential adverse health effect of Sheesha

Concerns about SS have gained increasing coverage in the media (11, 12, 45, 46). These articles have been predominantly framed to present SS in a negative light, highlighting the potential negative health consequences and the inconsistency around the exemption of SS from the Smoke-Free Ontario Act (12, 45, 46).

Accumulating data suggest that non-cigarette tobacco smoking is increasingly frequent, both in developed and developing countries (47). While knowledge on the carcinogenetic effects of smoking pipes, cigars and bidis^{1,2} is considerable (2), there is little information about the health effects associated with other forms of tobacco smoking. In particular, the International Agency for Research on Cancer (IARC) Monograph on tobacco smoking, published in 2004 (2), noted little evidence about the health effects of smoking chilium (clay pipe), cheroot, chutta, *khii yoo* (used in Northern Thailand), kiraiku (used in Kenya) or sheesha. This dearth of evidence appears to have persisted, with a review published in 2010 drawing attention to the need for research on the health effects of non-cigarette tobacco products (47). In 2003, it was estimated that more than 100 million people worldwide smoke sheesha daily (48). As noted by WHO (2005) “there is surprisingly little research addressing tobacco smoking using a waterpipe” (4), and there are data that SS is increasing in young people (49, 50) and in women (23, 50).

Although sheesha use has a long history in developing countries, little is known about the adverse health effects attributed to it (2). The few studies available have, in the main, focussed on cancers of various types in less developed countries, and overall the evidence has been appraised as inconclusive because of methodological problems (16). Investigation of the potential adverse health effects of SS through epidemiological study designs such as cohort case-control or cross sectional study is unfeasible in Canada in the short to medium term. This is largely due to the fact that sheesha is a new trend here and

¹ Bidis as defined by CDC are small, thin hand-rolled cigarettes imported to the United States primarily from India and other

² According to IARC Monograph states “Bidi smoking is the most common form of tobacco smoking in India and is also prevalent in other south-Asian countries and an emerging problem in the USA.”

based on the experience of cigarette smoking and other types of non-cigarette smoking, there would be expected to be a long time lapse between exposure to the smoke and developing the diseases attributed to it (2). This makes the investigation of early markers of adverse effects timely.

One study used the sister-chromatid exchange (SCE) assay in lymphocytes as surrogate measures to assess the adverse health effect of SS and found that the genotoxicity of SS is higher than that induced by regular cigarette smoking (51). However, limitations of this analysis are that neither the mechanism of SCE formation has not been established, nor has a direct association been established between SCE induction and an adverse cellular or health outcome (52). Over the last decade or so, considerable attention has been focussed on the potential value of gene expression analysis as a promising marker of exposure and/or early development of disease (53). Kaddah et al., (14) investigated matrix metalloproteinase gene expression in cells obtained by broncho-alveolar lavage from patients with chronic obstructive pulmonary disease and found that a reported history of sheesha use was associated with upregulated expression of these genes. In 2010 Rastam et al. (54) highlighted the intense need of sound knowledge driven from epidemiological, clinical and biological studies, and suggested that investigations using microarrays might be especially useful tool to assess the adverse health effects driven from SS (54). However, so far as we are aware, no such investigation has yet been reported.

In view of the lack of knowledge of the health effects of sheesha use, its widespread use in many parts of the world (55), and apparent rapid increases in its use in countries in which sheesha has not traditionally been used (56), there is an urgent need to evaluate potential early markers of the health effects of sheesha use. Therefore, we undertook a study of the effects of sheesha use on the expression of genes whose expressions are known to be altered in the development of tobacco-related cancers, one of the main groups of diseases that could be associated with sheesha use in the long-term. This is the main component of my thesis, and is reported in Chapter 2.

Some studies have been conducted in Asian and Middle Eastern populations, where sheesha has been long established in the culture, to assess the effects of SS on health outcomes. A few systematic reviews have addressed the health effects of sheesha tobacco smoking within a limited scope, either focusing on cardiorespiratory disorders (25, 29) or lung function (57). Akl et al., (16) conducted a comprehensive SR and meta-analyses to assess the effects of sheesha on multiple health outcomes. However, in considering exposure, for some articles, Akl et al., (16) categorized combined exposure to sheesha and other methods of smoking such as use of cigars or pipes as “non cigarettes type of smoke”. In some included studies, most of participants were at a high risk of developing cancer diseases due to occupational hazard. Most of the studies were assessed as providing “low” to “very low” quality of evidence. Moreover, since 2008 (when Akl et al., (16) conducted their literature searches) more than 50 articles have been published concerning the health effects of sheesha. Therefore, we carried out a new SR and meta-analyses to assess the impact of sheesha on human cancers. A priori, we considered that this component of the thesis was important in order to inform the selection of genes in our gene expression analysis (ideally to focus on genes that had altered expression in cancers associated with SS). In practice, we think this component has been more useful in considering the complementarities of the epidemiological evidence base with the results of the gene expression part of the study, and so report on this component in Chapter 3.

In Canada, the demographic characteristics of sheesha smokers are still unclear. Few data are available regarding the age of smokers, the cultural background or the exposure to sheesha practices and sheesha smoke. A study conducted in Toronto investigated some factors that may have affected the proliferation of SS there (6). To our knowledge no study on sheesha smoke has been conducted in Ottawa. Information is also needed about when sheesha smokers started to smoke, the frequency with which they smoke and why they adopted sheesha in a context in which the prevalence of cigarette smoker is decreasing. Therefore, we developed a questionnaire and implemented a pilot survey that gathered information on the characteristics of sheesha smokers. A detailed analysis of this component of my thesis is presented in chapter 4.

Aims of the proposed study

The proposed study has one primary, and two supplementary, aims.

Primary aim

The primary aim is to assess the effect of sheesha Massal smoking on level of gene expression in salivary cells among young sheesha smokers in Ottawa.

Supplementary aim: systematic review to assist in evaluating biological plausibility

The first supplementary aim was to conduct a SR to assess all types of cancers attributed to SS from the literature by conducting a meta-analysis. Results from this study could be used in decision-making about the genes whose expression would be determined and in the interpretation of the results from the gene expression analysis.

Supplementary aim: pilot data on sheesha smokers

The second supplementary aim was to generate a framework used to recruit hard to access people for aim one, and to obtain demographic and characteristics of participants who accepted to take the gene expression part of the study. Therefore, we collected data on smoking practices to shed light on the emerging context of SS and estimating the amount of exposure while assessing the effect of SS on health. As a secondary objective we proposed to collect information on beliefs and attitudes of sheesha smokers to understand why sheesha has gained popularity among students while the popularity of other form of smoking is decreasing among this section of population.

The study was approved by the Ottawa Hospital Research Ethics Board (OHREB).

Chapter 2. The effect of sheesha smoking on gene expression

Introduction

Research studies on smoking are increasingly interdisciplinary. The trend is to use multiple approaches to test hypotheses about different components of a “web of causation” instead of looking for a simple cause-effect relation (58). Determination of the chemical profile and the characteristics of the smoke inhaled from different kinds of smoking device are crucial to understand the nature of exposure. In addition, understanding the psychology underlining smoking behaviour can help to explain its mode of use and the frequency of exposure. Knowing who the smokers are and assessing their body’s reaction toward a specific kind of smoke gives an idea about the capability of the body to clear itself from what it is exposed to. Gathering all information presents a panoramic view that allows us to predict the adverse health effect of a specific kind of smoke as well as to set recommendations (5, 54).

In this study we focused on the potential carcinogenic effects of sheesha tobacco smoking. We selected Massal as a filling for sheesha based on a small survey that we conducted earlier (please refer to chapter 4), in which we found that Massal is the most popular filling used to smoke sheesha. Massal is a blend of tobacco, sugar, glycerin and a special compound of ingredients that gives the smoke a particular aroma (59).

There appears to be variability in the potential adverse effects of SS according to the method of smoking, the breathing pattern of the smokers (60), the nature of the smoking compound used (17), and the material from which the hose is constructed (61). Therefore, we can hypothesise that the smoke, during its journey through the different parts of sheesha, acquires some characteristics that influence its reaction in the smoker’s body.

Moreover, previous studies have called for research to rigorously assess the adverse health effects of SS (62, 63) and others have suggested the use of proxy measures, especially those based on microarrays (64), to address this need.

Gene expression as a surrogate marker

Gene expression is a cellular state in which a gene is in the process of making proteins in response to a signal such as occurs in response to an environmental exposure (65). Overexpression is a cellular state when the gene makes excess copies of proteins in response to exposure. Underexpression is a cellular state when the gene stops or decreases the process of making proteins in response to exposure. Although the same genetic material is present in all the cells in the body, different factors influence whether various types of cell overexpress or underexpress specific genes, other genes may not be expressed at all. Thus, the biological state and the type of cell might affect the expression level of certain genes in specific cells. Measuring the level of mRNA theoretically gives an indication about the cellular response to specific exposures. Potentially, therefore, mRNA level could be used to predict the long-term effect of a specific exposure and the risk of developing certain diseases (66, 67).

Gene chips, known as microarrays, are recognized to be useful clinical research tools. They were introduced in 1990s and since then, have been used in the investigation of disease process such as studying the etiology, the pathogenesis and predicting the treatment outcomes (66, 68). Microarrays are used to provide simultaneous information on the expression levels of many genes. Two types of gene chips are available, ready-made and custom-made. In this study, we used a custom-made RT-PCR array to assess the effect of SS on previously selected genes.

In this part of the study we aimed to assess the potential carcinogenic effects of Massal SS in the salivary cells of young sheesha smokers by:

- First, investigating the expression of certain genes involved in the pathways of tumors that have been previously linked to tobacco smoking.
- Second, assessing the alteration in the expression of genes related to xenobiotic metabolism.

Gene selection

Genes were prioritized for investigation of expression in two ways. The first involved (a) identifying cancers considered to be caused by smoking and (b) identifying genes known to have altered expression in people with these types of cancer. The second required selection of genes involved in xenobiotic metabolism.

Selection of genes involved in the pathways of smoking related tumors

Tobacco smoke has been classified as a Class I carcinogen by IARC, and it is estimated that it accounts for 22% of global cancer deaths (2). For nine specific types of cancer, the evidence for a causal role of tobacco smoking has been deemed sufficient (Class I); specifically oral cancer, lung cancer, laryngeal cancer, pancreatic cancer, esophageal cancer, breast cancer, bladder cancer, stomach cancer and cervical cancer (2, 4). We did not consider types of cancer for which the evidence was deemed “limited” or “insufficient”. Because it was identified as a potential concern in the SR regarding cancer related SS (Chapter 3), we have added nasopharyngeal cancer as a potential disease although in fact there proved to be no evidence of a positive association between SS and nasopharyngeal cancer. Therefore, the contribution of the SR regarding cancer related SS was more limited than expected. We therefore focused on the ten types of cancer associated with tobacco smoking in general. To identify genes known to have altered expression in these ten types of cancer, we searched the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (69). This resource assembles genomic, chemical, and systemic functional information about genes, and is widely used as a bioinformatics tool to integrate and interpret the large-scale datasets generated by genome sequencing and other high-throughput experimental technologies. KEGG (69) has been especially valuable in developing molecular network-based overviews of the effects of diseases, drugs, and environmental compounds on gene expression and regulation. From the home page of KEGG (69) we selected “Cancers” under “Human diseases”, it shows that KEGG (69) have information on the pathways of 55 human cancers, yet no pathways through gene expression were reported for laryngeal, stomach or cervical cancers. Therefore, only seven cancers related to smoking were considered further in the gene selection process.

Table 3 Genes selected on the basis of literature and genes included in the array

96-Well Custom PCR Array Template				
#	Gene whose expression has been associated with smoking-related cancer	Gene selected for inclusion in array	Gene RefSeq #	Long-term effect/ Pathway through
<i>Cancer pathway genes</i>				
1	p16	CDKN2A	NM002825	Loss of expression
2	INK4A	CDKN2A	NM000077	Loss of expression
3	c-MYC	NMI	NM012333	Over expression
4	n-MYC	NMI	NM004688	Over expression
5	STAT3	STAT3	NM003150	Expression
6	BCL2	BAK1	NM000633	Over expression
7	ERBB2	ERBB2	NM004448	Over expression
8	EGFR	EGFR	NM005228	Over expression
9	iNOS	NOS2	NM000625	Increase expression
10	PTGS2 ¹	PTGS2	NM000963	Over expression
11	FAS	FAS	NM000043	Increase expression
12	CCND1	CCND1	NM001188	Over expression
13	CNTD1 ²	CNTD1	NM173478	Suggested by the array manufacturer
<i>Genes involved in xenobiotic metabolism</i>				
14	CYP1B1	CYP1B1	NM000104	Alter expression
15	CYP1A1	CYP1A1	NM000499	Alter expression
16	GSTP1	GSTP1	NM000852	Alter expression
17	GSTT1	GSTT1	NM000853	Alter expression
18	GSTM3	GSTM2	NM000849	Alter expression
<i>Housekeeping genes</i>				
19	GAPDH	GAPDH	NM002046	Housekeeping
20	ACTB	ACTB	NM001101	Housekeeping

¹ Also known as COX2² is suggested by the array manufacturer for a proper development of the array

Selection of genes in the pathway of diseases related to tobacco smoking.

The genes selected are summarized in Table 3. We first discuss the selection of cancer pathway genes, by cancer type. In some instances (which are specified), the gene selected for inclusion in the RT-PCR array differs from the one identified in the literature review. This choice was made in discussion with the array manufacturer and the laboratory in which the assays were undertaken.

Oral cancer

In the pathway of oral cancer, we chose to investigate the expression of the following genes:

p16/INK4A (through loss of expression). In developing the array, we chose *CDKN2A* (cyclin dependent kinase, tumor suppressor gene). *CDKN2A*, located on chromosome 9p21, encodes two distinct proteins: *p16 INK4A* and *p14 ARF*.

EGFR (through overexpression).

c-MYC/n-MYC (through overexpression). *n-MYC* interactor (*NMI*) encodes a protein that interacts with *n-MYC* and *c-MYC* (two members of the oncogene Myc family). *NMI* was selected as representing *c-MYC* and *n-MYC*.

STAT3 (through expression) (69).

Lung cancer

Non-small-cell lung cancer (NSCLC) represents a heterogeneous group of cancers, consisting mainly of squamous cell (SCC), adeno (AC) and large-cell carcinoma. However, only the pathway of SCLC could be tracked through gene expression. We selected the gene *BCL2* which tracks SCLC pathway (through overexpression). In addition, the gene *BAK1* was selected because it encodes the Bcl-2 homologous antagonist/killer protein, a member of the BCL2 protein family whose function is to regulate apoptosis (69).

Pancreatic cancer

We selected the following gene *ERBB2* [HSA:2064] [KO:K05083] that could be tracked through gene expression as it has been found that it is involved in the pathway of pancreatic cancer (through overexpression).

Esophageal cancer

To assess esophageal cancer as a possible long-term outcome for SS we have selected the following genes.

EGFR (overexpression).

iNOS also known as *NOS2* (increased expression).

PTGS2 (also known as *COX2*) (overexpression).

FAS (increased expression) (69).

A correlation between up regulation of *BCL2* and esophageal cancer was found by McCabe (70). As discussed above for lung cancer, we also selected *BAK1* expression because it is likely to affect *BCL2* protein levels and apoptosis.

Breast cancer

Through the gene expression pathway, we selected the following genes:

c-MYC (through overexpression) . As explained above for oral cancer, *NMI* was selected as representing *c-MYC*.

ERBB2 (through overexpression).

CCND1 (through overexpression) (69).

Bladder cancer

We selected two genes involved in the pathway of bladder cancer through an alteration of the expression:

EGFR (through overexpression) and *ERBB2* (through overexpression) (69).

Nasopharyngeal cancer

We selected one gene involved in the pathway of nasopharyngeal cancer through an alteration of the expression:

BCL2 (through overexpression). As explained above for lung cancer, *BAK1* was selected as it is likely to affect *BCL2* function.

Selection of genes related to xenobiotic metabolism

The body eliminates potentially harmful compounds through sets of chemical reactions collectively known as xenobiotic metabolism. These reactions are controlled by enzymes encoded by xenobiotic metabolizing genes. Determining the expression of xenobiotic genes in individuals is a potential means of estimating their risk of developing tumors when exposed to harmful substances.

The xenobiotic detoxification process has been considered earlier to comprise two phases. However, recent studies tend not to differentiate between these two phases because the classification of the functional roles of the genes involved has become more complex (71, 72).

From genes of xenobiotic metabolism we have selected *CYP1A1*, *CYP1B1*, *GSTP1*, *GSTT1* and *GSTM2* (65). Available evidence supports the significant contribution of these genes in the carcinogen metabolism machinery. On the one hand, expression of these genes is induced by exposure to carcinogenic agent. On the other hand, they are highly expressed in cancer tissues such as breast, colon, lung, brain and testicular cancer. Expression of these genes have been previously studied to assess the response of tissue to carcinogenic exposures such as cigarette smoke (73).

From xenobiotic genes we have selected some genes previously found to be affected by cigarette smoking such as;

CYP1A1 [through overexpression]

Location : 15q24.1

Sequence :

Chromosome: 15; NC_000015.9 (75011883..75017877, complement)

This gene, *CYP1A1*, encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and its expression is induced by some polycyclic aromatic hydrocarbons (PAHs). The gene has been associated with lung cancer risk (74).

CYP1B1 cytochrome P450, family 1, subfamily B, polypeptide 1 [*Homo sapiens*]

Location : 2p22.2

Sequence :

Chromosome: 2; NC_000002.11 (38294746..38303323, complement)

Similar to *CYP1A1*, the *CYP1B1* gene encodes a member of the cytochrome P450 superfamily of enzymes. The enzyme encoded by this gene localizes to the endoplasmic reticulum and metabolizes pro-carcinogens such as polycyclic aromatic hydrocarbons and 17beta-estradiol. Previous articles have reported association between the expression of this gene and certain types of cancer, notably of the breast and lung (75-77).

Glutathione S-transferases (*GSTs*) are a family of enzymes that play an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. We selected these genes for three reasons. First,

human *GSTs* have an important role in human carcinogenesis (66). Second, they have previously been studied in assessing the effect of cigarette smoking on gene expression (78). Third, most of the xenobiotic metabolizing genes have been found to respond by overexpression as a result of exposure to cigarette smoking (78).

1. *GSTM3* glutathione S-transferase mu 3 [*Homo sapiens*]

Location : 1p13.3

Sequence :

Chromosome: 1; NC_000001.10 (110276554..110283660, complement

The gene *GSTM2* was selected to represent *GSTM3* in the array.

2. *GSTP1* glutathione S-transferase pi 1 [*Homo sapiens*]

Location : 11q13

Sequence :

Chromosome: 11; NC_000011.9 (67351066..67354124

3. *GSTT1* glutathione S-transferase theta 1 [*Homo sapiens*]

Location : 22q11.23

Sequence :

Chromosome: 22; NC_000022.10 (24376139..24384284, complement

Housekeeping genes encode for proteins that are constantly required by all the cells in a body to maintain their basic function. As such, they are always expressed irrespective of cell's condition. In our study we have selected two housekeeping genes, the gene encoding beta-actin (*ACTB*) and that encoding glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). These genes have been frequently used, in previous studies to normalize changes in expression of specific genes, because they are expressed at relatively constant levels in most normal and pathological conditions (79). Hence,

variation in expression of these genes may be an artifact of conditions of sample handling and of batch processing of arrays, and variation in the expression of other genes outside the range of variation of the housekeeping genes is typically interpreted as indicative of biological effect as distinct from artifact.

Selection of the tissue type investigated

Early signs of many diseases such as diabetes, AIDS, tuberculosis, or leukemia are usually found in the mouth (80, 81). Indeed the reciprocal effects between oral and general health, have stimulated researchers to investigate general health through the use of oral examination and biomarkers of cells found in the cheeks and saliva (65, 78, 82-84). The oral cavity is the first organ in the body that receives the inhaled amount of tobacco smoke. It is not surprising to see the wide range of adverse health effects in the mouth of tobacco smokers that are mainly attributed to tobacco smoking. Exposure to tobacco smoke alters gene expressions either through increasing or through decreasing the expression of specific genes in the epithelial cells of bronchial airway as well as in the oral mucosa (78). Moreover, a substantial proportion of DNA in saliva sample arises from lymphocytes secreted by the salivary gland. Indeed, Thied et al., 2000 showed a median of 74% (range 16%-96%) of DNA in saliva arises from lymphocytes (84). Therefore, gene expression levels in salivary cells, enabled by collection of saliva samples from sheesha smokers, could be used as non-invasive surrogate measures of the effects of SS.

Methodology

Human subjects

In this before-after comparison study, the city of Ottawa was selected to be the geographic area of study. The main inclusion criteria were that the individual was between the age of 18 and 25 and reported that they smoked sheesha. The study took place on one weekday and one weekend day in spring 2012. Participants were requested to abstain from cigarettes for minimum time of 12 hours if they were cigarette smokers. They were also asked not to take any anti-inflammatory drug (such as pain killers,

inhalers, allergy medication, etc.) for at least the day of the study and the night before. They were asked not to participate if they had toothache or other abnormal oral conditions. In addition, as recommended by Oragene for the use of their RNA collection kits, the participants were asked not to chew gum, eat or drink for at least the 30 minutes period prior to saliva sampling.

Recruitment

A snowball sampling technique was adopted. Participants, who were recruited from the University of Ottawa (uOttawa) -during the pilot survey for chapter 4- and identified themselves as potential candidates for the gene expression part of the study, were contacted and were asked to invite their peers for the study. Five participants came accompanied with their friends. In total 16 volunteers were identified to be eligible for this study. The nature of the project was explained and detailed information regarding the questionnaire and the gene expression were given for those who were eligible to participate in this study. The assurance of anonymity was provided, and participants were asked to read and sign information sheet and consent form regarding the questionnaire and saliva sampling. They were also asked to respond freely and truthfully to each question. All questionnaires, information sheets and consent forms were available in French and English (appendices A and B).

Data Collection on the oral health and the drug intake

Participants were asked to fill out two separate questionnaires, the primary questionnaire and the oral health questionnaire.

The primary questionnaire (available in appendices A and B) comprised 40 questions in a combination of open ended and multiple choice formats, and was designed primarily to enabled investigation of correlations between the characteristics of sheesha smokers and the performance of their gene expression in response to Massal smoking. The questions collected data on socio-demographic characteristics, lifestyle, beliefs about and attitudes towards sheesha, and the mode of smoking behavior. The questions on lifestyle included other psychoactive habits among sheesha smokers, such as cigarette smoking, alcohol

drinking and the use of smokeless tobacco. Participants were asked about their willingness to provide saliva samples.

An oral health questionnaire (appendices A and B) was specially developed to assess the eligibility criteria of the volunteers who were asked to provide saliva samples. It comprised 10 questions. Questions collected data on oral, dental health, overall health of the participants and their drug intake.

Saliva collection

After signing the information sheet and consent forms regarding the oral health questionnaire and saliva sample. Participants were asked to provide saliva sample according to the collections instructions provided with the Oragene•RNA kits. In brief volunteers used sugar to generate saliva and then spit into specific containers. The containers have a proprietary solution in the cap. When closed, the solution mixes with the saliva and preserves the mRNA. After providing the saliva samples participants were asked to smoke Massal (double apple = altifahtain). They were asked to provide their second saliva samples after one hour and a half of SS, as this was the average time of a SS session according to our participants (Chapter 4).

The saliva samples were collected and delivered by the researcher (HE-K) to the EndoTox Laboratory located at the uOttawa Smyth Campus. Dr. James Gomes and his laboratory staff extracted RNA from the saliva samples and undertook the array assays from which gene expression were generated using the CFX software (BioRad).

Analyses of gene expression from saliva samples

The saliva samples were collected from 16 subjects prior to smoking sheesha and after one hour and a half of smoking sheesha using RNA extraction tubes from Oragene (as per the manufacturer's protocol). During the laboratory process, samples from one subject were found to have insufficient material for analysis. We therefore excluded one subject from analysis. Therefore we were left with samples from 15 participants. Three

main steps were required to process the saliva samples, RNA extraction, cDNA synthesis, and plating (preparation of a 96-well PCR plate) to enable real time-PCR assay of expression of the genes selected *a priori* from consideration of cancer and xenobiotic metabolism pathways.

The saliva samples were first inactivated by heating them at 50⁰C for two hours prior to extracting RNA. To optimize the RNA yield from the saliva sample the sample was purified as suggested by the Oragene protocol (PD-PR-028). Three aliquots of 500μL each were transferred into three 1.5mL tubes so that a sufficient amount of RNA would be available. These tubes were then heated to 90⁰C for 15 minutes and then cooled to room temperature. A neutralizer solution supplied by the manufacturer with the sample collection kit was added and mixed thoroughly. The sample was then cooled over ice for 10 min. and then centrifuged at 13,000g for 3min. The supernatant was carefully transferred to another tube and the pellet was discarded.

The RNA was precipitated by adding two volumes of ethanol and incubating at -20⁰C for 30 min. The RNA was separated by centrifugation at 13,000g for 3 min. The precipitated RNA from all three aliquots was combined in 350μL of buffer RLT and 350μL 70% ethanol. The solution was transferred into a spin column with a collection tube. After washing the reagents with various buffers as per the RNAeasy cleanup kit protocol from Qiagen, the final RNA sample was collected in 25μL of water.

The concentration of the purified RNA was analyzed using Nanodrop. A typical yield of RNA from saliva sample was between 80-120 μg/ml. The RNA was then converted into cDNA using the RT2 First Strand kit (Qiagen) according to the manufacturer's instructions. Briefly, the mix was prepared by thawing the frozen cDNA and then the entire mixture was heated at 42⁰C after adding the reverse transcription mix. The reaction was then stopped by raising the incubation temperature to 95⁰C.

The gene expression assay was conducted using a custom designed qPCR array with 17 genes arranged into a 96 well plate using RT2 Profiler PCR array (CAPH11260D) (Qiagen). The assay was completed with housekeeping genes (*ACTB* and *GAPDH*) and

template controls and all manufacturers' instructions were followed in conducting the assay using CFX96 qPCR (BioRad).

The data was accrued using the data acquisition system in CFX96 and was analyzed using the CFX software (BioRad). Expression of individual genes was expressed after normalization to the housekeeping genes and adjusting for the negative and positive controls. For each subject there were two samples - before and after sheesha. The expression of genes before and after sheesha was examined by using the gene study protocol from CFX96. Raw data were also collected and linked to Qiagen data analyses software for analyses of gene expression using a different protocol; and change in gene expression before and after sheesha was examined. Results from EndoTox lab reported the fold changes for the selected genes for each of the 15 subjects are presented in appendix C.

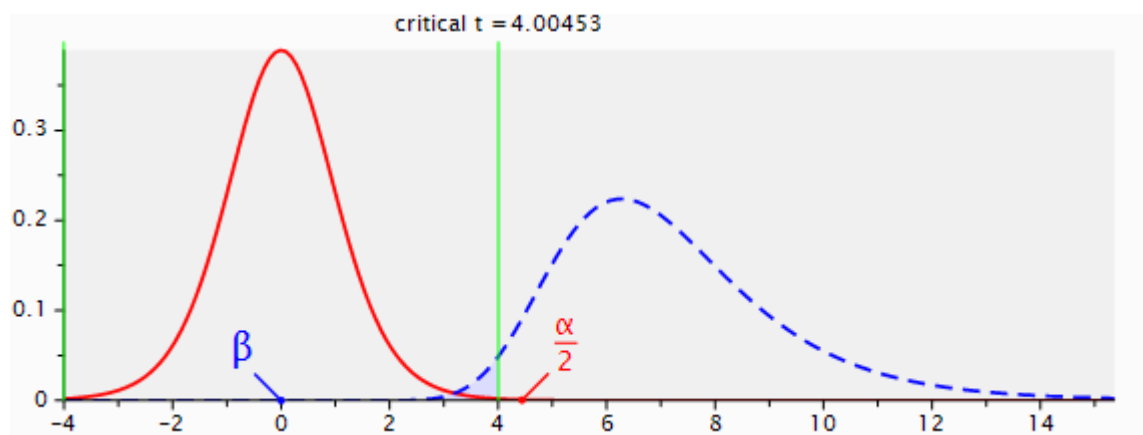
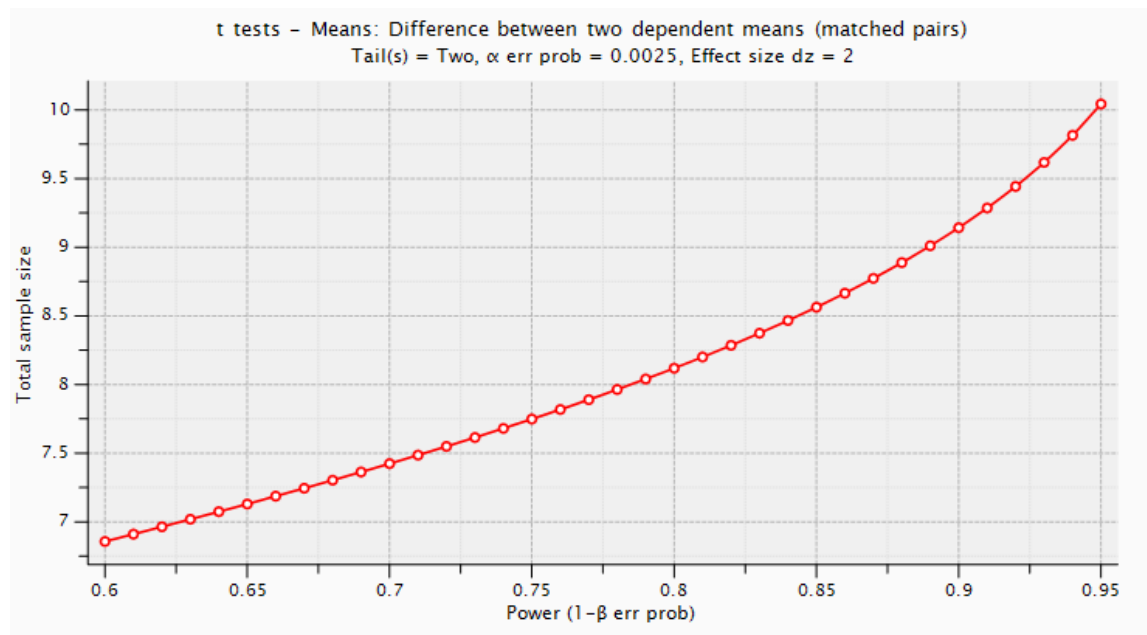
Sample size calculation

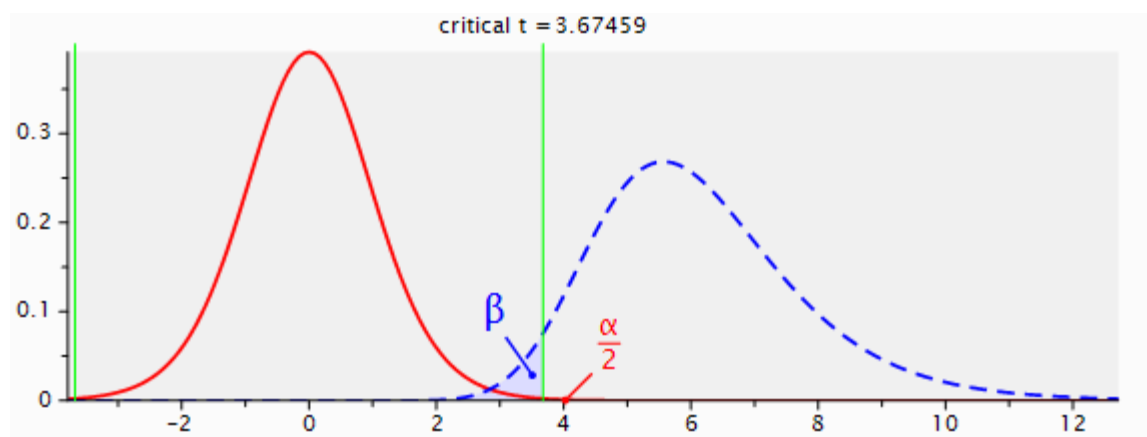
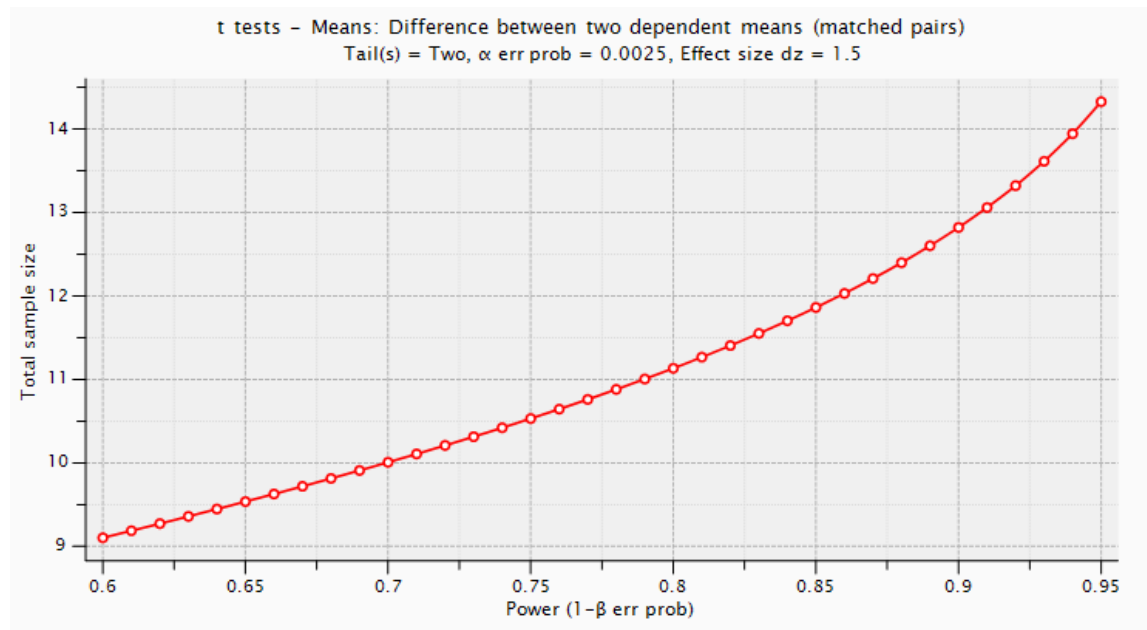
When we designed the study, we planned to use the t test to assess the effect of smoking on gene expression. We considered two effect sizes of sheesha on gene expression, first $\delta = 2$ as utilized by Lin et al., (85) second $\delta = 1.5$ from Gumus et al., (83). In the latter study (83), the effect of tobacco smoke on oral leukoplakia cell transcriptome was investigated by assessing buccal mucosal specimens from nine cigarette smokers and nine never smokers (with a history of smoking at least ten packs per year; they did not define the number of years of smoking). Therefore, this effect size was assumed to represent the long-term effect of cigarette smoking on gene expression.

To be more conservative we divided $\alpha/20$ as recommended by Bonferroni $\alpha = 0.05/20 = 0.0025$ (86).

Thus, considering both effect sizes first δ (2 or 1.5), we planned a priori to have more than 95% power from a sample size of 15 participants.

Figure 3 Correlation between power and sample size for t-test





Statistical analysis

Since planning the study, we have observed that the majority of recent published gene expression analyses have been based on normalized data by using housekeeping genes. This has in part been driven by the software used to do preliminary processing of the optical density data generated by the chemistry of the RT-PCR assay of the arrays. The softwares typically used recently to generate initial results from the assays (one example of which was used in this study) also generate fold-changes between the experimental condition (here after SS) and the reference condition (here before SS). *T*-tests are not applicable to such data since the assumptions for the *t*-test are violated. Furthermore, as has become standard practice in gene expression analysis, the assays were conducted in triplicate. We therefore decided to use average fold change weighted by the inverse of the variance of the biological samples results for each individual. This analytical approach is similar to that typically used for the meta-analysis of aggregate data in gene expression. It is an established method that integrates the results “effect size” across studies with average effect size obtained by weighting each “effect size” by its inverse variance. In this study, the effect size is the fold change that weighted by the inverse variance, as recommended by Sanchez et al., 2008 (87).

Thus, the expression of genes after smoking was compared to that before smoking sheesha. A reading of fold change equal one indicates that there is no change in the expression of selected gene. A less than one fold change indicates a decrease in the expression of the selected gene in saliva sample after smoking sheesha in comparison to that before smoking sheesha. However a greater than one fold change indicates an increase in the expression of the selected gene in saliva after smoking sheesha in comparison to the expression of the same gene in saliva before smoking sheesha. It was assumed that the expression of the two housekeeping genes would be unaffected by exposure and therefore their fold changes would be around one.

Results

Characteristics of participants

The characteristics of the participants are shown in Table 4. The age of the participants was in a range between 18 and 21 years. The female/male proportion was 6/9. Two of the participants were of Canadian origin, nine from the Middle Eastern region (one participant did not specify this precisely, but was inferred to be from that region on the basis of other information), one from Eastern Europe and two from West Africa. Only two participants completed the questionnaire in French; the others completed it in English. Nine out of fifteen participants had tried cigarettes at least once in their lifetime, but only one of them reported having become a regular cigarette smoker. SS is a habit that is practiced among our participants periodically: weekly, monthly or daily. Four out of fifteen are not regular sheesha smoker. The number of years of smoking sheesha ranged between 1 and 11 years. Our participants were not regular alcohol consumers; ten of them do not drink alcohol and the rest drink alcohol monthly.

The participants had good health, and the majority had been to a dentist within the past year (Table 5).

Table 4 Characteristics of sheesha smokers

Participants ID	Age	Sex	Background	Smoked Cigarettes	Description of alcohol consumption	# of years for smoking sheesha	Regular sheesha smoker
SF1	18	M	MEA	Yes	Do not drink	1	No
NA2	18	M	MEA	No	Do not drink	11	Yes
TB3	21	M	MEA	No	Do not drink	3	Yes
RR5	21	M	MEA	Yes	Do not drink	3	Yes
DT6	19	F	MEA	No	Do not drink	4	Yes
HE7	19	M	MEA	No	Do not drink	2	Yes
SM8	19	M	Canada	Yes	Every month	4	Yes
PP9	18	M	Canada	Yes	Every month	3	No
PB10	20	M	West Africa	No	Every month	2	No
KH11	18	F	West Africa	Yes	Do not drink	1	No
CD12	19	F	Europe	Yes	Every month	5	Yes
KB13	20	M	MEA	Yes	Every month	6	Yes
SAH15	20	F	MEA	Yes	Do not drink	2	Yes
SA16	19	F	MEA	No	Do not drink	7	Yes
AA17	18	F	MEA	Yes	Do not drink	1	Yes

Table 5 Oral health and reported medication used by participants

	Toothache	Bleeding gum	Dental treatment	see dentist	Currently taking any medication
SF1	No	No	No	2-3 months ago	No
NA2	No	Rarely	Yes	2-4 weeks ago	No
TB3	No	No	No	7-12 months ago	No
RR5	No	No	No	More than 12 months ago	No
DT6	No	No	No	4-6 months ago	No
HE7	No	No	No	4-6 months ago	No
SM8	No	Yes	No	4-6 months ago	No
PP9	No	No	Yes	2-3 months ago	No
PB10	No	Rarely	No	4-6 months ago	No
KH11	No	No	No	2-3 months ago	No
CD12	No	No	No	7-12 months ago	No
KB13	No	Rarely	No	2-4 months ago	No
SAH15	No	Rarely	No	More than 12 months ago	No
SA16	No	No	No	More than 12 months ago	No
AA17	No	No	No	More than 12 months ago	No

Results of statistical analysis

Fold changes in gene expression after as compared to before sheesha smoking are presented in Table 6. We used the weighted average method to integrate the results for each gene across the three replicate assays of each sample from the 15 participants. Thus, the analysis was weighted by the inverse of the variance between replicate assays for each sample. Fold-changes with large CIs have a lower weight, while the fold changes with small CIs have a larger weight.

The fold changes of housekeeping genes are: 0.153 for *GAPDH* with 95% CI between 0.137 & 0.169 and 0.739 for *ACTB* with 95% CI between 0.688 & 0.790. The fold changes across the 15 samples of genes selected from the cancer pathways ranged between: 0.018 for *ERBB2* and 0.604 for *STAT3*. The effect of sheesha smoking on the expression of genes related to xenobiotic metabolisms resulted in fold changes that ranged between 0.048 for *GSTT1* and 0.213 for *GSTP1*. In detail, the four genes *STAT3*, *PTGS2*, *CYP1A1* and *GSTP1* show fold changes that lie within the variance of fold change of housekeeping genes. However the rest of genes *CDKN2A*, *NMI*, *BAK1*, *ERBB2*, *EGFR*, *NOS2*, *FAS*, *CCDN1*, *CYP1B1*, *GSTT1*, and *GSTM2* present fold changes that are outside the range of variability observed for the housekeeping genes.

Table 6 Meta-analysis of fold changes with 95 % CI in gene expression for each gene across the 15 samples

96-Well Custom PCR Array Template						
#	Gene whose expression has been associated with smoking-related cancer	Gene selected for inclusion in array	Pathway through	Meta-Analysis of fold changes of each gene	95% CI	
					Lower bound	Upper bound
<i>Cancer pathway genes</i>						
1	<i>p16</i>	<i>CDKN2A</i>	Loss of expression	0.029	0.010	0.048
2	<i>INK4A</i>	<i>CDKN2A</i>	Loss of expression	0.029	0.010	0.048
3	<i>c-MYC</i>	<i>NMI</i>	Over expression	0.115	0.076	0.153
4	<i>n-MYC</i>	<i>NMI</i>	Over expression	0.115	0.076	0.153
5	<i>STAT3</i>	<i>STAT3</i>	Expression	0.604	0.531	0.676
6	<i>BCL2</i>	<i>BAK1</i>	Over expression	0.063	0.045	0.081
7	<i>ERBB2</i>	<i>ERBB2</i>	Over expression	0.018	-0.010	0.046
8	<i>EGFR</i>	<i>EGFR</i>	Over expression	0.019	0.002	0.036
9	<i>NOS2</i>	<i>NOS2</i>	Increase expression	0.046	0.021	0.070
10	<i>COX2</i>	<i>PTGS2</i>	Over expression	0.576	0.526	0.626
11	<i>FAS</i>	<i>FAS</i>	Increase expression	0.092	0.063	0.121
12	<i>CCND1</i>	<i>CCND1</i>	Over expression	0.065	0.031	0.100
13	<i>CNTD1</i>	<i>CNTD1</i>	Suggested by the Lab	0.314	0.080	0.547
<i>Genes involved in xenobiotic metabolism</i>						
14	<i>CYP1B1</i>	<i>CYP1B1</i>	Alter expression	0.147	0.001	0.292
15	<i>CYP1A1</i>	<i>CYP1A1</i>	Alter expression	0.174	0.102	0.246
16	<i>GSTP1</i>	<i>GSTP1</i>	Alter expression	0.213	0.139	0.286
17	<i>GSTT1</i>	<i>GSTT1</i>	Alter expression	0.048	0.034	0.062
18	<i>GSTM3</i>	<i>GSTM2</i>	Alter expression	0.101	0.048	0.154
<i>Housekeeping genes</i>						
19	<i>GAPDH</i>	<i>GAPDH</i>	Housekeeping	0.153	0.137	0.169
20	<i>ACTB</i>	<i>ACTB</i>	Housekeeping	0.739	0.688	0.790

Discussion

All 16 genes investigated showed significant decreases in gene expression with substantial differences in the magnitude of the decrease. The fold change between before and after an hour and a half of smoking sheesha ranged between 0.018 and 0.604, that is, between a 1.655-fold and 55-fold reduction in expression level.

Previous studies have investigated the impact of cigarette smoking on gene expression. All but one assessed the long-term effect of cigarette smoking on gene expression and showed significant **increases** in the expression of xenobiotic genes compared with controls who had never smoked using blood or oral mucosal samples from leukoplakia (78, 83, 88)

Only one study (65) considered the short-term effect of cigarette smoking on gene expression. Changes in expression of two genes involved in xenobiotic metabolism, *CYP1B1* and *GSTP1*, were investigated in exfoliated buccal mucosal cells in a single individual. In this never smoker, after smoking 4 cigarettes during a six-hour time period, *CYP1B1* expression increased to reach a peak level after 6 hours and then the level of expression gradually returned to the starting level after 18 hours. By contrast, in the same never smoker, *GSTP1* showed a slight decrease in expression over the 6-hour smoking period, followed by an increase to exceed the starting point, and then a gradual decrease to the starting level after 18 hours (65). Of note, our study differs from that of Spivack et al., (65) in that included more than a single individual, studied gene expression in cells of a different type, and included a broader range of genes, including housekeeping genes.

Patients with oral cancer lose the expression of *p16/INK4A* (69, 89). Hence, at first sight, it is somewhat concerning that sheesha smoke is associated with a reduction in expression of *p16/INK4A* gene which is correlated with oral cancer. Cody et al., 1999 (89) shows that approximately one third of all oral cancers have lost the expression of *p16/INK4A* gene. However, this has to be set against the overall pattern of findings that point to reduction in gene expression of all of the genes including the housekeeping genes.

Overall, this first study on the short-term effects of SS in young adults, shows an overall pattern of reduced expression of cancer related genes in salivary cells. However, further investigation on all the selected genes is recommended in order to understand and differentiate well between the short-term and long-term effects of exposure on the performance of these genes.

Strengths and limitations

So far as I am aware, this is the first study of gene expression change associated with sheesha use. Additional novel aspects are the focus on acute effects, the use of salivary cells, and the approach to selecting genes for investigation.

Sixteen genes have been selected for this study to assess the effect of SS on salivary gene expression. They have been selected based on the fact that they are affected by cigarette smoking as well as they are in the pathways of cancer diseases (90-95). We also included genes of xenobiotic metabolism in our investigation because cigarette smoking is known to be associated with altered expression of xenobiotic metabolizing enzymes including *CYP1A1*, *CYP1B1*, *GSTP1*, *GSTT1*, and *GSTM3* (66, 78, 83, 88, 96, 97).

Another strength of the study lies in the setting and design. The origin of participants is a good example. Our participants represented the typical community of sheesha smokers in Ottawa. We have ten participants from Middle Eastern region, two Canadian, one from East Europe and two from West Africa. The before - after comparison of the effects of SS enabled us to exclude inter-individual variation as an explanation of differences in gene expression.

A limitation is that to make the study feasible within the constraints of undertaking a master's degree thesis, only the expression of genes before and after an hour and a half of SS was considered. Serial samples to investigate the time duration of effect were not taken, but the only previous study of serial sample in a cigarette smoker of which we were aware was based only on a single participant (65).

We assessed the short-term effect of SS on gene expression and not the long-term effect, due to the novelty of the issue. Most of the participants reported having smoked sheesha for less than 5 years. One of them also reported being a habitual cigarette smoker. Investigation of the long-term effect of SS on gene expression would require a study with appropriate design, such as a cohort study (or less optimally, a cross-sectional study) comparing gene-expression in exposed and non-exposed individuals, and a bigger sample size.

We focused on genes whose expressions have been found to be altered in cancer in this study. Of note, since we planned the study, the number of types of cancer for which evidence that tobacco smoking is classified as sufficient to infer causation by IARC has increased – now colorectal, ovarian and childhood cancer have been added (98). In addition, smoking is related to many chronic diseases such as chronic obstructive pulmonary disease, heart disease and other acute diseases, and therefore we suggest a more comprehensive approach in future work.

We focused on gene expression to make our study feasible. We recognize that consideration of changes at multiple cellular levels, such as gene methylation, gene deletion and gene mutation, is likely to be relevant to disease occurrence.

GAPDH and *ACTB* are housekeeping genes and therefore were not expected to show substantial fold changes. Both of them were found to have a significant decrease in gene expression with fold changes of 0.153 for *GAPDH* with 95% CI 0.137 & 0.169 and a fold change of 0.739 for *ACTB* with 95% CI 0.688 & 0.790. One interpretation is that changes in expression level within the range of these fold changes, as was observed for *GSTP1*, *CYP1A1*, *PTGS2* and *STAT3*, are unlikely to be a consequence of sheesha. However, although these are widely used as housekeeping genes, differences in expression level have been observed across tissue types for *GADPH* (99). Somatic mutations in *ACTB* have recently been found to be associated with diffuse B-cell lymphoma, which also suggests greater effects of variability in this gene than hitherto identified (100). It may therefore be appropriate in future work to consider a broader range of housekeeping genes, particularly when novel tissue types (here salivary cells) are being investigated.

Chapter 3. Sheesha smoking and human cancers: a systematic review and meta-analysis

Background

Assessing the adverse health effects of sheesha tobacco smoking through systematic review (SR) is considered to be a key component of this project. Another component of the project focuses on gene expression, the investigation of which is increasingly being used as expression levels or profiles may prove to be effective surrogate measures to predict the side effects of some exposures. A difficulty is prioritizing the genes for which to measure expression, because not all the pathways of the diseases have been documented and pathogenesis involves many steps, such as gene methylation, gene expression and mutation. Sheesha has been in the culture of Asian and Middle Eastern people for a long time. Some studies have been conducted in those populations to assess the effects of SS on health outcomes. We wanted to evaluate and synthesize this work, in order to inform the selection of genes in our gene expression analysis (ideally to focus on genes that had altered expression in cancers associated with SS) and to consider the complementarity of the epidemiological evidence base with the results of the gene expression part of the study.

As mentioned in Chapter 1, a few SRs have addressed the health effects of sheesha tobacco smoking within a limited scope, focusing mainly on cardiorespiratory disorders (25, 29) or lung function (57). Akl et al., (16) conducted a comprehensive SR and meta-analyses to assess the effects of sheesha on multiple health outcomes. In appendix D, we present a summary of the results of Akl et al., (16), together with a critical appraisal of their work. Briefly, in considering exposure, for some articles, Akl et al., (16) categorized combined exposure to sheesha and other methods of smoking such as use of cigars or pipes as “non cigarettes type of smoke”. In some of the included studies, most of participants were at a high risk of developing cancer diseases due to occupational hazards. Moreover, unlike the pattern of use in Canada and other developed countries, most of the tobacco used in these studies was unprocessed and burned directly by

charcoal, rather than indirectly heated. Most of the studies were assessed as providing a low quality of evidence. Therefore, the applicability of their results to the consideration of the specific health effects of sheesha, which is the focus of our investigation, is questionable. In addition, since 2008 (when Akl et al., (16) conducted their literature searches), more than 50 articles have been published concerning the health effects of sheesha. Therefore, we carried out a new SR and meta-analyses to assess the impact of sheesha on human cancers by applying new protocol. We modified the inclusion and exclusion criteria used by Akl et al., (16) in order to improve the specificity of the results to SS.

Objective

The aim of this SR was to assess critically the associations between cancers of all types of human cancers and SS, and synthesize the results by meta-analysis.

Research Question

Are sheesha smokers at a higher risk of developing cancer in comparison to non-smokers?

Population

Our population are sheesha smokers in an age range from young adult to seniors. The idea behind the wide range of age is that previous studies that assessed the link between cigarette smoking and developing cancer revealed that there is a long time lapse between starting regular smoking and developing cancers. We excluded studies of occupational groups known to have high risk of cancer, notably underground miners known to have very high levels of exposure to radon.

Exposure, sheesha tobacco smoking

We are interested in assessing the carcinogenic effects of SS on humans. Only studies in which exposure to sheesha could be distinguished from other type of exposure to tobacco smoke are considered in this review.

Comparator: non-smokers

Non-smokers are the comparator. We defined non-smokers to comprise former smokers, those who stopped smoking for at least one year prior to the investigation, and never smokers. Never smokers were those who did not use any type of smoking material such as sheesha, cigarettes or pipes.

Outcomes

We searched for all type of cancers in humans.

Studies

Observational studies such as cohort studies, case-control studies and cross-sectional studies were sought.

Methodology

Eligibility criteria

Inclusion criteria

We included observational studies that examined the association between SS and human cancers, such as cohort studies, case-control studies and cross-sectional studies. Potentially eligible studies had to include a group of individuals smoking sheesha exclusively. We considered publications with raw data.

Exclusion criteria

We excluded case reports, case series, outbreak investigations, studies published as abstracts, and studies done on animals. We also excluded studies in which the effects of sheesha could not be distinguished from other types of smoking or other exposures (such as occupational hazards), studies where in which sheesha was used to smoke cannabis or other drugs, or when the outcome was a mediator such as Carcino-Embryonic Antigen (CEA) levels. We excluded articles published in languages other than English, French or Arabic.

Search strategy

On Oct 19, 2011, we ran the list of keywords for “waterpipe” (appendix E) prepared by Akl et al., (16) article, in the four databases “Medline, Embase, Pubmed, and Global Health”, and then we looked at references to track other synonyms for sheesha. One more keyword term (Argyle) was identified and added to our list to be used in our strategy. The list of key words, regarding the outcome, included cancer* OR neoplasm* OR tumor* OR tumour* OR carcinoma* OR Cancer. We reviewed the reference lists of the articles identified from these searches to check for other potentially relevant papers not identified in the electronic databases. We searched Proquest Dissertations and Theses to identify grey literature, specifically unpublished academic work.

Selection process

Two reviewers independently screened titles and abstracts resulting from the search to identify potentially eligible articles. We obtained the full text of citation for articles considered as potentially eligible by at least one of the two reviewers. Later, the two reviewers independently screened the full texts for eligibility. Hereby, we evaluated inter-reviewer agreement, while disagreements were resolved by discussion or by a third reviewer.

Data abstraction

One reviewer abstracted data from all of the included studies in the eligible folder using a pre-prepared data abstraction form (appendix F). A second reviewer checked data abstraction. They resolved disagreements with the help of a third reviewer.

The abstracted data included information about:

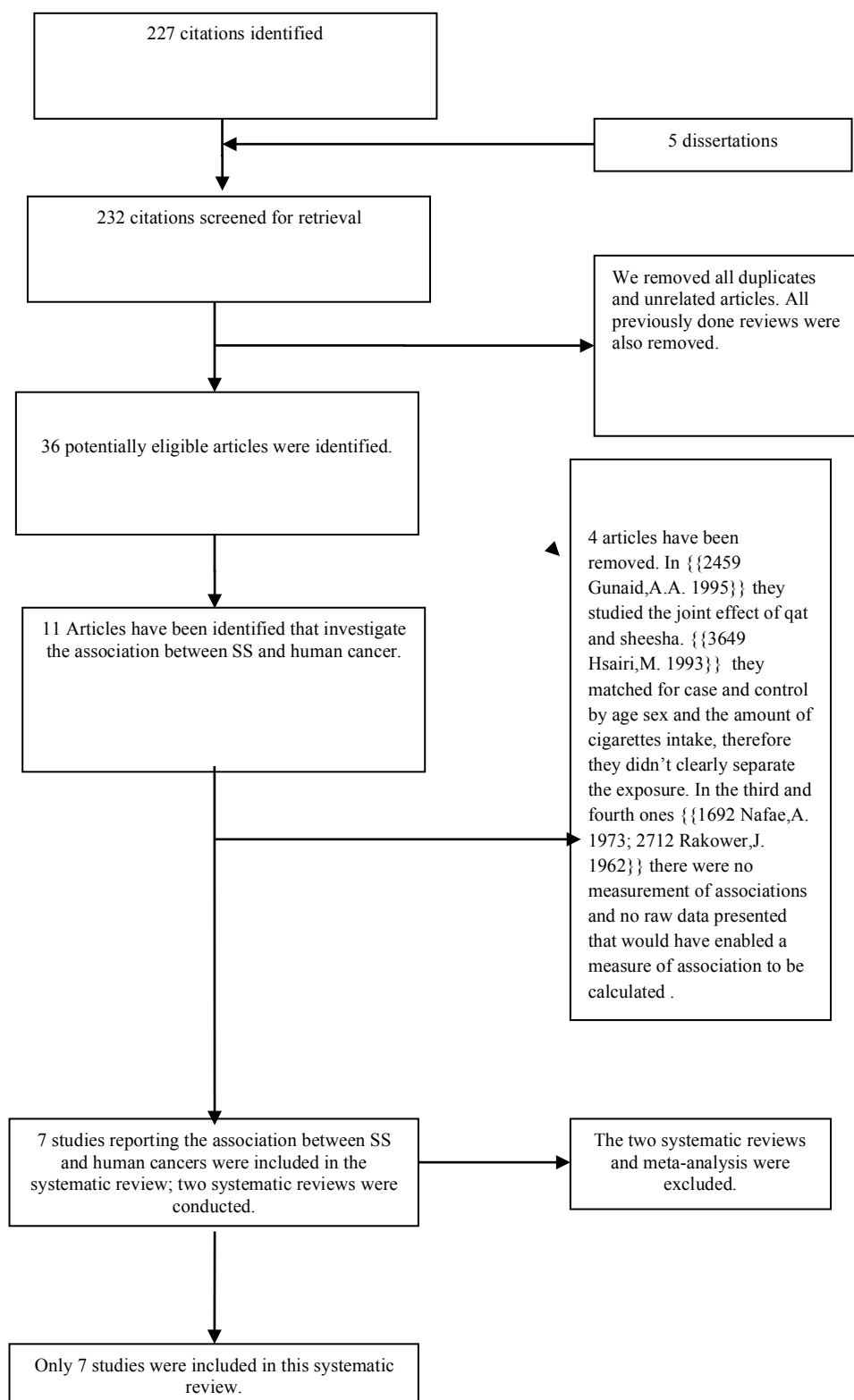
- A. Study design and funding.
- B. Population: setting and period, and participants' characteristics.

- C. Exposure: type, measurement tool, definition of non-smokers and exposure levels of participants.
- D. Outcomes: measurement tools.
- E. Methodologic features: handling of confounding, participation rate, and missing data.
- F. Statistical results: presenting the OR, Pooled data.

Data analysis

We conducted a new SR and meta-analysis to assess the impact of sheesha on human cancers. We pooled data for each cancer separately. The limitation of the included studies did not allow us to run sensitivity analysis. We tested results for homogeneity across studies using I^2 test and used the following interpretation of the value of I^2 : 0% to 49%, heterogeneity might not be important; 50% to 79%, moderate heterogeneity; 80% to 100%, severe heterogeneity. Pooled results were reported for meta-analyses of all I^2 value. When I^2 indicated moderate or severe level of heterogeneity possible explanatory factors was explored. Differences across included studies were assessed such as time period, population characteristic and methodological issues.

We applied Downs and Black's checklist (101) to assess the methodological quality of included studies.

Figure 4 The study flow diagram

Results

We identified 227 citations: 85 articles from Medline databases, 108 articles from Embase, 28 articles from Global Health, one article from Ovid and 5 articles from CINAHL. We removed duplicate citations and articles that were not related to SS such as environmental, electrical, viral, research and some articles related to inducing anaesthesia in children. Among 227 peer-reviewed articles we identified 36 articles potentially relevant for our systematic review. Review of the reference lists of these 36 articles did not identify other potentially relevant papers. Five dissertations were retrieved. However none of these dissertations was directly related to sheesha and cancer. After retrieving the potential eligible articles, 7 met the eligibility criteria. Figure 4 shows the study flow. The main reasons for excluding articles are presented in appendix G.

Overview of included studies

The seven included studies assessed the associations between SS and lung cancer (two studies (27, 102), esophageal cancer (three studies (103-105)), nasopharyngeal cancer (one study (106)), and bladder cancer (one study (107)).

Lung cancer

The two studies of lung cancer were individually matched, hospital based, case-control studies, both of which were carried out in India. One was conducted in the Kashmir Valley (Eastern India) (27), and the other in Chandigarh (North West of India) (102) (further details, Table 7).

The study in Chandigarh (102) was based on a single hospital. We have not been able to determine if this is the only or the main hospital where all cases are referred for treatment. Cases were confirmed by cytological or histological examination of the

material obtained from the primary site or metastatic lymph node/pleural fluid with an obvious primary lesion in the lungs detected radiologically. Two hospital controls were selected for each patient from among the visitors and attendants. The study in the Kashmir Valley was based on the main referral centre for the pulmonary and oncology cases for the region, yet they didn't reported the participation rate of potentially eligible cases (27). Cases were histologically confirmed. It was stated that controls were generally relatives of the patients; no further information on control selection was provided.

In the Chandigarh study (102) it was stated that smokers used a mixture of crude tobacco and jaggery "coarse, unrefined sugar" on the head of the sheesha. No other information on the nature of sheesha exposure was provided. Data collection was done by trained personnel who interviewed subjects in the hospital. It is not stated if the interview schedule had been validated.

In the Kashmir Valley study (27) sheesha "Jajeer" was documented as containing processed tobacco mixed with molasses. It was stated that the head, when filled with the mixture, is lit with live charcoal, but it was not specified whether the mixture was separated from charcoal by using tinfoil. However, if the charcoal were in direct contact with the mixture, the head would burn quickly, so it seems likely that tinfoil separated mixture from charcoal. In the Kashmir study, data collection was done using a validated questionnaire administered by personal interview. It was not specified whether the interviewers were trained.

Definition of exposure groups

Gupta et al., (102) defined non-cigarette-smokers as individuals having exposure of less than 1 cigarette per day for less than 1 year. They did not provide an equivalent definition of non-sheesha-smokers. However, they consider sheesha to be equivalent to 4 cigarettes.

Koul et al., (27) did not define non-smokers.

Exposure level of participants

Gupta et al., (102) categorized sheesha exposure status as 1-4 sheesha sessions per day. They also presented a table showing the OR and 95 % confidence interval of lung cancer for duration of smoking in men. They presented OR and 95 % CI of lung cancer for average consumption of cigarette-equivalents per day in men. They also calculated a cumulative consumption of each tobacco product according to average consumption of cigarette-equivalents multiplied by the duration of smoking. Cigarette-equivalents were calculated by applying a weight of 1 to cigarettes, 0.5 to bidis and 4 to sheesha with no scientific support. Tobacco drawn from sheesha came from heating tobacco and molasses blend and not combusting tobacco as in the case of cigarettes smoking. Thus, the mainstream smoke from sheesha is different from that of cigarettes in its components and characteristics. Therefore the combination of exposure used in this study lost information on the different types of exposure.

Koul et al., (27) categorized sheesha exposure status into exclusive sheesha smokers.

Treatment of potential confounding factors

Gupta et al., (102) selected their controls to match their cases by age and sex. Results were adjusted for age and education. Other potential confounding factors, including other forms of tobacco consumption, were considered but no adjustment was made for any of them.

Koul et al., (27) matched for age and sex. It was stated that logistic regression was used in analysis, but the factors adjusted for were not specified.

Overall, there was a significant positive association between SS and developing lung cancer (pooled OR 5.03, 95% CI 3.57-7.09), with moderate heterogeneity ($I^2 = 71\%$). The OR was somewhat higher than that observed by Akl et al., (16) (OR 2.12, 95% CI 1.32-3.42). The study of Gupta et al. (102) was included by Akl et al., (16) plus five other studies that didn't meet our inclusion criteria. Akl et al., (16) did not include the study of Koul et al., (27) as it was published in 2011.

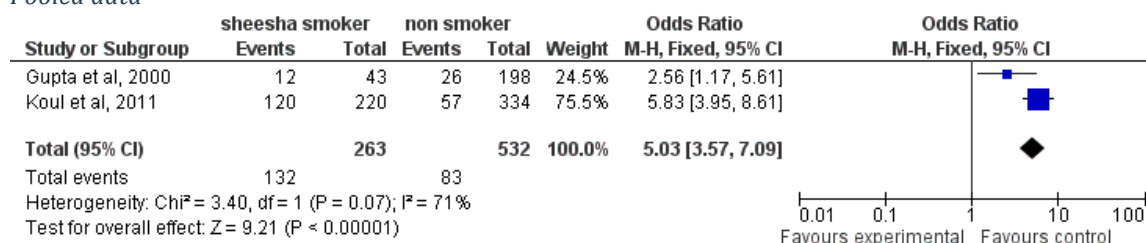
The OR for the association between SS and lung cancer in Kashmir valley was approximately two times higher than that found in Chandigarh. Previous studies showed that Kashmiri people have high incidence rates of all cancers. Kashmir is the northwestern region of the Indian subcontinent. Its economy depends on agriculture, and the per capita income in the state of Jammu and Kashmir is low (108). Many inhabitants of the Kashmir Valley consume hot tea (109) in a way that differs from other parts of the world in that it is made by boiling a mixture of water, tea, salt, spices and a pinch of sodium bicarbonate for more than one hour and then sipped while extremely hot; this might increase their risk of cancers (110). By contrast, Chandigarh, located in northern India, is known as "The City Beautiful". The city was reported in 2010 to be the "cleanest" in India, based on a national government study. Chandigarh has high per capita incomes, second only to Goa in India (109). These differences might contribute to the differences in ORs found between residence of Kashmir valley and those of Chandigarh. Apart from the difference in settings, there is a 10-year time difference between these studies which might contribute to this difference in OR. Moreover, Gupta et al., (102) provided information on risks associated with different levels of exposure to sheesha smoking e.g., the OR for developing lung cancer with the exposure of 1-4 sheesha sessions per day is (OR 1.94, 95% CI 0.85-4.44), the overall OR of developing cancer while smoking sheesha is (OR 2.56, 95% CI 1.17-5.61). In contrast, Koul et al., (27) did not specify exposure so precisely. For exclusive sheesha smokers OR of having lung cancer is (5.83, 95% CI 3.95-8.6). This might also contribute to the difference in OR seen between these two studies.

Table 7 Characteristics of the included studies assessing the effects of SS on lung cancer

Study	Population Place/Period	Exposure / Comparison / Measurement tools
- Gupta 2000 Matched case-control study. - Funding sources: International agency for research on cancer , Lyon France, and the International Cancer related technology transfer fellowship award.	- Incident cases recruited from the Department of Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh. 265 patients 235 males and 30 females - Controls: 435 male and 90 female visitors and attendants of the patients; two controls per case. - In Chandigarh India, Northern India - From January 1995 to June 1997.	- Type: SS using a mixture of crude tobacco and jaggery "coarse, unrefined sugar" as filling. - Developed tool, not standardized used a not validated questionnaire. Data collection were done by trained personnel who interviewed subjects in the hospital. - They combined different tobacco products by applying as 1 to cigarette and 4 to sheesha. - Non-smokers defined as individuals having exposure to less than 1 cigarette per day for less than 1 year.
- Koul et al., 2011. Case-control study. - Funding sources: not reported.	- Incident cases of histologically proved lung cancer, based on the main referral centre for the pulmonary and oncology cases for the region, and the participation rate of potentially eligible cases was high. 251 cases, 209 males and 42 females. - Controls: 400, 328 males and 72 females - Kashmir valley of the Indian subcontinent. - From June 2005 to December 2006.	- Type: SS using "Jajeer" filling a mixture of tobacco processed with molasses. - Developed tool, standardized used validated questionnaire administered by personnel interviews. It was not specified whether the interviewers were trained. - No definition of non-smokers

Outcome	Methodological features	Results	Pooled data
• Lung cancer • Cases confirmed on cytological and histological examination of the material obtained from primary site or metastatic lymphnode /plural fluid with obvious primary lesion in the lung detected radiologically. • Blinding of adjudicator not reported	• Selection bias: hospital based incident cases, community based controls selected from the same study base as cases . • Information bias: no standardization in exposure with objective outcome evaluation, no definition for regular sheesha smokers. Non-smokers were defined as individuals having exposure of less than 1 cigarette per day for less than 1 year. • Participation rate was not reported. Missing data on 197 of exposures was reported. • Cumulative consumption was reported per years of smoke. • Missing data for 197 cases and 232 controls were reported • Confounding factors: they matched for age and sex. Results were adjusted for age and education. • Rated 28 by Downs and Black.	• OR 2.56 (95% CI 1.17-5.61)	• pooled OR 5.03, 95% CI 3.57-7.09), with (I² = 71%).
• Lung cancer • Cases confirmed by histological confirmation. • Blinding of adjudicator not reported	• Selection bias: Hospital based incident cases. Controls were generally relatives of the patients. • Information bias: no standardization in exposure. Objective outcome evaluation, no definition for regular sheesha smokers. Non smoker category was not defined. • Participation rate was not reported. • No missing data was reported. • No classification of exposure was reported. • Confounding factors: Matched for age and area of residence, adjusted for education, religion and other confounding factors. • Rated 26 by Downs and Black.	• OR=5.83 with 95% CI(3.95-8.6)	

Pooled data



Esophageal cancer

Three studies, all of which were of case-control design met our inclusion criteria, one in Iran, in which cases and controls were matched for age, sex and place of residence (103), and two in the in Kashmir valley in India, one of which had frequency matching by area of residence (equal number of cases and controls were selected from rural, urban and semi-urban settings) between cases and controls (104) and the other in which there was matching for age and sex (83). In all three studies, diagnosis was histologically confirmed. None of the studies gave information on participation rates.

The Iranian study included five cities - Gonbad, Minoodasht, Kalaleh, Azadshahr, and Ramian - in eastern Golestan province. In Gonbad City, the largest in eastern Golestan Province, participants were recruited in the Atrak Clinic, the largest in eastern Golestan Province and the only specialised clinic covering Esophageal Squamous Cell Carcinoma (ESCC) (103). In the other cities, local physicians who were asked to refer any suspected upper gastrointestinal tract cancers to the Atrak Clinic. Two population based control subjects, matched on age, sex and area of residence were sought for each case; for ~10 % only one matched control was recruited. Thus, among a total of 300 cases recruited, 271

had two matched controls and 29 had one, resulting in a total of 571 healthy controls. Information on sheesha exposure and other factors was obtained using a pretested questionnaire administered by trained interviewers.

There appears to be overlap in the time period and population between the two studies in the Kashmir Valley (104, 105). In one of these studies, cases with histologically confirmed squamous cell carcinoma of oesophagus were selected from a tertiary care institution (104), while in the other, they were recruited from the Department of Gastroenterology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, the main referral centre for the pulmonary and oncology cases in the Kashmir valley (105). In the study of Khan et al., the name of institution and whether this was the only institute in the region in which EC would be diagnosed, or whether local physicians had been instructed to refer cases to the institute was not specified. Only cases with mid esophageal involvement and histopathology of squamous cell carcinoma were included. On the one hand, this could be considered a strength in that exclusion of high or low esophageal cancer would exclude tumours that originated in the stomach or in the head and neck region, but on the other a weakness because of exclusion of genuine ESCC cases. It was stated that healthy subjects were included as controls; no other information on control selection was provided (104). In all, 100 cases and 100 controls were recruited. It was stated that a predesigned questionnaire was used to determine exposure, but there was no mention as to whether the questionnaire had been validated or the mode of administration. In the study of Malik et al., (105) cases were unrelated ethnic Kashmiri residents. Controls were selected from among medical staff and patients who came for routine check up at the Institute in which cases were ascertained, to match cases by age and sex. Selection criterion for controls included no evidence of malignancy as well as no prior family history of cancer. 135 cases and 195 controls were recruited. Information on age, occupation, demographic features, the use of hot noon chai (salted tea), the consumption of alcohol, and usage of sheesha to smoke tobacco were collected via personal interviews. It is not clear if the interview schedule was structured or validated or if the interviewers had been specifically trained. In both of the Kashmir Valley studies,

participants were categorized as having a positive or a negative smoking history, but the definitions were not specified. Cumulative exposure was not considered.

The description of exposure in each of the three studies is limited. In the Iranian study, it was stated that only the nass filling comprised tobacco, ash and lime (103). In this study, ever smokers comprised current and former smokers. Current smokers are those who had practiced any kind of smoking habit within one year before the interview. Former smokers are those who had stopped their smoking habits at least 1 year prior to the interview. In one of the studies in the Kashmir Valley, it was stated that “Hukka smoking is a type of tobacco smoking in which tobacco smoke passes through a water pipe, and it is very common in kashmiri population” (105). In the other study, neither sheesha, nor the tobacco used, was described (104). In considering the Kashmir Valley studies, we have considered the sheesha exposure to be similar to that described by Koul et al., (27) (see section on lung cancer above).

Treatment of potential confounding factors

In the Iranian study, in addition to matching cases and controls on age, sex, place of residence, adjustment was made for education (as a marker of socioeconomic status) and ethnicity (103). Khan et al., (104) provided information on the distribution of cases and controls by potential confounders such as occupation, indoor home pollution, education, family history of esophageal carcinoma, and consumption of meat, dried pickled vegetables and hot tea. No adjustment was made for any of these factors, even though the distribution of occupation, and reported consumption of fruit, meat and fish differed significantly between cases and controls. In the study of Malik et al., (105) adjustment was made for age and sex, but other factors such as consumption of hot salted tea were not taken into account.

Statistical results

Difference between ORs

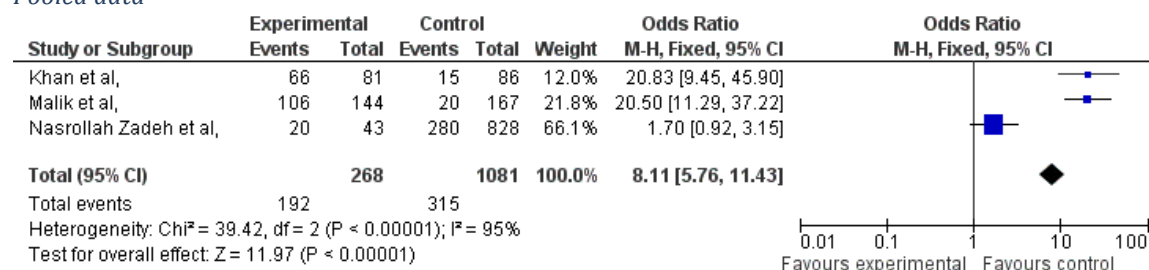
We found that the OR, of the association between SS and esophageal cancer, in Kashmir valley is higher than that found in Iran. Kashmir Valley is a place known with high incidence rates of all cancers; the society is considered to be fairly closed with unusual patterns of lifestyle exposures, notably the consumption of hot salted tea. The two articles too recent to have been included in the SR by Akl et al., (2010) (104, 105) suffered from information bias. The study conducted by Khan et al., (104) suffered from selection bias as well. Neither of these articles appeared to consider hot salted tea as a potential confounder.

Table 8 Characteristics of the included studies assessing the effects of SS on esophageal cancer

Study	Population Place/Period	Exposure / Comparison / Measurement tools
Nasrollahzadeh et al., 2008 case-control study Funding sources: funded by Digestive Disease Research Center: Tehran University of Medical Sciences (DDRC/TUMS); and the National Cancer at National Institute of Health.	Study area included Gonbad, Minoodasht, Kalleh, Azadshahr, and Raminian in eastern Golestan province of Iran. Cases: 300 esophageal squamous cell carcinoma cases, 50% males, Controls: 571 controls, two population-based matched control subjects per case for 90% of cases From December 2003 to June 2007	Type: SS, no more information on the tobacco used was provided Sheesha studied as cumulative consumption as sheesha per year with median exposure level for included subjects is 32 sheesha per year.
Khan et al., 2011. Funding sources was not mentioned.	Study area Kashmir Valley, India. Cases: 100 of mid esophageal squamous cell carcinoma. Controls: 100 healthy subjects. From January and December 2008.	Type: SS using "Jajeer" filling a mixture of tobacco processed with molasses smoking. No definition of non-smokers or smokers. Cumulative consumption not reported, only positive and negative history of smoking was reported.
Malik et al., 2010. Funding sources: funded by Indian Council of Medical Research (ICMR), New Delhi	Study area Kashmir Valley, India. Cases: 135 of esophageal cancer Controls: 195 healthy controls.	Type: SS using "Jajeer" filling a mixture of tobacco processed with molasses smoking. Current smokers are those who had practiced any kind of smoking habit within one year before the interview. Former smokers are those who had stopped their smoking habits at least 1 year prior to the interview.

Outcome	Methodological features	Results	Pooled data
- Eosophageal squamous cell carcinoma. - Measurement tool: histopathologically confirmed ESCC. - Blinding of adjudicator not reported.	- Selection bias: series of incident cases. Controls were population based. - No information bias: used validated questionnaire, through two trained interviewers. - Definition of ever-sheesha smokers: are those who smoked sheesha at least weekly for a period of 6 months or more. Current sheesha smokers are those who had smoked sheesha within 1 year before the interview. - Former smokers were those who had stopped their habit at least 1 year prior to the interview. - Participation rate not reported. - Confounding factors: matched for age, sex and residence. Adjusted for education, ethnicity and total intake of fruit and vegetables. - Rated 26 by Downs and Black. 31	OR=OR of hookah smokers compared to never smokers is 1.85 CI (0.95-3.58) OR for sheesha users for less than 3 times per day compared to never smokers is 1.31 CI (0.58-2.96). OR of sheesha smokers for more than three times per day compared to never smokers is 3.57 with CI (1.17-11.0). OR of sheesha smokers with median smoke time more than 19 years is 2.45 with CI (1.09-5.49), for a median time of smoke for less than 19 years is 1.11 CI (0.38-3.3) OR for cumulative sheesha use more than 32 sheesha per years is 2.04 with CI(0.84-4.99) OR for less than 32 sheesha per year is 1.66 with CI (0.65-4.22) OR of sheesha users who started smoking in median age less than 40 years is 2.31 with CI (0.98-5.48) for median age more than 40 years OR is 1.38 with CI (0.51-3.69).	pooled OR 5.03, 95% CI 3.57-7.09), with ($I^2 = 71\%$).
- Mid esophageal squamous cell carcinoma. - Cases confirmed by histological confirmation. - Blinding of adjudicator not reported	- Selection bias: Hospital based incident cases. No information on controls selection was provided. - Information bias Developed tool: data collection using not validated questionnaire. Mode of administration not reported. - Objective outcome evaluation - Definition for regular sheesha smokers. Non smoker category was not defined. - Participation rate not reported. - No missing data was reported. - Mentioned possible confounder ex occupation, indoor air pollution and some food... but they did not run statistical analysis. - Rated 31 by Downs and Black.	OR=20.83 with 95% CI(9.45-45.9)	
- Esophageal cancer. - Cases confirmed histologically. - Blinding of adjudicator not reported	- Selection bias: Hospital based incident cases. - Information bias: no standardized data collection and not using questionnaire. - Participation rate not reported.. - Missing data not reported. - Rated 27 by Downs and Black	OR=20.50 with 95% CI (11.29-37.22)	

Pooled data



The pooled OR is 8.11 with 95 % CI (5.76- 11.43) compared to 1.85 with 95% CI (0.2- 1.23) found by Akl et al., (16).

The I² test indicated a high of 95% heterogeneity among studies, which can in large part be attributed to selection bias, information bias and variable methods of control for potential confounders. Nonetheless, the differences might in part be attributed to the differences in place and population between Iran and the Kashmir Valley.

We have only three studies and we have enough evidence to believe that the Iranian study is of much better quality than the other two, and the other two may overlap therefore sensitivity analysis would not add value to our systematic review.

Bladder cancer

Only one article, a multicenter case-control study in the area of Greater Alexandria, Egypt during the period January 1994 - July 1996, met our inclusion criteria (106). The characteristics of this study are summarized in Table 9. Recruitment was based on a network of teaching and general hospitals. Cases were 151 male cases of histologically confirmed invasive bladder cancer aged 31 to 74 years, diagnosed during the year preceding interview. Controls were 157 men aged 32 to 74 years admitted for a wide spectrum of acute non-neoplastic, non-urinary tract, non-smoking-related conditions to the same network of hospitals and coming from the same area (Alexandria Governorate). Exposures were determined by interview by trained interviewers using a non-validated questionnaire. More than 95% of potentially eligible cases and controls were interviewed. Neither the sheesha nor the tobacco used were described in this article. The participants

were categorized as having never or ever smoked tobacco, including cigarettes, pipe, cigar, sheesha and hashish. The category of ever smokers is divided into Ex-smokers and current smokers. Cumulative exposure was reported.

Confounding factors

The increased risk for ever SS compared with never smoking (OR 2, 95 % CI 0.7-5.4) apparent on crude analysis was no longer apparent after adjustment for age, education, type of house, history of schistosomiasis and high risk occupation, plus tobacco smoking for sheesha and hashish smoking with (OR 0.8, 95% CI 0.2-4.0).

Table 9 Characteristics of included study assessing the effect of SS on bladder cancer

Study	Population Place/Period	Exposure / Comparison / Measurement tools	Outcome
• Bedwani et al., 1997 • Funding sources: funded by National Research Council • Applicazioni cliniche della ricerca oncologica (CNR-ACRO project; and Italian association for Cancer Research	• Incident cases recruited from multiple centres. • Cases: 151 male incident cases of invasive bladder cancer and 157 males as controls • In greater Alexandria, Egypt • From January 1994 to July 1996	• Type: SS • Participants categorized as ever or never sheesha smoker. • No exposure level or cumulative consumption were reported.	• Invasive bladder cancer • Cases identified by clinician in the oncology and radiotherapy department and histologically confirmed cases • Blinding of adjudicator not reported.

Methodological features	Results	Pooled data
• Selection bias: hospital based incident cases of bladder cancer confirmed within the year preceding interview, controls were hospital based controls. • Information bias: objective outcome evaluation with no standardized measurements. Participation rate was > 95 • Confounding factors all participants were male so no confounding	• OR 0.8, 95% CI 0.2-4.0	• No pooled OR

Nasopharyngeal carcinoma

One article, a matched case-control study based on five hospitals in Algeria, Morocco and Tunisia during the period January 2002 - March 2005, in which controls were frequency-matched with cases on centre, age, sex, and childhood household type (urban/rural), met our inclusion criteria (106). The characteristics of this study are summarized in Table 10. 636 cases diagnosed between 2001 and 2004 were included. Prevalent cases accounted for 25% of total cases. Cases were identified by clinicians in the oncology and radiotherapy departments of each hospital, and details of histology were sought from patient's records. Not all patients had histological confirmation of the disease. There were 615 controls, of whom 61% were hospitalized patients and the remainders were recruited from among friends and families of patients with conditions other than NPC. Exposure information was collected by interview by trained physicians. More than 90% of potentially eligible cases and controls were interviewed.

Participants were categorized as never or ever smokers; no information on the daily consumption of sheesha and age of starting smoking was collected and therefore cumulative exposure was not determined. Analysis was stratified by sex and centre with adjustment for age, SES and dietary factors. For undifferentiated NPC carcinoma, the OR of ever sheesha smokers compared to never smokers was 0.5 with 95% CI (0.2 -1.24).

Table 10 Characteristics of included study assessing the effect of SS on nasopharyngeal cancer

Study	Population Place/Period	Exposure / Comparison / Measurement tools
• Feng et al., 2009 • Funding sources: funded by the Association for International Cancer Research (grant number 03-252).	• Incident cases recruited from multiple centres. • Cases 636 cases of nasopharyngeal cancer and 615 controls from the same hosopitals as cases but not related diseases. • Not all patients histologically confirmed • In Algeria, Morocco and Tunisia. • From January 2002 to March 2005	• Type: SS • Participants categorized as ever or never sheesha smoker. • No exposure level or cumulative consumption were reported

Outcome	Methodological features	Results	Pooled data
- Nasopharyngeal carcinoma - Cases identified by clinician in the oncology and radiotherapy department. - Blinding of outcome adjudicator was not reported.	- Selection bias: series of hospital based incident cases, controls were hospital based or family and friend of cases. - Information bias; objective outcome evaluation with no standardized measurements. - Participation rate was > 90 - Confounding factors; Matched for hospital, age, sex and household type (urban or rural), adjusted for	0.49 with 95% CI (0.2 - 1.23).)	No pooled OR

Discussion

Our SR focuses more on the effect of SS solely, as we adopted different list of exclusion and inclusion criteria. Thus, we excluded all articles that combine the effect of sheesha with other form of tobacco smoking. We also excluded studies done in China among miner workers, as we are aware of the effect of radon on lung cancers. Moreover, these workers tended to be sheesha and pipe smokers simultaneously.

Since the time when Akl et al., (16) conducted their research one more article concerning SS and lung cancer have been published and two articles conducted in Kashmir Valley concerning the effect of SS on esophageal cancer. The meta-analysis revealed a statistically significant positive association between smoking sheesha and developing esophageal cancer. The result found by Akl et al., (16) was positive but not significantly associated. Concerning bladder cancer our result was the same found by Akl et al., (16) as no study on bladder and nasopharyngeal cancer with SS had been conducted since then. The OR of developing nasopharyngeal cancer for ever sheesha smokers compared to never smokers is 0.5 with 95% CI (0.2 -1.24). The same result was marked by Akl et al., (16). We applied Down and Black checklist which is considered as a validated measurement tool, used for both clinical and observational studies. Down and Black assess all the details in the study however Akl et al., (16) used the GRADE rating system, it is more generalized and dramatically reduces the rate of the observational studies. All the included studies rated relatively good in Downs and Black checklist.

Strengths

The main strength of our meta-analysis is that we followed the approach used by the Cochrane Collaboration for conducting a SR (111). We used a sensitive and comprehensive search strategy. We searched more than three data bases; Ovid MEDLINE, Embase classic and Embase from 1947 until present, Pubmed, and Global Health current on cabdirect. We searched through the grey literature by looking for any dissertation that assessed the effect of SS on developing cancer. We also reviewed the references of retrieved articles to look for any missed one. Two independent reviewers applied the inclusion and exclusion criteria on selection process and came out to an

agreement by a discussion or by the help of a third reviewer. Data abstraction was carefully processed and reviewed.

We applied more rigorous inclusion and exclusion criteria than Akl et al., (16) that reflect to some extent the population and the exposure of interest. Potentially eligible studies had to include a group of individuals smoking sheesha exclusively. Our outcome of interest was tumours in humans such as lung cancer, bladder cancer, nasopharyngeal cancer and esophageal cancer. We excluded case report, case series, outbreak investigations, studies published as abstracts, and studies done on animals. We also excluded studies not distinguishing sheesha from other type of smoke or exposure (such as occupational hazard), studies where they use the sheesha to smoke Cannabis or other drugs, or when the outcome is a mediator such as Carcino-Embryonic Antigen (CEA) levels in sheesha smokers, and not considered as a disease.

Compared with previous systematic reviews, we increased the scope of our SR by searching in three languages English, French, and Arabic. Indeed, these three languages are the dominant languages in the Middle East and North Africa, from where sheesha spread to western countries.

Limitation

Although sheesha caught the attention of many researchers lately and hundreds of articles have been published on sheesha so far. Practically, researches on sheesha are still in their preliminary phases. No new cancer related to SS had been added to those identified by Akl et al., (16). Most of the included studies suffer from many issues. Hereby we ended up with SR of such a limited quality. The association between SS and developing cancer diseases is still insignificant most of the time. The main indications of risk are from work done in India – in three out of 4 studies in the Kashmir Valley (are all three in the part of the Valley that is in India – Pakistan and China also have jurisdictions in the valley). In fact, this impaired the main aim of this SR that was to guide the choice of genes for the gene expression component or to properly interpret that component.

Chapter 4. Pilot survey on sheesha smokers

Background

A specific objective of this section of the study was to generate a framework to recruit people for the gene expression analysis part of the study as well as to obtain demographic information on SS and to document the characteristics of participants who accepted to take part in the gene expression study.

As a secondary objective we proposed to collect information on mode of SS, health concerns, beliefs, attitudes of sheesha smokers, and to assess how SS relates to other psychoactive habits. We also sought to gain information on why sheesha has gained popularity among young adults in the “non-traditional” community of Ottawa, while the prevalence of other forms of smoking is decreasing among this section of the population (112). Here we define “non-traditional community” as one in which sheesha is not part of their culture.

Methodology

Sampling

As the study sought to engage younger sheesha smokers, we identified the University student population as an appropriate sampling frame. Moreover, previous studies have identified that University students are the most highly engaged in SS (9, 113, 114) and another study indicates sheesha bars as primary destinations for young people to socialize with friends (10).

Participants were eligible for inclusion if the individual was between the age of 18 and 25 and had ever smoked sheesha. Consistent with the exploratory nature of the study, our sample size was modest (115). Our target was to achieve a final sample of 30 participants. In order to engage a population that comprised active sheesha smokers from a range of cultural backgrounds and those of the relevant age ranges two different

non-probability sampling approaches were selected: (1) sampling from the University population (age focused) and (b) sampling through a local sheesha bar (behavior focused).

Recruitment

Recruitment took place between January and March 2012. Initial recruitment took the form of recruitment advertisements that were posted within the Faculty of Medicine at uOttawa as well as at the uOttawa Main Campus, Smyth Road Campus and at Carleton University, sheesha bars, grocery shops that sell Massal and Herbal fillings for sheesha smokers, and through community centres. In all cases potential participants responded by contacting the researcher (HE-K) by email. If interested respondents met the inclusion criteria they were invited to attend a meeting to complete the survey in a face-to face setting.

However, low levels of response required us to adapt recruitment. We subsequently took an in-person approach to recruitment and set up a table near the Health Promotion Centre (HPC) at the main Campus of the (uOttawa) where we were offered this opportunity. Students came by the table to inquire about the study and if they met the inclusion criteria, were provided information about the study and invited to complete the survey.

Following comments from several participants, we also approached potential candidates at a local sheesha bar that was identified by several respondents. In this instance a researcher (HE-K) approached patrons (following consent to do so by the proprietor) who fit the age criterion and invited them to participate in the study.

Procedure

In all instances, if initial contacts showed interest in the study they were provided with an information sheet and consent form that gave details of the study aims. In each case, the nature of the project was explained and detailed information regarding the

questionnaire and the second part of the study (gene expression analyses) was given for those who were willing to participate. Assurance of anonymity was provided, and participants were asked to read and sign an information sheet and consent form before completing the questionnaire. They were also asked to respond freely and truthfully to each question. Copies of the information sheet, consent form and questionnaire can be found in appendices A and B.

If participants remained interested then in-person meetings were arranged in order to go through the survey. In order to assess the questionnaire instrument, this was completed face-to-face to gain feedback on the feasibility of completing the survey as well as gaining feedback on useful additions or modifications.

The meetings took place in our office located at the (uOttawa) which provides a quiet and a private environment or, due to practical constraints, at the sheesha bar and at the HPC. On average surveys were completed in approximately 30 minutes.

Measures

Survey instrument

We developed two separate questionnaires, a primary questionnaire that focused on demographic information and sheesha practice, and an oral health questionnaire to be utilized alongside the genomic analyses.

The primary questionnaire comprised 40 questions in a combination of open ended and multiple choice formats. Questions related to socio-demographic characteristics and lifestyle as well as beliefs and attitudes towards sheesha and mode of smoking behavior. Other variables were collected such as whether they received help or intended to stop SS and whether it is difficult to quit SS. Information about other psychoactive habits among sheesha smokers - such as cigarette smoking, alcohol drinking and the use of smokeless tobacco - were collected. In order to explore health-related beliefs we

asked participants to assess the risk of SS and then compare it with that of cigarette smoking.

In addition to the closed questions, and as part of the questionnaire-development aim of the study, we asked participants to respond to open-ended questions and to provide comments.

While completing the questionnaire a number of participants spontaneously offered thoughts or additional comments based on the questionnaire content – as opposed to structural aspects. These comments occasionally built on data collected as part of the questionnaire – such as providing additional details regarding motivation for SS. In order to better contextualize the results of the closed questions we captured these additional comments through taking “field notes”. However, comments were not recorded verbatim and the field notes are used here only to provide additional context to the responses to open- and closed -question responses. Notes were only taken after obtaining additional verbal consent from participants.

As such, written comments from participants are documented between quotation marks next to ID numbers and their sex. Comments from our field notes are presented as general supporting documentation. In accordance to our consent form, participants had the right to skip any question. Also non-regular smokers were specifically asked to skip questions such as mode and type of smoking, their intention to quit SS, whether they received advice or help to stop smoking sheesha and their parents smoking behavior. We reported such skipped questions as NA in the results section.

The oral health questionnaire comprised 10 questions. Data collected from this questionnaire were used to assess the eligibility of participants to provide saliva samples. Results from the oral health questionnaire are presented in chapter 2.

Data analysis

Results were a combination of multiple choice questions and open-ended questions. Responses to open questions were subjected to qualitative description (116) in which “straight descriptions of the content are desired” (Sandelowski, 2000, p339). This approach differs from alternative approaches to qualitative data analysis, such as grounded theory, as our goal was not the development of theory to describe the practice of SS, but merely a descriptive analysis of the comments provided. Consequently, the description is more literal as opposed to interpretive. As such, this approach is akin to what Sandelowski has described as the “who”, “what” and “where” of the qualitative data (116). Coding was undertaken by two reviewers (HE-K, SN) and comments categorized according to the areas of interest of the study or the substantive area of concern. All comments were coded independently by the reviewers and then discussed. Following discussion descriptive codes were revised until a final agreed set was arrived at.

Data from closed ended questions were presented with frequency tables, prepared using the SAS version 9.2 (**SAS 9.2**; *Statistical Analysis System*).

In this pilot study our goal was not to make a statistical inference or external statistical generalization. Instead it was to obtain an insight into a particular phenomenon, the emerging state of SS. Accordingly, in the analysis we bring together some quantitative aspects and some qualitative comments that help us understand the questionnaire data.

We considered that it was important to stratify some responses according to whether the participants had backgrounds in which sheesha was part of the culture (6). We operationalized this background as the participant having been born in the Middle Eastern region, including Lebanon (117), Syria (118), Jordan (119), Egypt (120), Iraq (121), Iran (122), Turkey (123) and other (124). The Arabian Gulf (i.e. countries in the Gulf Co-operation Council - GCC) (125-127), Pakistan (128) and India (27). If they had been born in Canada, we classified this background based on the reported place of birth of at least one of their parents having been in these countries.

Results

Demographics

Thirty volunteers completed the primary questionnaire, of whom 14 were female and 16 were male (Table 11). Two participants were recruited through advertisements at uOttawa, five through the sheesha bar and the remainder (23) through the in-person recruitment at the HPC.

The majority of respondents (83.3 %) completed the primary questionnaire at the uOttawa while the rest completed it at the sheesha bar. Slightly more than quarter of participants (27 %) were between 18 and 19 years, (43.3 %) of participants were between the age of 20 and 21 years, the rest, (30 %), were 22 years of age or over. Ninety percent of the participants were students and two thirds (66.67 %) of those who completed the questionnaire were born in Canada. Slightly more than half of the participants (53.33 %) were of Asian and Middle Eastern origin.

Table 11 Sociodemographic characteristics of sheesha smokers in Ottawa

	Number	Frequency
Age		
18-19 y	8	26.67
20 -21y	13	43.33
22-23 y	8	26.6
24-25	1	3.33
Gender		
Female	14	46.66
Male	16	53.33
Language		
English	28	93.33
French	2	6.66
Born in Canada		
Yes	20	66.67
No	10	33.33
Mother born in Canada		
Yes	8	26.66
No/NA	22	73.33
Father born in Canada		
Yes	6	20.00
No/NA	24	80.00
Participant born in, or at least one of their parents born in, Middle Eastern or Asian countries		
Yes	16	53.33
No/NA	14	46.67

Sheesha smoking practices

All of the participants had tried smoking sheesha as it was one of our inclusion criteria. A high percentage of participants (70 %) had tried sheesha for the first time when they were sixteen years of age or older, whereas (23.33 %) had tried it between the age of twelve and fifteen, the rest (6.66 %) had tried it at the age of eleven years. More than half of the respondents (60 %) reported that they smoked sheesha monthly but less than weekly, a substantial percentage (33.33 %) reported that they smoked sheesha weekly, whereas the rest (6.66 %) reported that currently they did not smoke sheesha. A regular sheesha smoker is defined here as the one who had smoked sheesha at least once a month for six consecutive months. Almost three quarters (73.33 %) of our participants categorized themselves as regular sheesha smokers. Approximately one third of our respondents (33.33 %) were regular patrons of sheesha bars. Half of the respondents were not regular patrons of sheesha bars, while the rest (16.66 %) preferred not to answer this question. A high percentage (66.66 %) of the respondents reported that they shared the same sheesha; some (35.00 %) of them did not use a plastic extension and thus they shared the same mouthpiece to inhale the smoke (Table 12).

From the point of view of some respondents, the mode of smoking sheesha is different from that of cigarettes, where some of them explained that they do not inhale the smoke while smoking sheesha; they take puffs, hold it in their mouth and then exhale it, so it doesn't go into their lungs. From their point of view that is different from cigarette smoking, where in order to enjoy cigarette smoking, smokers inhale a deep smoke that goes right into their lungs. One subject suggested adding more questions related the practice and mode of SS;

“How does someone smoke sheesha? Some take “puffs” some inhale, how do you prepare the sheesha; some squeeze the tobacco and rinse it quickly under water”.
(JA19, Male)

The most popular filling used for SS was Massal, with 63.33 % of participants indicating that they used it to smoke sheesha. By contrast, only 3.33 % smoked herbal filling and the rest (33.33 %) did not know what they are smoking. Most of the respondents used one filling in each session (53.33 %). A high percentage (69.99 %) of participants reported that each sheesha setting lasted between one and two hours. A small percentage preferred not to respond to these two questions (data shown in Table 12).

Table 12 Mode of sheesha smoking

	Number	Frequency
Age when first tried sheesha		
11 y	2	6.66
12-13 y	3	10
14-15 y	4	13.33
16+	21	70
Regular sheesha smoker		
Yes	22	73.33
No	8	26.66
Frequency of sheesha use		
Monthly	18	60
Weekly	10	33.33
Daily	0	0
Do not smoke sheesha	2	6.66
Regular patron of sheesha bars		
Yes	10	33.33
No	15	50
NA	5	16.66
Type of timbac used		
Herbal	1	3.33
Massal	19	63.33
Unsure	10	33.33
Number of fillings in each session		
1	16	53.33
2-3	8	26.66
More than 3	1	3.33
NA	5	16.66
Do you share the same sheesha / Do you use plastic extension?		
Yes/ Yes	13	43.33
Yes/ No	7	23.33
Yes/ NA	1	3.33
No / No	4	13.33
NA	5	16.66
On average how long does each session last		
1 hour	11	36.66
2 hours	10	33.33
More than 2 hours	4	13.33
NA	5	16.66

The relationship of sheesha smoking to other psychoactive habits

Trying cigarette smoking was more attractive to the respondents than trying smokeless tobacco. Slightly less than three quarters (73.33 %) reported having ever tried smoking cigarettes at least once in their lifetime. When we asked them to categorize themselves as regular cigarette and regular sheesha smokers, slightly more than quarter (30 %) reported that they were regular cigarette as well as regular sheesha smokers, (43.33 %) that they were regular sheesha smokers yet not regular cigarette smokers. (10 %) regular cigarette smokers but not regular sheesha smokers and (16.66 %) that they did not regularly smoke either cigarettes or sheesha. Less than quarter of the respondents (20.00 %) had ever tried smokeless tobacco (at least once in their lifetime). Slightly more than half (56.66 %) reported that they do not drink alcohol. Two of the participants reported that the reason they did not drink was that they were Muslim and respected the Islamic prohibition on drinking alcohol.

Responding to the question “was (were) any question(s) you think it would be better not to ask, if so, why?” two subjects mentioned that alcohol drinking was very personal information;

“Drinking alcohol; very personal” (RT 17, Male).

Table 13 Psychoactive habits and the association between cigarette and sheesha smoking

	Number	Frequency
Do you drink alcohol?		
Yes	13	43.33
No	17	56.66
Have you ever tried cigarette smoking?		
Yes	22	73.33
No	7	23.33
NA	1	3.33
Are you a regular cigarette smoker?		
Yes	12	40
No	18	60
Are you a regular cig smoker/ regular sheesha smoker?		
Yes/ Yes	9	30
Yes/ No	3	10
No/ Yes	13	43.33
No/ No	5	16.66

Health concerns and beliefs

The overwhelming majority of sheesha smokers (93.33 %) reported that sheesha might be or is definitely harmful to their health. In addition, approximately three quarter of the participants (76.67 %) did not hold the belief that the water in the tank filters the noxious fumes. The most popular filling was Massal, with (63.33 %) reporting that they used it as the filler, whereas (50 %) of respondents reported that they did not use nicotine-free filling “herbal hukkah”. It appears that some people perceive the term “herbal” as equivalent to healthy product, with a small percentage (13.33 %) reporting that they used “herbal hukkah” because it is safer than the Massal one.

Although the majority (93.33 %) reported that sheesha might be or definitely harmful to their health, when we asked them to compare the adverse health effects of sheesha to that of cigarette smoking the majority of respondents (73.33 %) indicated that they thought cigarette smoking is equally, or more harmful to their health than SS (Table 14). This was reflected by the thoughts of one participant who shared her story with us; her husband used to smoke cigarettes prior to their first baby, but didn’t want the newborn to be subject to a second hand smoke, and so shifted from cigarettes into sheesha as a healthy alternative way of smoking. This view – that sheesha was less harmful than cigarettes – was shared by several other respondents. In particular one respondent argued that none of the official organizations warned about the possible adverse health effects of sheesha, in contrast to the situation for cigarettes.

Indeed, the majority of sheesha smokers (83.33 %) felt that it would not be difficult for them to quit smoking sheesha. Access to sheesha, and the procedures involved in SS, was indicated as a partial reason for this:

“In my opinion quitting cigarettes is harder and need an extra effort, because cigarettes are found everywhere and they are easy to get. Hookah, in order to smoke it you have to go to certain places and even if you have one at home you have to prepare it and clean it after usage.” (BA7, Male).

Moreover, this was supported by the lack of access to nicotine tobacco, as one respondent mentioned:

“Because the city doesn't allow timbak with nicotine to be sold” (CS12, Male).

None of the respondents indicated that he or she has received help from any official organization to quit smoking sheesha, and only five participants noted that they received advice from family member or friend to quit smoking sheesha.

Table 14 Smokers' beliefs

	Number	Frequency
Do you think shisha smoking is harmful to your health?		
Probably / Definitely Yes	28	93.33
Probably / Definitely No	2	6.67
Why do you consume nicotine free timbak?		
Safer	4	13.33
Available	5	16.66
I don't use nicotine free timbak	15	50.00
N/A	6	20.00
Comparing the risk of sheesha to that of cigarettes		
Cigarettes>sheesha	16	53.33
Cigarettes=sheesha	6	20.00
Cigarettes<sheesha	8	26.67
The water in the tank acts as filter		
Yes	7	23.33
No	12	40.00
Don't know	11	36.67
Have you ever received help or advice to help you stop smoking sheesha?		
Yes, from a program or professional	0	0.00
Yes, from a family member or friend	5	16.66
Do you think it is difficult to quit sheesha smoking?		
Probably / definitely Yes	5	16.67
Probably / definitely No	25	83.33

Social and cultural aspects of sheesha

Friends played a central role in influencing their peers to smoke sheesha, with over 80 % (83.33 %) indicating that they were introduced to sheesha by their friends, regardless of whether sheesha was part of their culture (Table 15). Even though sheesha was always available at the house of those who have it in their culture, yet participants still reported that friends introduced sheesha to them. In some cultures sheesha is offered to the guests to welcome them. A student from Iraq explained that he has been aware of sheesha since he was a little boy. His parents are non-sheesha-smokers however they used to provide sheesha to their guests at home. Later in life his friends introduced sheesha to him.

“sheesha was always found at home but not often smoked; SS was done by guests at our house” (MM 22, Male)

Almost two thirds (63.33 %) of participants indicated that they thought sheesha was an important social aid that helped them to feel more comfortable during social gathering. This was supported open-text comments that emphasized the role of sheesha in creating a social ambiance and a friendly spirit which makes them enjoys their session.

“Sheesha does not necessarily make you feel comfortable but it creates a friendly spirit”. (BP28, Male)

This was emphasized by a patron of the sheesha bar who noted differences between the way sheesha and cigarettes are smoked:

“To me sheesha is better than cigarette as it is difficult to make [...] and it is enjoyed by environment rather than used as stress relief method”. (MD26, Female).

Others suggested to add more questions related to the social setting;

“More questions related to the social setting. Example: I only smoke in social setting I don’t drink when I smoke. It is a good way of relaxing. I feel the questions asked don’t help me reflect that.” (CS12, Male)

Nonetheless, SS, in the context of social setting, influences participant’s risky behavior to partake the same sheesha with friends. Where (70 %) of respondents indicated that they share their sheesha and (61.9 %) of them do not use plastic extension.

In their comments, participants -Canadian as well as immigrant- attempted to highlight the importance of cultural background that is behind SS, in response to the open ended question “Do you think there is a question (s) that would help us understand more about waterpipe smoking among young people that we should ask and haven’t asked? Please specify”.

“May be ask the cultural background” (PS1, Male)

“Signing the importance of culture; I am from Ivory Cost, I have lived in Tunisia for six years I prefer smoking sheesha occasionally than smoking cigarettes because it is in their culture”. (BP28, Male)

According to our findings, sheesha bars are predominantly crowded with clients having sheesha in their cultures “came from Middle Eastern or Asian origin”. Those who have sheesha in their culture are more likely to be patron of sheesha bars than those who do not have sheesha in their culture and were able to report the name of sheesha bar. Where (43 %) of those who have sheesha in their culture indicated that they are patron of sheesha bars, comparing to (33 %) of those who do not have it in their culture. Five out of 30 did not complete this question.

Table 15 Attitudes of sheesha smokers in Ottawa

	Number	%
Who introduced sheesha to you?		
Parents/ Others	5	16.67
Friends	25	83.33
Do you think that sheesha is an important social aid that helps you feel more comfortable during social gathering?		
Yes	19	63.33
No/ it doesn't make any difference	11	36.66
Have sheesha in your culture/ are you a sheesha patron?		
Yes/ Yes	16/7	43
No/ Yes	12/3	33
Do you share the same sheesha / Do you use the same plastic extension?		
Yes/ Yes	13	43.33
Yes/ No	7	23.33
Yes/ NA	1	3.33
No / No	4	13.33
NA	5	16.66

Discussion

In this exploratory study we collected information from a purposive sample of young adults who smoke sheesha. Participants were largely students and recruited through a University setting, although a minority was recruited through a sheesha bar.

Health concerns

The overwhelming majority of sheesha smokers (93.33 %) perceived sheesha as a behavior in that might be, or is definitely, harmful to their health. Indeed, the view that the water filters out the noxious fumes was not common among our participants. On the other hand, when they were asked to compare cigarettes to sheesha, the majority of respondents (73.33 %) thought that cigarette smoking is equally or more harmful to their health than SS. In addition, none of the respondents indicated that he or she has received help or advice from a program or a professional organization to quit SS. Consequently, a majority considered sheesha to be an as safe or safer way to enjoy tobacco smoking. The same finding was highlighted by Roskin et al., (6) when they concluded;

“In the absence of public health information, students have fallen back on superficial experiences to form views that waterpipe smoking is less harmful than other forms of smoking and it is currently much more acceptable in student society than other forms of smoking”.

This was reflected in perceptions regarding the ease with which participants could quit SS.

A high percentage (70 %) of participants reported sharing their sheesha and some of them do not use plastic extension. These findings are in line with what was reported by Asfar et al., (129) who found that sharing the same hose is a common practice among sheesha smokers. This might put sheesha smokers on a higher risk of transmitting communicable diseases such as hepatitis B, common cold and upper airway infection (42, 43). In practice, sheesha smokers used to heat the mouthpiece by the lit charcoal if

they wanted to share it without using a disposable plastic extension. However the effectiveness of this method is quite questionable.

Elements contributing to increasing popularity of sheesha smoking among youth in Ottawa

In the present study, respondents emphasized the role of culture and society on promoting and partaking of SS. The perception of SS is not equal throughout different societies. In most Middle Eastern countries, notably the Lebanon, Syria and Saudi Arabia, sheesha is embedded in the culture. However, according to immigrants from some other regions such as Algeria and Morocco, sheesha is not accepted. The cultural background is an important factor in initiating SS, as described by previous study (6). Thus, the cultural background is the base that provides sheesha and makes it available, but the social aspect is the dynamo that helps to increase the popularity of SS among all sections of Canadian youth, immigrant or white, with most of them acquiring the habit from friends and almost two thirds indicating that sheesha helps people feel more comfortable in social gatherings. Roskin et al., (6) conducted a similar qualitative study. They have identified some elements that stand behind the widespread of sheesha use in England and Canada, such as the affordable social gathering, especially how sheesha makes students feel more comfortable.

Other elements appeared to contribute to the fast proliferation of sheesha use in Ottawa such as the gap in regulations (for example; the availability of Herbal filling over the shelves and the presence of such a large number of sheesha bars that offer sheesha indoors to their customers) and the absence of health education programs concerning the potential adverse health effects of SS. In addition, none of the participants indicated having received advice from any official organization to help them quit SS. Although the warning signs concerning tobacco smoking are found almost everywhere, including sheesha bars, it seems that people understand the sign to be solely intended for cigarette smoking. In fact, the logo of nonsmoking is a lit cigarette crossed by red line. This suggests an unbalanced handling of different forms of tobacco smoking by agencies involved in tobacco regulation. The consequences of the mismanagement of tobacco

smoking might have the potential to build a stigma concerning cigarette smoking but not other forms of tobacco smoking. This may explain why a substantial (43.33 %) proportion of participants were regular sheesha smokers but not regular cigarette smokers. Also, among those who do not have sheesha in their culture, some accepted sheesha but not cigarettes.

The Herbal filling and the exemption of sheesha bars

Two kinds of fillings are used here in Canada -the Herbal (with no tobacco) and the Massal (that contains tobacco). Some participants encountered difficulties while trying to estimate the number of SS sessions per month. They explained that SS is a seasonal practice that increases during the summer and leisure times while it decreases during the winter. On the other hand, whilst most respondents indicated that they felt they could quit sheesha if they wanted to, yet they hadn't actually thought to quit, most also indicated that they use the Massal tobacco. The smoke that is emitted from Massal contains nicotine (130), the additive substance found in cigarettes which creates a state of physical and psychological dependency. Therefore it might be hard for them to quit SS. The contradiction might be explained that some patrons are overestimating their ability to quit SS due to the potential physical dependency created by the nicotine, or the frequency with which they smoke sheesha was not enough to create dependency. Abughosh et al., 2012 conducted a study in US to assess the desirability of student to quit smoking sheesha and found that sheesha smokers have low level of desire to quit SS (131).

In general, most of our participants do not use the "Herbal" filling, although some of them considered that it would be an alternative safe way to smoke, where (13.33 %) reported that they smoke "Herbal" because it is safer from their point of view. Smoking sheesha using "Herbal" filling emits tar and noxious fumes, but not nicotine. The absence of nicotine from cigarettes does not decrease their carcinogenicity. According to an IARC publication in 2004 (2), de-nicotinized cigarettes, similar in their components to the regular cigarette apart from the absence of nicotine, still contain the

carcinogenic compounds found in regular cigarettes. Therefore, we encourage conducting a study concerning the adverse health effects of sheesha “Herbal” filling. When reliable evidence becomes available, it might be important to amend the Act to a more secure level that includes all kinds of smoke, in order to avoid loopholes.

Limitations

The main limitation of this pilot study is the sample size. Only thirty people were included. Whilst limited in terms of the inferences that can be drawn for the general population, from a qualitative perspective it provides great insight into the attitudes of sheesha smokers. Although we only actively recruited from two locations, the participants were from different origins, reflecting the architecture of a contemporary Canadian society.

Strength

The qualitative analysis of the study helps us to understand SS in a multicultural society.

Recommendation for future study

It is important to conduct a study among representative sample of this population in order to gain results that are generalizable.

Chapter 5. Discussion

Bridging the different parts of the project

SS is gaining attention from legislators, NGOs and other interested parties. It is therefore, a pertinent topic of research for public health and public health policy.

This project focused on sheesha tobacco smoking as an exposure and cancers as potential outcomes. The thesis has three separate components: (I) a gene expression study as an innovative early marker of the effect of sheesha; (II) a systematic review, undertaken to synthesize available evidence about the effects of sheesha on cancer, a potential long-term outcome of SS; (III) a pilot survey to both select participants for the gene expression study, and explore the characteristics of sheesha smokers. As such, the thesis involves a process of triangulation, using multiple methods in order to explore the phenomenon in question (132).

While studies have assessed the effect of cigarette smoking on gene expression (66, 78), to our knowledge this is the first study that addresses the effect of SS on the expression of cancer-related genes. Gene expression signature of a disease is the assessment of the performance of many genes in a specific diseased patients and comparing them with that of a healthy individuals. An important applicability of this method is the tracking the pathway of diseases. Thus, using a biomarker such as expression of one or more genes to predict the adverse health effects of any exposure is a promising method (133). In addition it potentially enables information on emerging health concerns, such as sheesha use in young people in countries in which sheesha use was previously very uncommon, to be obtained relatively rapidly. This would allow at least interim policies to be developed before evidence of long-term effects of high quality accrues. However, this method is

relatively new, as reflected for example that in the main international database on gene expression (KEGG) (69) gene expression maps so far are only available for 55 diseases.

In theory, systematic reviews are placed on top of hierarchy of evidence-based science. However, the results that emerge from a SR depend primarily on the quality, quantity and transparency of reporting of included studies, and this proved to be a challenge in consideration of the associations between sheesha and cancer evaluated in this thesis.

The survey method is potentially very informative regarding the characteristics, beliefs and attitudes of sheesha smokers and the method of use. Furthermore, it was conducted with a view to providing preliminary data for a larger survey.

Recapitulating the main results

Gene expression

SS significantly reduces the expression of both xenobiotic metabolism genes and other genes found in the pathways of tobacco related cancer diseases. The fold change between before and after an hour and a half of smoking sheesha ranged between 0.018 and 0.604, that is, between a 1.655 times and 55 fold reduction in expression level, with CI that do not cross 1.

Systematic review

Applying the Cochrane Collaboration methodology for conducting a systematic review, we were able to assess four cancers: lung, esophageal, bladder, and nasopharyngeal cancer. In order to focus more on the effect of SS on carcinogenic outcomes, we applied more defined inclusion and exclusion criteria than in previous systematic reviews. Out of a total of 232 citations identified as potentially eligible by our search strategy, seven articles met our inclusion criteria. Two studies were included in assessing the relation between the lung cancer and SS. The pooled OR was 5.03 (95% CI 3.57-7.09), indicating

a significant association between SS and lung cancer. Regarding the association between SS and esophageal cancer, three articles met our inclusion criteria. The pooled OR was 8.11 (95 % CI 5.76- 11.43). Only one article on bladder cancer Bedwani et al., (107) met our inclusion criteria. The adjusted OR of developing bladder cancer compared for ever sheesha smokers compared to never smokers is 0.8 (95% CI 0.2-4.0). One article met our inclusion criteria while assessing nasopharyngeal carcinoma. The OR of ever sheesha smokers compared to never smokers is 0.5 (95% CI 0.2 -1.24).

From the above results it seems that sheesha may have a large effect on lung and esophageal cancers, but not on the other cancers. However, a major limitation of the existing studies is that the internal validities of the included studies are questionable. Most of the included studies had a high risk of bias, including selection bias, non-differential and differential information bias and incomplete control for potential confounders. The extent to which these sources of bias affected the observed magnitudes of association is difficult to determine because of incomplete reporting.

Pilot survey

The pilot survey enabled us to find volunteers for the gene expression part of the research. It led us to focus on “Massal” smoking in that part of the research, and provided an estimate of the average length of a SS session. The study also gave us an indication of the characteristics of sheesha smokers, shed light on the perceived risk of SS and why sheesha has gained so much popularity among young adults in Ottawa. It suggested factors that may act as contributors to proliferate the use of sheesha in Ottawa. It also brought attention to the importance of putting SS in the priority list of policy makers. For example, it suggested that there is a widespread misbelief that sheesha is a way of smoking that is safe for young adults. It also differentiated between the Massal and Herbal fillings while highlighting the misbelief that surround the Herbal and its potential adverse health effects.

Convergence or divergence of results

Results from pilot data informed the gene expression part by knowledge considered as crucial to assess the effect of SS on cancer as an outcome. It shed light on the elements that contribute to the wide spread use of sheesha in Ottawa such as novelty of the issue, the friendship, the scarcity of evidence on the side effect of SS and the lack of awareness from official organizations about the adverse health effect of SS. The SR component of this thesis only identified 7 studies relating to sheesha and carcinogenic effects. Moreover, each study suffers from serious limitations with regard selection bias, non-differential and differential information bias and incomplete control for potential confounders.

In the gene expression study, “Massal” was the filling used, in line with data from a small survey that we conducted earlier (please refer to chapter 4). Thus, in Ottawa, the most commonly used filler for sheesha is Massal, a blend of tobacco, glycerin and aromatic ingredients. It is interesting that in the Kashmir Valley, in which the risks for cancers of the lung and esophagus associated with sheesha were highest, the filling (“Jajeer” as documented by Koul et al., (27)) contains processed tobacco mixed with molasses. Thus, the type of exposure in the Kashmir Valley is similar to that used in Ottawa and investigated in the gene expression component of the thesis.

Although our study shows an overall pattern of significant reduction in gene expression of cancer related genes in salivary cells after one hour and a half of smoking sheesha, the conclusion is still elusive. Further investigation on all the selected genes is recommended in order to understand and differentiate well between the short-term and long-term effects of exposure on the performance of these genes.

In order to accurately assess the impact of SS on the health of smokers, we should take into consideration other factors such as the availability of sheesha and the frequency of smoking it. Sheesha is mostly smoked occasionally, during leisure time and summer. However, Massal contains nicotine; hereby it is highly susceptible to cause psychological

or physical dependence. When this is attained, the temporary effect of SS on gene expression is likely to increase, resulting in prolonged alteration of the expression of cancer related genes, which might affect the risk of developing cancer or other diseases.

Further investigations on the expression of cancer related genes, in relation to short-term exposure to carcinogenic factors, are crucial to explain the convergence or divergence of results from SR and gene expression component of the study.

The strength and limitation of the study

Strength

A main strength of this study is the mixed methods approach. This provides a more holistic appreciation than investigating using single approach method. In fact, this is a powerful technique that promotes validation of data yielded from three different approaches through cross verification by studying the convergence of these results. To our knowledge, this is the first on SS that combines three different parts to present sound knowledge from different perspectives. It combines the result of the SR to the practical way of surveillance that assess the method of use and the belief that surround sheesha to the laboratory result. We aimed from this study to predict the carcinogenic adverse health effects related to Massal SS before we experience the actual burden of diseases.

Limitation

We have already mentioned the limitations related to each section. However there are also some over-arching limitations. We did not assess the effects of the Herbal filling which does not contain tobacco. This type of filling is exempt from the non-smoking act and this has enabled the opening of sheesha bars in North America.

The sample size is an important limitation to mention in the pilot data on sheesha smokers where we have thirty volunteers completed the questionnaire and most of them are from uOttawa. However in this component of the study, volunteers were from different origins, reflecting the architecture of a contemporary Canadian society.

Although changes in the expression of some genes are established to be in the pathway of cancer causation and expression is known to be altered as a result of cigarette smoking, there is no direct correlation between the length of perturbation of gene expression and developing cancer.

Applicability

Gap in the literature and recommendations for future research

Although the evidences from SR were deemed insufficient; SS yields high quantity of potentially deleterious smoke from the mainstream of sheesha such as carbon monoxide, tar and nicotine in comparison to that conveyed from the mainstream of cigarette smoking. (1,2). The gene expression profile conducted by Kaddah et al., 2009 (14) suggested that SS induces expression of metalloproteinases in BAL as much as in smoking cigarettes. Our results show that sheesha reduces the expression of genes related to some cancer diseases that were previously linked to cigarette smoking. Hereby, as suggested here it is recommended to increase researches that study the link between SS and tobacco related cancer diseases based on high quality epidemiological studies.

It is not appropriate anymore to combine different modes of smoke while assessing exposure to smoke. It is time to separate between sheesha smokers, cigarette smokers and sheesha and cigarette smokers. Find consensus on definitions and methods used to assess the adverse health effects of SS. Find a common definition of regular sheesha smoker and non-smokers. Apply equivalency to the dose and different types of tobacco used in SS according to the content and quality of tobacco used. Appropriately classify sheesha smokers in terms of dose and time since they started SS.

The Downs and Black list is a good tool used to evaluate cross-sectional studies. However it did not reflect all limitations found in the included studies of SR.

There is an urgent need for high quality evidence coming from well conducted epidemiological studies among societies that have sheesha use established in their culture for a long time. This would be complemented by conducting epidemiological studies based on proxy measures among both societies those who have sheesha in their cultures and those in which sheesha use has not been established but increased recently. These studies should be characterized with high standards and low biases. This research will permit us to build portfolio of evidence rapidly.

As listed before we recommended to assess the short-term effect of cigarette smoking on cancer related genes in order to comprehend well the short-term effect of cigarette smoking on the performance of cancer related genes.

SS is a public health concern that cannot be managed by targeting one of the stakeholders. Three main stakeholders are involved in this issue; sheesha smokers, the government and the healthcare professionals. Nevertheless, we don't know whether the health care professionals are ready to accommodate our finding concerning the possible adverse health effect of SS and integrate them in their routine work such as while collecting patient's history, when giving post operative recommendations or in their anti-smoking activities. Nevertheless, knowledge dissemination is required in order that people properly perceive that sheesha is a tool that conveys tobacco smoke.

There is a significant paucity of data that substantially undermines the potential for intervening or creating evidence-based policy in this area. There is, therefore, a serious need to exponentiate researches on SS to develop an evidence base with which to inform policy in order to avoid politicized, reactive responses that may result not only in poor public health policy, but also poor public health.

There is a need to develop and pilot test a common questionnaire used to assess sheesha tobacco smoking and characteristics of sheesha smokers. It should classify and differentiate between sheesha smokers and cigarette smokers with classification of sheesha smokers into smokers, second hand smokers and both. It should be easily tailored to accommodate differences among population and to be adopted in new studies. The questionnaire should be tested for its validity and practicality.

Key messages

The potential adverse health effects of sheesha smoking should not be underestimated; Tobacco smoking, regardless of its mode of delivery, affects susceptibility to cancer.

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Appendices

Appendix A: Primary and oral health questionnaires – French versions

Questionnaire principal

Feuille de renseignements et Formulaire de consentement

L'incidence de la consommation de la chicha sur l'expression génétique des muqueuses

Chercheur principal : *****, Ph.D., 613-562-5800, poste *****

Financement : Financement actuellement recherché

Introduction

Vous êtes invité à prendre part à ce projet de recherche en répondant à un questionnaire parce que vous vous êtes identifié comme un consommateur de la chicha et que vous êtes âgé de 18 à 25 ans.

Veillez prendre connaissance de la Feuille de renseignements et du Formulaire de consentement à l'intention du patient, et n'hésitez pas à poser toutes vos questions avant de décider si vous souhaitez ou non prendre part à cette étude de recherche. Nous vous invitons à discuter de votre décision avec les membres de votre famille, vos amis et votre équipe de soins de la santé.

Contexte

Récemment, on aura constaté dans les pays occidentaux une augmentation inexplicquée de la consommation de la chicha, particulièrement chez les jeunes adultes. L'augmentation de la popularité de la chicha chez cette population est possiblement attribuable à la croyance que la chicha s'avérerait la façon la plus saine de fumer le tabac. À ce jour, nous ne disposons pas d'une base de connaissance suffisamment solide au sujet des effets indésirables sur la santé associés à la chicha.

Objectif de l'étude

On vous invitera à répondre à un questionnaire comportant certains renseignements personnels, et certaines de vos opinions et de vos croyances sur la consommation de la chicha. Ce questionnaire a pour but de mieux comprendre les caractéristiques, les antécédents de consommation et les habitudes des consommateurs de la chicha qui sont âgés de 18 à 25 ans.

Nous recueillerons l'information recueillie à partir de 30 questionnaires afin de générer un cadre conceptuel qui sera appelé à être utilisé pour recruter des personnes dans la deuxième partie de cette étude. La deuxième partie de cette étude aura pour but d'évaluer les effets indésirables sur la santé qui sont associés à la fumée de la chicha, par l'entremise d'étude sur l'expression génétique des tissus. Le questionnaire conçu et évalué aux fins de cette étude pourrait également être employé à l'avenir pour évaluer la prévalence et les déterminants de la consommation de la chicha chez les jeunes personnes, suivant la distribution de celui-ci à une portion représentative de cette population.

Durée de l'étude

Le questionnaire devrait prendre environ 30 minutes de votre temps.

Effets secondaires ou risques possibles

Votre participation à cette étude ne comporte aucun risque ou effet secondaire.

Bienfaits reliés à l'étude

Il est possible que vous ne retiriez aucun avantage direct en prenant part à cette étude. Votre participation à cette recherche permettrait au chercheur d'en apprendre davantage au sujet des effets indésirables associés à la fumée de la chicha.

Retrait de l'étude

Vous avez le droit de retirer votre consentement en tout temps, sans que votre décision n'entraîne de pénalités.

Coûts relatifs à l'étude

Vous ne serez pas rémunéré pour votre participation à cette étude de recherche. Toutefois, on vous remboursera les frais de stationnement et d'autobus, ainsi que toute dépense reliée à cette étude.

Confidentialité

Tout renseignement personnel sur la santé sera maintenu confidentiel, à moins que la loi n'exige leur divulgation. Des représentants du Conseil d'éthique en recherches de L'Hôpital d'Ottawa, ainsi que de l'Institut de recherche de l'Hôpital d'Ottawa, pourront procéder à l'examen de vos

dossiers médicaux originaux, sous la supervision du personnel du docteur ***** , uniquement à des fins de vérification.

Aucune publication ou présentation résultant de cette étude ne pourra servir à vous identifier. Aucune information pouvant servir à vous identifier ne sera transmise à l'extérieur de l'Université d'Ottawa. Tout renseignement transmis à l'extérieur de l'Université sera codé à l'aide d'un numéro d'étude indépendant.

Seuls le docteur ***** ou son personnel pourront accéder au lien entre votre nom et le numéro de l'étude indépendant. Ce lien et les dossiers de l'étude seront entreposés en lieu sûr et séparément. Ces dossiers seront conservés pour une période de 15 années suivant la fin de l'étude. Tout dossier papier sera entreposé dans un cabinet ou un bureau verrouillé à l'Université d'Ottawa.

Le lien et les dossiers de l'étude seront entreposés en lieu sûr et séparé. Le tout sera conservé pour une période de 15 ans suivant la fin de l'étude. Les fichiers électroniques quant à eux seront stockés et protégés par un mot de passe, auquel seuls le docteur***** ou son personnel auront accès. Une fois la période de rétention terminée, tous les dossiers papier seront déchiquetés ou jetés aux rebuts confidentiels, et les fichiers électroniques seront supprimés.

Participation volontaire

Votre participation à cette étude s'effectue sur une base volontaire.

Questions au sujet de l'étude

Pour toute question au sujet de l'étude, veuillez communiquer avec ***** , candidate à la maîtrise, soit par courriel (*****@uottawa.ca) ou téléphone, au 613-562-5800, poste ****. Si vous souhaitez parler au chercheur principal, le docteur ***** , veuillez composer le 613-562-5800, poste ****.

Le Conseil d'éthique en recherches de L'Hôpital d'Ottawa (CÉRHO) a révisé ce protocole. Le CÉRHO est chargé de l'ensemble des aspects éthiques de toutes les études de recherche menées auprès de sujets humains effectuées à L'Hôpital d'Ottawa. Pour toute question au sujet de vos droits à

titre de sujet de recherche, veuillez communiquer avec le président du Conseil d'éthique en recherches de L'Hôpital d'Ottawa, au 613-798-5555, poste 14902.

Formulaire de consentement

L'incidence de la consommation de la chicha sur l'expression génétique des muqueuses

Consentement à la participation à la recherche

Je reconnais que l'on sollicite ma participation à une étude de recherche en vue d'évaluer les effets indésirables sur la santé associés à la consommation de la chicha. ***** m'a fourni les renseignements au sujet de cette étude.

J'ai pris connaissance des 6 pages de cette Feuille de renseignements et de ce Formulaire de consentement à l'intention du patient. On a répondu à toutes mes questions de manière satisfaisante.

Je consens volontairement à prendre part à cette étude en répondant à ce questionnaire. On me remettra un exemplaire signé de cette Feuille de renseignements et de ce Formulaire de consentement.

Signatures

Nom du participant (en caractères d'imprimerie)

Signature du participant

Date

Énoncé du chercheur (ou de la personne chargée d'obtenir le consentement)

J'ai expliqué soigneusement au participant de la recherche la nature de l'étude susmentionnée. Pour autant que je sache, le participant apposant sa signature à ce consentement reconnaît la nature, les exigences, les risques et

les avantages que comporte sa participation à l'étude. Je reconnais ma responsabilité envers le soin et le bien-être du participant susmentionné, le respect des droits et des désirs de ce dernier, et le déroulement de cette étude, conformément aux directives et aux règlements relatifs à la bonne pratique clinique.

Nom du chercheur/délégué (en caractères d'imprimerie)

Signature du chercheur/délégué

Date

Questionnaire principal

chicha

Récemment, une augmentation de la consommation de chicha a été observée dans certains pays, en particulier chez les jeunes adultes. Nous aimerions en savoir plus sur l'utilisation de la chicha au Canada et, à cette fin, il serait vraiment utile que vous remplissiez ce questionnaire.

Nous vous suggérons de lire le questionnaire ci-dessous avant de le remplir. À la fin du questionnaire, nous sollicitons vos commentaires et votre rétroaction au sujet du questionnaire.

Les informations provenant de votre questionnaire seront analysées avec celles obtenues de questionnaires remplis par d'autres personnes. Si vous souhaitez connaître les résultats de notre étude, veuillez nous le faire savoir à la question que nous posons à la fin du questionnaire.

Merci d'avance.

Les informations figurant sur cette page seront conservées de manière confidentielle. Nous vous demandons de fournir certaines coordonnées (p. ex. nom, numéro de téléphone) que nous allons utiliser pour rester en contact avec vous, au cas où l'on vous sélectionnerait pour fournir des échantillons de salive. Ces informations seront conservées en lieu sûr et dans un endroit séparé des autres données que vous nous fournirez. Il sera impossible d'identifier les réponses que vous fournirez dans les rapports et les publications que nous produirons à la suite de cette étude.

1. Numéro d'identification (fourni par le chercheur)

2. Êtes-vous étudiant?

☐ Non ☐ Oui

Si oui, veuillez fournir le nom de votre établissement.

Veuillez remplir les informations suivantes :

1. Quel est votre âge? _____

2. Quel est votre sexe?

☐ Homme ☐ Femme.

3. Êtes-vous né au Canada ?

☐ Oui ☐ Non. **Si non, veuillez indiquer dans quel pays vous êtes né.** _____

4. Votre mère est-elle née au Canada?

☐ Oui ☐ Non ☐ Je préfère ne pas répondre.

Si non, veuillez indiquer dans quel pays elle est née.

5. Votre père est-il né au Canada?

☐ Oui ☐ Non ☐ Je préfère ne pas répondre.

Si non, veuillez indiquer dans quel pays il est né.

6. Quel est votre le plus haut niveau de scolarité?

☐ Je n'ai pas de diplôme d'études secondaires.

☐ J'ai un diplôme d'études secondaires.

☐ Je n'ai pas de diplôme d'études collégiales.

☐ J'ai un diplôme d'études collégiales.

☐ J'ai un diplôme universitaire.

☐ J'ai un diplôme postuniversitaire.

7. Avez-vous déjà essayé de fumer ou avez-vous déjà fumé (que ce soit seulement une ou deux inhalations)?

Cigarette ☐ Oui ☐ Non

Chicha ☐ Oui ☐ Non

Produit sans fumée (tabac à mâcher) ☐ Oui ☐ Non

8. Avez-vous déjà fumé des cigarettes pendant une période d'au moins six mois?

☐ Oui ☐ Non

9. Comment décririez-vous votre consommation d'alcool?

☐ Habituellement, je bois de l'alcool tous les mois (au moins une fois par mois mais pas toutes les semaines).

☐ Habituellement, je bois l'alcool toutes les semaines (au moins une fois par semaine, mais pas tous les jours).

☐ Habituellement, je bois de l'alcool tous les jours (au moins une fois par jour ou la plupart des jours du mois).

☐ Habituellement, je bois de l'alcool lorsque je fume la chicha.

☐ Habituellement, je bois de l'alcool lorsque je fume des cigarettes.

☐ Je ne bois pas d'alcool.

10. Quel âge aviez-vous lorsque vous avez essayé la chicha la première fois?

☐ 7 ans ou moins

☐ 8 ans

☐ 9 ans

☐ 10 ans

☐ 11 ans

☐ 12 ans

☐ 13 ans

☐ 14 ans

☐ 15 ans

☐ 16 ans ou plus

11. Qui vous a initié à la chicha?

☐ Parent(s)

☐ Ami(s)

☐ Autres

12. Lequel des énoncés suivants décrit le mieux votre consommation de la chicha?

☐ Habituellement, je fume la chicha tous les mois (au moins une fois par mois mais pas toutes les semaines).

- ☐ Habituellement, je fume la chicha toutes les semaines (au moins une fois par semaine, mais pas tous les jours).
- ☐ Habituellement, je fume la chicha tous les jours (au moins une fois par jour ou la plupart des jours du mois).
- ☐ Je ne fume pas la chicha.

13. Avez-vous déjà fumé la chicha régulièrement (au moins une fois par mois pendant six mois consécutifs)?

- ☐ Oui ☐ Non

Si non, veuillez passer à la question n° 26.

14. Quel niveau de scolarité aviez-vous atteint lorsque vous avez commencé à fumer la chicha régulièrement?

- ☐ Secondaire
☐ Collège
☐ Université

15. En moyenne, combien de rondes de chicha avez-vous remplies et allumées lors de chaque séance que vous en fumez?

- ☐ 1
☐ 2-3
☐ Plus de 3

16. En moyenne, combien de temps dure chaque session?

- ☐ 1 heure
☐ 2 heures
☐ Plus de 2 heures

17. Êtes-vous un habitué des bars à chicha?

- ☐ Oui ☐ Non

18. Si oui, veuillez nous indiquer l'endroit que vous fréquentez.

19. Au cours des 30 derniers jours, quel type de *timbak* (tabac utilisé dans la chicha) avez-vous fumé?

- ☐ Mélange d'herbes sans nicotine
- ☐ Tabac Massal
- ☐ Asfahani/Ajami
- ☐ Je n'ai aucune préférence/je ne suis pas certain

Veuillez indiquer le nom de la marque et l'arôme

20. Habituellement, est-ce que vous partagez le chicha avec d'autres personnes?

☐ Non

☐ Oui

Si oui, est-ce que vous utilisez des tubes en plastique?

☐ Oui

☐ Non

21. Est-ce que vos parents fument?

Mère

☐ cigarettes

☐ Oui

☐ Non

☐ Je ne sais pas

☐ chicha

☐ Oui

☐ Non

☐ Je ne sais pas

Père

☐ cigarettes

☐ Oui

☐ Non

☐ Je ne sais pas

☐ chicha

☐ Oui

☐ Non

☐ Je ne sais pas

22. Avez-vous déjà pensé à arrêter de fumer la chicha?

☐ Oui

☐ Non

23. Avez-vous l'intention de cesser de fumer la chicha?

☐ Pas du tout

☐ Le mois prochain

☐ Dans les 6 prochains mois

☐ Dans l'avenir

24. Avez-vous déjà reçu de l'aide ou des conseils pour vous aider à arrêter de fumer la chicha?

☐ Oui, de l'aide d'un programme ou d'un professionnel

☐ Oui, d'un ami

☐ Oui, d'un membre de ma famille

☐ Non

25. Si vous consommez du *timbak* sans nicotine, pourquoi le faites-vous?

☐ Je n'utilise aucun *timbak* sans nicotine

☐ C'est moins dangereux que le *timbak* avec nicotine

☐ C'est moins cher que le *timbak* avec nicotine

☐ C'est disponible

☐ Autres – Veuillez préciser

26. Pensez-vous que fumer la chicha est dangereux pour votre santé?

- ☐ Absolument pas
- ☐ Probablement pas
- ☐ Probablement
- ☐ Absolument

27. Pensez-vous que l'eau de la chicha filtre les substances potentiellement nocives?

- ☐ Oui
- ☐ Non
- ☐ Je n'en ai aucune idée

28. Comment comparez-vous les risques de la cigarette à celle de la chicha?

- ☐ L'effet nocif des cigarettes sur la santé est supérieur à celui de la chicha
- ☐ L'effet nocif des cigarettes sur la santé est inférieur à celui de la chicha
- ☐ L'effet nocif des cigarettes sur la santé est le même que celui de la chicha

29. Pensez-vous que fumer la chicha aide les gens à se sentir à l'aise lors de célébrations, de fêtes ou d'autres rassemblements sociaux?

- ☐ Oui, cela les rend plus à l'aise.
- ☐ Non, cela les rend moins à l'aise.
- ☐ Non, cela n'a aucun effet.

30. Une fois que quelqu'un a commencé à fumer la chicha, pensez-vous qu'il est difficile d'arrêter?

- ☐ Absolument pas
- ☐ Probablement pas
- ☐ Probablement
- ☐ Absolument

31. Seriez-vous prêt à fournir un échantillon de salive?

- ☐ Oui ☐ Non

Veuillez indiquer votre nom, votre numéro de téléphone et votre courriel

Commentaires et rétroaction

Merci encore de remplir notre questionnaire. Nous vous serions très reconnaissants de nous fournir des commentaires et de la rétroaction.
Les questions étaient-elles toutes claires et faciles à comprendre?

Parmi les deux expressions suivantes, laquelle est la plus appropriée :
« fumer la chicha » ou « boire de la chicha »?

Quel est le meilleur terme correspondant à la chicha (p. ex. narguilé, pipe à eau, gouza, houka)?

Avez-vous trouvé un choix de réponse approprié pour chacune des questions posées?

Parmi les questions posées, est-ce que vous croyez qu'une ou plusieurs sont inappropriées. Si oui, pourquoi?

Est-ce que nous devrions reformuler certaines questions?

Pensez-vous qu'une ou plusieurs autres questions pourraient nous aider à mieux comprendre la consommation de chicha chez les jeunes? Veuillez préciser.

Avez-vous d'autres commentaires?

Je voudrais obtenir une copie des résultats de cette étude.

☐ Oui ☐ Non

Veuillez nous fournir votre adresse de courriel?

Questionnaire relatif à la santé buccale et aux échantillons de salive

Feuille de renseignements et Formulaire de consentement

L'incidence de la consommation de la chicha sur l'expression génétique des muqueuses.

Chercheur principal : ***** Ph.D., 613-562-5800, poste ****

Commanditaire : Commanditaire actuellement recherché

Introduction

Vous êtes invité à prendre part à ce projet de recherche en répondant à un bref questionnaire et en fournissant deux échantillons de salive parce que vous vous êtes identifié comme un consommateur de la chicha et que vous êtes âgé de 18 à 25 ans.

Veillez prendre connaissance de la Feuille de renseignements et du Formulaire de consentement à l'intention du patient, et n'hésitez pas à poser toutes vos questions avant de décider si vous souhaitez ou non prendre part à cette étude de recherche. Nous vous invitons à discuter de votre décision avec les membres de votre famille, vos amis et votre équipe de soins de la santé.

Contexte

Récemment, on aura constaté dans les pays occidentaux une augmentation inexplicable de la consommation de la chicha, particulièrement chez les jeunes adultes. L'augmentation de la popularité de la chicha chez cette population est possiblement attribuable à la croyance que la chicha s'avérerait la façon la plus saine de fumer le tabac. À ce jour, nous ne disposons pas d'une base de connaissances suffisamment solide au sujet des effets indésirables sur la santé associés à la chicha.

Procédures de l'étude

Une étude de comparaison avant-après est proposée pour évaluer l'incidence à court terme de la consommation de la chicha sur l'expression génétique. Cette partie de l'étude s'effectuera au site. Treize sujets ont été recrutés pour répondre à un bref questionnaire de santé oral, lequel devrait prendre environ cinq minutes. Nous vous demanderons également de fournir deux échantillons de salive : un avant de fumer la chicha, et un autre après avoir fumé la chicha (environ 45 minutes plus tard).

Durée de l'étude

L'ensemble de cette étude devrait prendre environ six mois à compléter. Toutefois, votre participation même avec nous exigera environ une demi-heure pour la première partie, et seuls les sujets invités à la deuxième partie auront ensuite à passer une autre heure avec nous.

Effets secondaires ou risques possibles

Votre participation à cette étude ne comporte aucun risque ou effet secondaire.

Bienfaits reliés à l'étude

Il est possible que vous ne retiriez aucun avantage direct en prenant part à cette étude. Votre participation à cette recherche permettrait au chercheur d'en apprendre davantage au sujet des effets indésirables associés à la fumée de la chicha.

Retrait de l'étude

Vous avez le droit de retirer votre consentement en tout temps, sans que votre décision n'entraîne de pénalités.

Coûts relatifs à l'étude

Vous ne serez pas rémunéré pour votre participation à cette étude de recherche. Toutefois, on vous remboursera les frais de stationnement et d'autobus, ainsi que toute dépense reliée à cette étude.

Confidentialité

Tout renseignement personnel sur la santé sera maintenu confidentiel, à moins que la loi n'exige leur divulgation. Des représentants du Conseil d'éthique en recherches de L'Hôpital d'Ottawa, ainsi que de l'Institut de recherche de l'Hôpital d'Ottawa, pourront procéder à l'examen de vos dossiers médicaux originaux, sous la supervision du personnel du docteur *****, uniquement à des fins de vérification.

Aucune publication ou présentation résultant de cette étude ne pourra servir à vous identifier. Aucune information pouvant servir à vous identifier ne sera transmise à l'extérieur de l'Université d'Ottawa. Tout renseignement transmis à l'extérieur de l'Université sera codé à l'aide d'un numéro d'étude indépendant.

Seuls le docteur ***** ou son personnel pourront accéder au lien entre votre nom et le numéro de l'étude indépendant. Ce lien et les dossiers de l'étude seront entreposés en lieu sûr et séparément. Ces dossiers seront conservés pour une période de 15 années suivant la fin de l'étude. Tout dossier papier sera entreposé dans un cabinet ou un bureau verrouillé à l'Université d'Ottawa.

Le lien et les dossiers de l'étude seront entreposés en lieu sûr et séparé. Le tout sera conservé pour une période de 15 ans suivant la fin de l'étude. Les fichiers électroniques quant à eux seront stockés et protégés par un mot de passe, auquel seuls le docteur ***** ou son personnel auront accès. Une fois la période de rétention terminée, tous les dossiers papier seront déchiquetés ou jetés aux rebuts confidentiels, et les fichiers électroniques seront supprimés.

Participation volontaire

Votre participation à cette étude s'effectue sur une base volontaire.

Questions au sujet de l'étude

Pour toute question au sujet de l'étude, veuillez communiquer avec *****, candidate à la maîtrise, soit par courriel (*****@uottawa.ca) ou téléphone, au 613-562-5800, poste ****. Si vous souhaitez parler au chercheur principal, le docteur *****, veuillez composer le 613-562-5800, poste ****.

Le Conseil d'éthique en recherches de L'Hôpital d'Ottawa (CÉRHO) a révisé ce protocole. Le CÉRHO est chargé de l'ensemble des aspects éthiques de toutes les études de recherche menées auprès de sujets humains effectuées à L'Hôpital d'Ottawa. Pour toute question au sujet de vos droits à titre de sujet de recherche, veuillez communiquer avec le président du Conseil d'éthique en recherches de L'Hôpital d'Ottawa, au 613-798-5555, poste 14902.

Formulaire de consentement

L'incidence de la consommation de la chicha sur l'expression génétique des muqueuses.

Consentement à la participation à la recherche

Je reconnais que l'on sollicite ma participation à une étude de recherche en vue d'évaluer les effets indésirables sur la santé associés à la consommation de la chicha. ***** m'a fourni les renseignements au sujet de cette étude.

J'ai pris connaissance des 6 pages de cette Feuille de renseignements et de ce Formulaire de consentement à l'intention du patient. On a répondu à toutes mes questions de manière satisfaisante. Si je décide plus tard au cours de l'étude de retirer mon consentement, il me sera possible de le faire en tout temps.

Je consens volontairement à prendre part à cette étude en complétant un questionnaire de santé oral et en fournissant deux échantillons de salive, soit un avant de fumer la chicha, et un autre après avoir fumé la chicha. Ceci devrait prendre environ 45 minutes de mon temps, y compris le temps requis pour une séance de chicha.

On me remettra un exemplaire signé de cette Feuille de renseignements et de ce Formulaire de consentement.

Signatures

Nom du participant (en caractères d'imprimerie)

Signature du participant

Date

Énoncé du chercheur (ou de la personne chargée d'obtenir le consentement)

J'ai expliqué soigneusement au participant de la recherche la nature de l'étude susmentionnée. Pour autant que je sache, le participant apposant sa signature à ce consentement reconnaît la nature, les exigences, les risques et les avantages que comporte sa participation à l'étude. Je reconnais ma responsabilité envers le soin et le bien-être du participant susmentionné, le respect des droits et des désirs de ce dernier, et le déroulement de cette étude, conformément aux directives et aux règlements relatifs à la bonne pratique clinique.

Nom du chercheur/délégué (en caractères d'imprimerie)

Signature du chercheur/délégué

Date

Questionnaire sur la santé bucco-dentaire

1. Numéro d'identification (fourni par le chercheur)

2. Avez-vous un mal de dents?

☐ Oui☐ Non

Si oui, est-ce que vous éprouvez de la douleur en mangeant ou en buvant quelque chose?

☐ Chaud☐ Froid☐ Les deux

Pouvez-vous indiquer quelle dent?

3. Vos gencives saignent-elles lorsque vous vous brossez les dents?

☐ Oui, habituellement☐ Non, rarement☐ Oui, rarement☐ Non

4. Recevez-vous actuellement un traitement dentaire?

☐ Oui☐ Non

5. À quand remonte votre dernière visite chez le dentiste?

☐ Semaine dernière☐ 2-4 semaines☐ 2-3 mois☐ 4-6 mois☐ 7-12 mois☐ Plus de 12 mois

6. Prenez-vous des médicaments?

☐ Oui☐ Non

Si oui, veuillez indiquer le nom et la fréquence de la prise de celui-ci _____

Appendix B: Primary and oral health questionnaires – English versions

Primary questionnaire

Information Sheet and Consent Form

The impact of smoking sheesha on mucosal gene expression

Principal Investigator: *****, PhD; 613-562-5800 ext. ****

Funding: presently being sought

Introduction

You are being asked to participate in this research project by completing a questionnaire because you have identified yourself as a sheesha smoker between the ages of 18-25.

Please read this Information Sheet and Consent Form carefully and ask as many questions as you like before deciding whether to participate in this research study. You can discuss this decision with your family, friends and your health-care team.

Background,

Recently, Western countries witnessed an unexplained sharp rise in sheesha smoking especially among young adults. The increase in popularity of sheesha among young people is possibly attributed to the belief that sheesha is the healthiest way to smoke tobacco. So far we have no solid knowledge about the adverse health effects of sheesha.

Purpose of the Study

You are being asked to fill out a questionnaire regarding personal information, attitude and belief about the sheesha smoking. The purpose of this questionnaire is to understand the characteristics, pattern of use, and habits of sheesha tobacco smokers who are between the ages of 18-25.

We will use the information gathered from 30 questionnaires to generate a framework to be used to recruit people in the second part of this study. The second part aimed to assess the adverse health effect of sheesha tobacco smoke by seeking gene expressions performance. It is also intended that the questionnaire developed and tested for this purpose could be used in the future to assess the prevalence and determinants of sheesha use in young people, after distributing it to representative sample of this population.

Study Duration

The questionnaire should take approximately 30 minutes to complete.

Possible Side Effects and/or Risks

There are no risks or side effects when participating in this study.

Benefits of the Study

You may not receive any direct benefit from your participation in this study. Your participation in this research may allow the researchers to gain a better understanding of the adverse effects of sheesha smoking.

Withdrawal from the Study

You may withdraw from this project at any time and there will be no penalty to you.

Study Costs

You will not be paid to participate in this research study. However you will be reimbursed for parking or bus tickets, and for any expenses that are directly related to this study.

Confidentiality

All personal health information will be kept confidential, unless release is required by law. Representatives of the Ottawa Hospital Research Ethics Board, as well as the Ottawa Hospital Research Institute, may review your original records under the supervision of Dr. ***** for audit purposes.

You will not be identifiable in any publications or presentations resulting from this study. No identifying information will leave the premises of the University of Ottawa. All information which leaves the premises of the University will be coded with an independent study number.

The link between your name and the independent study number will only be accessible by Dr. ***** and his research staff. All completed questionnaires will be stored in a locked filing cabinet and kept for 15 years after completion of the study. All paper records will be stored in a locked file cabinet at the University of Ottawa. The link and study files will be stored separately and securely. Both files will be kept for a period of 15 years after the study has been completed. All electronic records will be stored and protected by a user password, again only accessible by Dr. ***** and his research staff. At the end of the retention period, all paper records will be disposed of in confidential waste or shredded, and all electronic records will be deleted.

Voluntary Participation

Your participation in this study is voluntary.

Questions about the Study

If you have questions about this study, please contact *****, MSc candidate by e-mail at *****@uottawa.ca or telephone 613-562-5800 extension ****. If you would like to speak to the principal investigator, Dr *****, please call 613-562-5800 extension ****.

The Ottawa Hospital Research Ethics Board (OHREB) has reviewed this protocol. The OHREB considers the ethical aspects of all research studies involving human subjects at The Ottawa Hospital. If you have any questions about your rights as a research subject, you may contact the Chairperson of the Ottawa Hospital Research Ethics Board at 613-798-5555, extension 14902.

Consent Form

The impact of smoking sheesha on mucosal gene expression

Consent to Participate in Research

I understand that I am being asked to participate in a research study about assessing the adverse health effect of sheesha smoking. This study has been explained to me by *****.

I have read the 6 page Information Sheet and Consent Form. All my questions have been answered to my satisfaction.

I voluntarily agree to participate in this study by completing the questionnaire.

A copy of the signed Information Sheet and Consent Form has been provided to me.

Signatures

Participant's Name (Please Print)

Participant's Signature

Date

Investigator Statement (or Person Explaining the Consent)

I have carefully explained to the research participant the nature of the above research study. To the best of my knowledge, the research participant signing this consent form understands the nature, demands, risks and

benefits involved in participating in this study. I acknowledge my responsibility for the care and well being of the above research participant, to respect the rights and wishes of the research participant, and to conduct the study according to applicable guidelines and regulations.

Name of Investigator/Delegate (Please Print)

Signature of Investigator/Delegate

Date

Primary questionnaire

Recently, an increase in sheesha use has been observed in some countries, especially among young adults. We would like to know more about sheesha use in Canada, and it would really help us if you would complete this questionnaire.

We suggest you read through the following questionnaire first, before completing it. At the end of the questionnaire, we have asked for your comments and feedback on the questionnaire.

The information from your questionnaire will be analysed together with the information from questionnaires completed by other people. If you would like to know the results of our study, please let us know in the question we ask at the end of the questionnaire.

Thank you in advance.

Information on this page will be kept confidential. We are asking you to provide some contact information (e.g. name, telephone number) that we will use to keep in touch with you, should we select you to provide us saliva sample. This information will be stored separately and securely from the other data you give us. It will not be possible to identify your responses in the reports and publications we will produce from this study.

3. ID number: (provided by researcher)

4. Are you a current student?

☐ No ☐ Yes

If yes, please provide the name of your institution

Please provide the following information:

32. What is your age?

33. Are you:

☐ Male ☐ Female

34. Were you born in Canada?

☐ Yes ☐ No. **If no, please tell us in what country you were born**

35. Was your mother born in Canada?

☐ Yes ☐ No ☐ Prefer not to answer

If no, please tell us in what country she was born

36. Was your father born in Canada?

☐ Yes ☐ No ☐ Prefer not to answer

If no, please tell us in what country he was born

37. What is the highest level of education you have completed?

- ☐ Didn't finish high school
- ☐ High school grad
- ☐ Didn't finish college
- ☐ College grad
- ☐ University grad
- ☐ Post grad degree

38. Have you ever tried or experimented with smoking (even one or two inhalations)?

Cigarette ☐Yes ☐No
 Sheesha ☐Yes ☐No
 Smokeless (chewing tobacco) ☐Yes ☐No

39. Have you ever smoked cigarettes for a period of at least six months?

☐Yes ☐No

40. How would you describe your alcohol consumption?

☐Usually, I drink alcohol monthly (at least once a month but less than weekly).

☐Usually, I drink alcohol weekly (at least once a week but less than daily).

☐Usually, I drink alcohol daily (at least once a day or on most days of the month).

☐Usually, I drink alcohol when I smoke sheesha.

☐Usually, I drink alcohol when I smoke cigarettes.

☐I do not drink alcohol.

41. How old were you when you first tried a sheesha?

☐7 years old or younger

☐8 years old

☐9 years old

☐10 years old

☐11 years old

☐12 years old

☐13 years old

☐14 years old

☐15 years old

☐16 years old or older

42. Who introduced sheesha use to you?

☐Parent(s)

☐Friend(s)

☐Other(s)

43. Which of the following best describes your sheesha use?

☐ Usually, I smoke a sheesha monthly (at least once a month but less than

weekly)

☐ Usually, I smoke a sheesha weekly (at least once a week but less than

daily)

☐ Usually, I smoke a sheesha daily (at least once a day or on most days of the month.

☐ I do not smoke sheesha.

44. Have you smoked sheesha regularly (at least once a month for six consecutive months in the past)?

☐ Yes ☐ No

If no please go to #26

45. In which level of education were you when you started to smoke a sheesha regularly?

☐ High School student

☐ College student

☐ University student

46. On average, how many fillings (headfuls, nafas, or ras) do you light in each session?

☐ 1

☐ 2-3

☐ More than 3

47. On average how long does each session last?

☐ 1 hour

☐ 2 hours

☐ More than 2 hours

48. Are you a regular patron of sheesha bars?

☐ Yes ☐ No

49. If yes, could please tell us the location.

50. In the past 30 days, what brand of timbak (tobacco filling used in sheesha) did you smoke?

☐ Nicotine free herbal product

☐ Tobacco Massal

☐ Asfahani/ Ajami

☐ I do not have a preference / unsure

Please specify the brand name and aroma _____

51. Do you usually share the same sheesha with others?

☐ No

☐ Yes

If yes, do you use plastic extension?

☐ Yes

☐ No

52. Do your parents smoke?

Mother

☐ cigarettes

☐ Yes

☐ No

☐ I don't know

☐ sheesha

☐ Yes

☐ No

☐ I don't know

Father

☐ cigarettes

☐ Yes

☐ No

☐ I don't know

☐ sheesha

☐ Yes

☐ No

☐ I don't know

53. Have you ever thought of stopping sheesha use?

☐ Yes

☐ No

54. Do you intend to stop using the sheesha?

☐ Not at all

☐ In the next month

☐ In the next 6 months

☐ In the future

55. Have you ever received help or advice to help you stop smoking sheesha?

☐ Yes, from a program or professional

☐ Yes, from a friend

☐ Yes, from a family member

☐ No

56. If you consume nicotine free timbak, do you use it because

☐ I do not use nicotine free timbak

☐ It is safer than timbak with nicotine

☐ It is cheaper than timbak with nicotine

☐ It is available

☐ Other reasons please specify

57. Do you think sheesha smoking is harmful to your health?

☐ Definitely not

☐ Probably not

☐ Probably yes

☐ Definitely yes

58. Do you think the water in the sheesha filters potentially harmful substances?

☐ Yes

☐ No

☐ I have no idea

59. How do you compare the risk of cigarette smoking to that of sheesha smoking?

☐ Adverse health effect from cigarettes is higher than the adverse health effect from sheesha

☐ Adverse health effect from cigarettes is lower than the adverse health effect from sheesha

☐ Adverse health effect from cigarettes is equal to the adverse health effect from sheesha

60. Do you think that smoking a sheesha helps people feel comfortable at celebrations, parties, or in other social gatherings?

☐ Yes, it makes them feel more comfortable.

☐ No, it makes them feel less comfortable.

☐ It doesn't make any difference.

61. Once someone has started smoking a sheesha, do you think it would be difficult to quit?

☐ Definitely not.

☐ Probably not.

☐ Probably yes.

☐ Definitely yes.

62. Would you be willing to provide a saliva sample?

☐ Yes. ☐ No. If yes please your name, telephone number, and email

Your comments and feedback

Thank you again for completing our questionnaire. We would really appreciate it if you could give us some comments and feedback.

Were all the questions clear/easy to understand?

What is the most appropriate term to be used “Smoking sheesha or “drinking sheesha”?

What is the best term applied to Sheesha, for example Argyleh, Waterpipe , Gooza, Hookah, Hubble Bubble ?

Did you find the appropriate optional response for all of the questions asked?

Was (were) there any question(s) you think it would be better not to ask and, if so, why?

Is (are) there any question(s) that needs to be rephrased?

Do you think there is a question(s) that would help us understand more about waterpipe smoking among young people that we should ask and haven't asked? Please specify.

Do you have additional comments?

I would like to have a copy of the results of this study.

☐ Yes ☐ No

If yes please provide us your email address

Oral health questionnaire and saliva sample

Information Sheet and Consent Form

The impact of smoking sheesha on mucosal gene expression

Principal Investigator: ***** , PhD 613-562-5800 ext. ****
Sponsor: Funding pending

Introduction

You are being asked to participate in this research project by completing a brief questionnaire and providing two saliva samples because you have identified yourself as a sheesha smoker between the ages of 18-25.

Please read this Information Sheet and Consent Form carefully and ask as many questions as you like before deciding whether to participate in this research study. You can discuss this decision with your family, friends and your health-care team.

Background

Recently, Western countries witnessed an unexplained sharp rise in sheesha smoking especially among young adults. The increase in popularity of sheesha among young people is possibly attributed to the belief that sheesha is the healthiest way to smoke tobacco. So far we have no solid knowledge about the adverse health effects of sheesha.

Study Procedures

A before-after comparison study is proposed to assess the short-term impact of smoking sheesha on gene expression. This part of the study is done on site. Thirteen people have been recruited to complete a brief oral health questionnaire, which should take approximately five minutes to complete. We also ask that you provide two saliva samples: one prior to smoking sheesha and another right after smoking one sheesha (approximately 45 minutes later).

Study duration

The entire study supposes to take around 6 months. However individual involvement will spend with us approximately half an hour in the first part and only those who have been allocated to the second part will be asked to spend one more hour with us.

Possible Side Effects and/or Risks

There are no risks or side effects when participating in this study.

Benefits of the Study

You may not receive any direct benefit from your participation in this study. Your participation in this research may allow the researchers to gain a better understanding of the adverse effects of sheesha smoking.

Withdrawal from the Study

You may withdraw from this project at any time and there will be no penalty to you.

Study Costs

You will not be paid to participate in this research study. However you will be reimbursed for parking or bus tickets, and for any expenses that are directly related to this study.

Confidentiality

All personal health information will be kept confidential, unless release is required by law. Representatives of the Ottawa Hospital Research Ethics Board, as well as the Ottawa Hospital Research Institute, may review your original records under the supervision of Dr. ***** for audit purposes.

You will not be identifiable in any publications or presentations resulting from this study. No identifying information will leave the premises of the University of Ottawa. All information which leaves the premises of the University will be coded with an independent study number.

The link between your name and the independent study number will only be accessible by Dr. ***** and his research staff. All completed questionnaires will be stored in a locked filing cabinet and kept for 15 years after completion of the study. All paper records will be stored in a locked file cabinet at the University of Ottawa. The link and study files will be stored separately and securely. Both files will be kept for a period of 15 years after the study has been completed. All electronic records will be stored and protected by a user password, again only accessible by Dr. ***** and his research staff. At the end of the retention period, all paper records will be disposed of in confidential waste or shredded, and all electronic records will be deleted.

Voluntary Participation

Your participation in this study is voluntary.

Questions about the Study

If you have questions about this study, please contact *****, MSc candidate by e-mail at *****@uottawa.ca or 613-562-5800 extension ****. If you would like to speak to the principal investigator, Dr *****, please call 613-562-5800 extension ****.

The Ottawa Hospital Research Ethics Board (OHREB) has reviewed this protocol. The OHREB considers the ethical aspects of all research studies involving human subjects at The Ottawa Hospital. If you have any questions about your rights as a research subject, you may contact the Chairperson of the Ottawa Hospital Research Ethics Board at 613-798-5555, extension 14902.

Consent Form

The impact of smoking sheesha on mucosal gene expression

Consent to Participate in Research

I understand that I am being asked to participate in a research study about assessing the adverse health effect of sheesha smoking. This study has been explained to me by
*****.

I have read the 4 page Information Sheet and Consent Form. All my questions have been answered to my satisfaction. If I decide at a later stage in the study that I would like to withdraw my consent, I may do so at any time.

I voluntarily agree to participate in this study by completing the oral health questionnaire and providing a saliva sample before smoking sheesha and a second saliva sample right after smoking one sheesha. This might take 45 minutes of my time, including the time spent during one session of sheesha smoking.

A copy of the signed Information Sheet and Consent Form has been provided to me.

Signatures

Participant's Name (Please Print)

Participant's Signature

Date

Investigator Statement (or Person Explaining the Consent)

I have carefully explained to the research participant the nature of the above research study. To the best of my knowledge, the research participant signing this consent form understands the nature, demands, risks and benefits involved in participating in this study. I acknowledge my responsibility for the care and well being of the above research participant, to respect the rights and wishes of the research participant, and to conduct the study according to applicable guidelines and regulations.

Name of Investigator/Delegate (Please Print)

Oral health Questionnaire

1. ID number: (provided by researcher)

2. _____
Do you currently have a toothache/feel pain in any tooth?

☐ Yes ☐ No,

if yes does it hurt when you eat or drink something

☐ Hot ☐ Cold ☐ Both

Could you please indicate which tooth

3. Do your gums bleed whenever you brush your teeth?

☐ Yes, usually

☐ Yes, seldom

☐ Yes, rarely

☐ No

4. Are you currently undergoing dental treatment?

☐ Yes

☐ No

5. When did you last see a dentist?

☐ Last week

☐ 2-4 weeks ago

☐ 2-3 months ago

☐ 4-6 months ago

☐ 7-12 months ago

☐ More than Twelve months ago

6. Are you currently taking any medication?

☐ Yes ☐ No, if yes please write down its name and how often
you take it _____

Appendix C: Fold changes in gene expression after sheesha smoking compared with before – average across three repeat assays, by subject

Position	Gene Symbol	Fold Change AA17			Fold Change CD12			Fold Change DT6		
		After vs before smoking	95% CI	Comments*	After vs before smoking	95% CI	Comments	After vs before smoking	95% CI	Comments
1	PTN	0.37	(0.06, 0.68)	B	0.42	(0.00001, 1.55)	B	0	(0.00001, 0.00)	A
2	CDKN2A	0.03	(0.00001, 0.08)	OKAY	0.49	(0.00001, 1.33)	B	0	(0.00001, 0.00)	OKAY
3	CCND1	0.41	(0.13, 0.69)	OKAY	1.45	(0.00001, 4.98)	B	0.03	(0.00001, 0.08)	OKAY
4	NMI	1.22	(0.96, 1.48)	A	0.95	(0.00001, 3.00)	B	1.57	(0.91, 2.23)	OKAY
5	STAT3	0.44	(0.35, 0.53)	OKAY	1.45	(0.00001, 5.97)	B	3.09	(1.63, 4.55)	A
6	BCL2	0.31	(0.07, 0.55)	OKAY	0.51	(0.00001, 1.40)	B	0.18	(0.08, 0.28)	OKAY
7	ERBB2	0.01	(0.00001, 0.04)	OKAY	0.4	(0.00001, 1.05)	B	0	(0.00001, 0.00)	OKAY
8	EGFR	0.1	(0.04, 0.16)	OKAY	0.46	(0.00001, 1.22)	C	0	(0.00001, 0.00)	OKAY
9	NOS2	0.19	(0.07, 0.31)	OKAY	0.52	(0.00001, 1.60)	B	0	(0.00001, 0.00)	A
10	PTGS2	0.39	(0.29, 0.49)	OKAY	1.7	(0.00001, 8.23)	B	2.48	(1.33, 3.63)	OKAY
11	FAS	0.75	(0.52, 0.98)	A	0.79	(0.00001, 2.75)	B	0	(0.00001, 0.00)	OKAY
12	BAK1	0.13	(0.04, 0.22)	OKAY	0.44	(0.00001, 1.15)	B	0.04	(0.02, 0.06)	A
13	CNTD1	0.16	(0.00001, 0.72)	B	0.69	(0.00001, 1.78)	B	0	(0.00001, 0.00)	OKAY
14	CYP1B1	0.18	(0.00001, 0.81)	B	0.46	(0.00001, 1.22)	C	0	(0.00001, 0.00)	OKAY
15	CYP1A1	0.13	(0.05, 0.21)	OKAY	0.59	(0.00001, 1.67)	B	0	(0.00001, 0.00)	A
16	GSTP1	0.23	(0.12, 0.34)	OKAY	0.4	(0.00001, 1.61)	B	0.15	(0.02, 0.28)	OKAY
17	GSTT1	0.05	(0.03, 0.07)	OKAY	0.46	(0.00001, 1.22)	C	0.04	(0.02, 0.06)	OKAY
18	GSTM2	0.49	(0.00001, 1.06)	B	0.34	(0.00001, 1.22)	B	0.01	(0.01, 0.01)	OKAY
19	GAPDH	1.09	(0.84, 1.34)	A	1.97	(1.51, 2.43)	OKAY	0.11	(0.08, 0.14)	OKAY
20	ACTB	0.92	(0.71, 1.13)	OKAY	0.51	(0.39, 0.63)	OKAY	9.19	(6.75, 11.63)	A
21	HGDC	0.02	(0.00001, 0.06)	OKAY	0.36	(0.00001, 1.01)	B	0	(0.00001, 0.00)	A

Position	Gene Symbol	Fold Change HE7			Fold Change KB13			Fold Change KH11		
		After vs before smoking	95% CI	Comments*	After vs before smoking	95% CI	Comments	After vs before smoking	95% CI	Comments
1	<i>PTN</i>	1.94	(0.00001, 4.63)	B	0.17	(0.08, 0.26)	OKAY	7.78	(0.00001, 25.94)	B
2	<i>CDKN2A</i>	0.79	(0.00001, 2.36)	B	0.38	(0.12, 0.64)	A	5.98	(0.00001, 23.47)	B
3	<i>CCND1</i>	0.55	(0.00001, 1.47)	B	0.07	(0.02, 0.12)	OKAY	12.51	(0.00001, 37.43)	B
4	<i>NMI</i>	1.15	(0.00001, 2.88)	OKAY	9.23	(7.30, 11.16)	A	13.74	(0.00001, 35.89)	B
5	<i>STAT3</i>	0.84	(0.00001, 2.28)	OKAY	2.63	(2.18, 3.08)	A	33.59	(0.00001, 172.22)	B
6	<i>BCL2</i>	0.09	(0.02, 0.16)	OKAY	1.71	(0.00001, 7.21)	B	5.51	(0.00001, 12.91)	B
7	<i>ERBB2</i>	5.27	(2.66, 7.88)	OKAY	0.09	(0.00001, 0.31)	OKAY	9.85	(0.00001, 28.63)	B
8	<i>EGFR</i>	0.01	(0.00001, 0.08)	B	0.01	(0.00001, 0.03)	A	8.6	(0.00001, 25.63)	B
9	<i>NOS2</i>	5.27	(0.00001, 15.80)	B	0.11	(0.05, 0.17)	A	9.51	(1.73, 17.29)	OKAY
10	<i>PTGS2</i>	1.07	(0.31, 1.83)	OKAY	8.78	(6.12, 11.44)	A	2.97	(0.00001, 12.09)	B
11	<i>FAS</i>	11.99	(0.00001, 57.57)	A	0.21	(0.00001, 0.76)	B	5.05	(0.00001, 15.62)	B
12	<i>BAK1</i>	0.9	(0.00001, 2.97)	B	0.31	(0.23, 0.39)	A	3.53	(0.00001, 16.28)	B
13	<i>CNTD1</i>	0.2	(0.00001, 0.70)	B	0	(0.00001, 0.00)	A	8.01	(0.00001, 20.80)	B
14	<i>CYP1B1</i>	0.43	(0.00001, 1.34)	B	0.09	(0.00001, 0.25)	OKAY	6.63	(0.00001, 20.32)	B
15	<i>CYP1A1</i>	17.8	(9.80, 25.80)	OKAY	1.08	(0.00001, 5.07)	B	30.22	(0.00001, 149.65)	B
16	<i>GSTP1</i>	2.64	(0.00001, 13.20)	A	18.43	(0.00001, 85.95)	A	13.43	(0.00001, 38.38)	B
17	<i>GSTT1</i>	23.32	(12.20, 34.44)	OKAY	0.43	(0.28, 0.58)	OKAY	10.39	(0.00001, 31.12)	C
18	<i>GSTM2</i>	0.15	(0.00001, 0.33)	B	0.53	(0.31, 0.75)	OKAY	4.85	(0.00001, 12.49)	B
19	<i>GAPDH</i>	1.37	(0.89, 1.85)	A	0.11	(0.09, 0.13)	A	2.53	(0.37, 4.69)	B
20	<i>ACTB</i>	0.73	(0.48, 0.98)	OKAY	9.39	(7.82, 10.96)	OKAY	0.4	(0.06, 0.74)	B
21	<i>HGDC</i>	5.97	(0.00001, 14.09)	B	0.66	(0.00001, 1.79)	B	4.11	(0.00001, 11.64)	B

Position	Gene Symbol	Fold Change NA2			Fold Change PB10			Fold Change PP9		
		After vs before smoking	95% CI	Comments*	After vs before smoking	95% CI	Comments	After vs before smoking	95% CI	Comments
1	PTN	2456.69	(0.00001, 34810.48)	B	0.02	(0.00001, 0.04)	A	0.03	(0.00001, 0.12)	OKAY
2	CDKN2A	0.96	(0.00001, 12.43)	A	0.04	(0.00001, 0.15)	B	0.01	(0.00001, 0.04)	OKAY
3	CCND1	19745219	(0.00001, 220307136.83)	OKAY	0.23	(0.00001, 0.60)	B	0.13	(0.00001, 0.59)	B
4	NMI	8.17E+08	(0.00001, 10643683640.33)	A	1.34	(0.00001, 3.97)	B	0.16	(0.00001, 0.73)	A
5	STAT3	15146698	(0.00001, 121322903.88)	A	1.11	(0.00001, 2.26)	B	0.17	(0.00001, 0.75)	A
6	BCL2	0.26	(0.00, 0.52)	B	0.22	(0.09, 0.35)	OKAY	0.35	(0.00001, 1.65)	B
7	ERBB2	1.36E+09	(0.00001, 16917834527.18)	A	0.34	(0.00001, 0.71)	B	0.03	(0.00001, 0.14)	OKAY
8	EGFR	15927318	(0.00001, 164080239.82)	OKAY	3.23	(0.00001, 12.65)	B	0.02	(0.00001, 0.07)	OKAY
9	NOS2	759663	(0.00001, 10728237.88)	A	0.1	(0.03, 0.17)	OKAY	0.01	(0.00001, 0.04)	OKAY
10	PTGS2	149.34	(0.00001, 1926.42)	OKAY	1.5	(0.82, 2.18)	OKAY	0.2	(0.00001, 0.91)	OKAY
11	FAS	3.97E+09	(0.00001, 28840099395.44)	A	0.96	(0.00001, 3.52)	B	0.22	(0.00001, 1.01)	A
12	BAK1	1131653	(0.00001, 12158231.39)	A	0.18	(0.07, 0.29)	OKAY	0.02	(0.00001, 0.10)	OKAY
13	CNTD1	2036274	(0.00001, 28203463.08)	OKAY	0.55	(0.00001, 2.28)	B	0	(0.00001, 0.00)	OKAY
14	CYP1B1	207.94	(0.00001, 3476.21)	OKAY	1	(0.00001, 3.20)	B	0	(0.00001, 0.00)	OKAY
15	CYP1A1	7.25	(0.00001, 83.18)	A	0.17	(0.00001, 0.39)	OKAY	0.15	(0.00001, 0.65)	B
16	GSTP1	3.52	(0.00001, 7.95)	B	1.61	(0.00001, 4.19)	B	0.05	(0.00001, 0.23)	A
17	GSTT1	18.64	(0.00001, 232.57)	OKAY	2.03	(0.00001, 6.42)	B	0.02	(0.00001, 0.09)	OKAY
18	GSTM2	3743.05	(0.00001, 54223.26)	B	0.05	(0.00001, 0.12)	B	0.03	(0.00001, 0.14)	B
19	GAPDH	0.34	(0.00001, 0.84)	B	0.37	(0.23, 0.51)	A	5.6	(0.00001, 24.92)	A
20	ACTB	2.95	(0.00001, 7.26)	B	2.69	(1.65, 3.73)	A	0.18	(0.00001, 0.80)	OKAY
21	HGDC	2.68E+09	(0.00001, 35679097782.29)	A	0.12	(0.00001, 0.49)	B	0.01	(0.00001, 0.05)	OKAY

Position	Gene Symbol	Fold Change RR5			Fold Change SA16			Fold Change SAH15		
		After vs before smoking	95% CI	Comments*	After vs before smoking	95% CI	Comments	After vs before smoking	95% CI	Comments
1	PTN	0.84	(0.00001, 3.78)	B	14.89	(0.00001, 53.06)	B	3.48	(0.26, 6.70)	OKAY
2	CDKN2A	0.52	(0.00001, 2.01)	B	60.71	(0.00001, 228.25)	B	6.19	(0.00001, 21.34)	B
3	CCND1	0.44	(0.00001, 1.26)	B	26.98	(0.00001, 76.01)	B	0.4	(0.06, 0.74)	B
4	NMI	9.06	(0.00001, 19.28)	OKAY	0.04	(0.00001, 0.08)	OKAY	2.42	(1.78, 3.06)	OKAY
5	STAT3	0.81	(0.00001, 2.47)	B	0.25	(0.00, 0.50)	OKAY	1.28	(0.97, 1.59)	OKAY
6	BCL2	0.97	(0.00001, 2.81)	B	9.33	(0.54, 18.12)	B	0.44	(0.20, 0.68)	A
7	ERBB2	0.46	(0.00001, 1.43)	B	3.22	(0.00001, 12.60)	B	5.48	(0.00001, 15.82)	B
8	EGFR	0.54	(0.00001, 3.27)	B	0.94	(0.00001, 3.80)	B	7.17	(0.00001, 26.97)	OKAY
9	NOS2	1.27	(0.00001, 6.97)	B	19.99	(0.00001, 70.90)	B	24.85	(0.00001, 59.47)	OKAY
10	PTGS2	0.74	(0.00001, 3.68)	B	0.19	(0.00001, 0.61)	A	1.29	(1.15, 1.43)	OKAY
11	FAS	0.62	(0.00001, 1.65)	B	0.03	(0.00001, 0.06)	OKAY	1.33	(1.17, 1.49)	A
12	BAK1	4.62	(0.00001, 21.34)	B	2.16	(0.00001, 6.55)	B	5.52	(3.08, 7.96)	OKAY
13	CNTD1	0.93	(0.00001, 2.29)	B	0.35	(0.00001, 0.72)	B	75.45	(0.00001, 167.05)	OKAY
14	CYP1B1	0.25	(0.00001, 0.96)	B	0.75	(0.00001, 2.86)	B	56.1	(0.00001, 177.37)	OKAY
15	CYP1A1	0.67	(0.13, 1.21)	B	19.01	(0.89, 37.13)	OKAY	1.08	(0.53, 1.63)	B
16	GSTP1	0.66	(0.00001, 1.43)	B	22.49	(0.00001, 100.40)	B	2.43	(1.19, 3.67)	OKAY
17	GSTT1	0.6	(0.00001, 1.58)	C	38.15	(0.00001, 144.08)	B	60.34	(0.00001, 281.95)	OKAY
18	GSTM2	0.85	(0.00001, 2.66)	B	0.97	(0.00001, 2.42)	B	0.47	(0.01, 0.93)	B
19	GAPDH	0.58	(0.00001, 1.24)	B	0.65	(0.40, 0.90)	A	1.44	(1.29, 1.59)	OKAY
20	ACTB	1.73	(0.00001, 3.69)	B	1.53	(0.95, 2.11)	B	0.7	(0.63, 0.77)	OKAY
21	HGDC	5.63	(0.00001, 21.75)	B	2.16	(0.11, 4.21)	B	13.86	(0.00001, 30.94)	OKAY

Position	Gene Symbol	Fold Change SF1			Fold Change SM8			Fold Change TP3		
		After vs before smoking	95% CI	Comments*	After vs before smoking	95% CI	Comments	After vs before smoking	95% CI	Comments
1	PTN	42.63	(0.00001, 125.51)	OKAY	15.41	(0.00001, 33.65)	OKAY	0.54	(0.00001, 1.44)	B
2	CDKN2A	21788.06	(6059.45, 37516.67)	OKAY	0.75	(0.08, 1.42)	B	0.13	(0.00001, 0.45)	B
3	CCND1	57.13	(0.00001, 136.54)	B	3.28	(0.00001, 8.08)	B	0.48	(0.06, 0.90)	B
4	NMI	465.05	(269.36, 660.74)	OKAY	1.68	(1.26, 2.10)	OKAY	1.01	(0.70, 1.32)	A
5	STAT3	17.16	(11.11, 23.21)	OKAY	0.87	(0.59, 1.15)	OKAY	0.87	(0.63, 1.11)	OKAY
6	BCL2	71.82	(10.56, 133.08)	OKAY	4.22	(0.94, 7.50)	OKAY	0.24	(0.12, 0.36)	OKAY
7	ERBB2	14.06	(0.00001, 43.01)	B	4.25	(0.00001, 11.60)	B	0.62	(0.03, 1.21)	B
8	EGFR	227.35	(0.00001, 984.95)	B	0.21	(0.00001, 0.64)	B	0.3	(0.00001, 1.10)	B
9	NOS2	42.26	(0.00001, 116.83)	B	1.32	(0.00001, 3.68)	B	0.18	(0.00001, 0.61)	B
10	PTGS2	7.88	(3.51, 12.25)	OKAY	0.46	(0.39, 0.53)	OKAY	0.68	(0.50, 0.86)	OKAY
11	FAS	80.8	(23.16, 138.44)	OKAY	0.39	(0.00001, 0.83)	A	1	(0.60, 1.40)	A
12	BAK1	103.16	(0.00001, 218.19)	B	1.16	(0.75, 1.57)	A	3.04	(0.28, 5.80)	A
13	CNTD1	43.07	(0.00001, 228.42)	B	4.34	(0.00001, 12.68)	B	0.24	(0.00001, 0.93)	B
14	CYP1B1	263.43	(0.00001, 898.28)	B	3.08	(0.00001, 11.93)	B	2.02	(0.35, 3.69)	B
15	CYP1A1	146.4	(0.00001, 365.62)	OKAY	4.11	(1.90, 6.32)	OKAY	0.39	(0.00001, 0.90)	B
16	GSTP1	5.1	(0.00001, 13.35)	A	4.6	(1.85, 7.35)	OKAY	0.65	(0.32, 0.98)	A
17	GSTT1	2219.86	(0.00001, 9040.14)	OKAY	1.46	(0.76, 2.16)	A	1.69	(0.00001, 3.89)	A
18	GSTM2	15.05	(0.00001, 33.11)	B	29.37	(0.00001, 81.97)	OKAY	0.32	(0.00001, 0.65)	B
19	GAPDH	1.08	(0.37, 1.79)	A	0.75	(0.60, 0.90)	A	1.12	(0.91, 1.33)	A
20	ACTB	0.93	(0.32, 1.54)	OKAY	1.33	(1.07, 1.59)	OKAY	0.89	(0.73, 1.05)	OKAY
21	HGDC	1812.48	(0.00001, 4479.92)	B	7.36	(0.00001, 22.64)	B	0.23	(0.01, 0.45)	OKAY

* In the comments;

A: indicates Best Results.

B: indicates Average Results.

C: indicates Weak Results.

***Appendix D: Summary and appraisal of previous systematic review
on health effects of sheesha***

A summary of the results found by Akl et al.

Akl et al. {{3457}} identified 24 studies and included them in the review. They assessed the effects of sheesha tobacco smoking on lung cancer, bladder cancer, oesophageal cancer, nasopharyngeal cancer, oral dysplasia, pregnancy outcomes, periodontal disease, infectious diseases, respiratory illness and infertility.

- Six studies that assessed the link between sheesha smoking and lung cancer were included in the review.

The meta-analysis was run on four of the six included studies. Based on 909 cases and 1753 controls from those four studies, there was a significant positive association between sheesha smoking and lung cancer OR 2.12 (95% CI 1.32-3.42), with little heterogeneity among the studies. Unlike the pattern of use in Canada and other developed countries, the tobacco in these studies was unprocessed and burned directly by charcoal, rather than indirectly heated. In addition most of sheesha smokers were miners who smoke pipe at the same time.

- One study assessed the link between sheesha tobacco smoking and oesophageal cancer.

In a single study included in the systematic review, which was assessed as providing a low quality of evidence, there was a positive association between sheesha smoking and oesophageal cancer OR =1.85 (95% CI 0.95-3.58).

- One study assessed the effect of sheesha tobacco smoking and nasopharyngeal cancer.

Akl et al. {{3457}}, found an inverse association between sheesha tobacco smoking and nasopharyngeal cancer OR= 0.49 (95% CI 0.2-1.23).

- One study assessed the effect of sheesha tobacco smoking and bladder cancer.

Akl et al. {{3457}} found an inverse associations between sheesha tobacco smoking and bladder cancer OR= 0.8 (95% CI 0.2-4.0).

- Five studies assessed the effect of sheesha tobacco smoking on periodontal disease and dry socket.

In four studies conducted in the same group of participants, or a subgroup of these, sheesha smoking was positively associated with periodontal disease OR ranged between 3 to 5 for different outcomes. However the fifth study assessed alveolar osteitis, also called dry socket, as an outcome. It is defined as failure to reserve the blood clot after tooth extraction. This blood clot is essential for the process of normal healing). An OR of 3.7 was observed for alveolar osteitis after tooth extraction. These studies were assessed as low quality.

- Three studies assessed the effect of sheesha tobacco smoking on low birth-weight.

In three studies of low birth-weight, assessed as low quality, a significant positive association was observed OR =2.12, (95% CI 1.08-4.18).

- Three studies assessed the effect of sheesha tobacco smoking on hepatitis C.

Three cross-sectional studies were included. The pooled OR for the association between sheesha tobacco smoking and hepatitis C was 0.98 with (95 % CI 0.8-1.21). These studies were assessed as providing very low quality of evidence.

- One study assessed the effect of sheesha tobacco smoking on respiratory illness.

The estimated OR was 2.3 with (95% CI 1.1-5.1). The included study was assessed as providing a very low quality of evidence.

- One study assessed the effect of sheesha tobacco smoking on infertility:

With very low quality of evidence the OR inferred from this study was 2.5 with (95% CI 1.0-6.3).

- One study assessed the effect of sheesha tobacco smoking and oral dysplasia:

Based on a single study which was assessed as providing a low quality of evidence, *Akl et al.* {{3457}} found that sheesha smoking was positively associated with oral dysplasia OR= 8.33 (95% CI 0.78-9.47), but this finding is difficult to interpret because participants chewed tobacco and smoked sheesha simultaneously.

Are the results of this systematic review valid?	
1. Was the review question clearly and explicitly stated? Was it a focused review question?	It was a focus review question yet it was not clearly and explicitly stated.
2. What was the review question?	It is implicitly stated: what are the effects of waterpipe tobacco smoking on health outcomes?
3. Did the SR include a methods section that described:	
a) A comprehensive, exhaustive search strategy for finding and including all the relevant studies? What databases and sources were searched? Was the search adequate? Were non-English studies excluded?	A comprehensive, exhaustive search strategy for finding and including all the relevant studies was provided in the Method section (Appendix 1) was attached to identify all terms used for the search. They searched MEDLINE (1950 onwards; access via OVID), EMBASE (1980 onwards; access via OVID) and ISI the Web of Science with no language restrictions.
b) The criteria used to decide which studies to include in the review (i.e. were the inclusion criteria reported and justified?) Was study selection reproducibly done (i.e. by two	The criteria used to decide which studies to include in the review were written in the eligibility criteria section: observational studies (i.e. cohort studies, case-control studies and cross-sectional

	series, outbreak investigations and abstracts. They also excluded studies assessing physiological outcomes [e.g. Forced Expiratory Volume in 1 Second (FEV1)], assessing waterpipe use for non-tobacco smoking purposes (e.g. marijuana smoking), not distinguishing waterpipe smoking from other forms of smoking, and not reporting any measure of association. Two reviewers independently screened the title and abstract of identified citations for potential eligibility using a standardized screening guide.
c) How the reviewers assessed the validity (i.e. quality) of each study included in the review? Was study quality assessment reproducibly done (i.e. by two independent reviewers)? Were all the included studies of high methodologic quality?	They appraised the methodological quality of included studies. They also used the GRADE approach to rate the overall quality of evidence for each outcome. Two reviewers independently studied the quality assessment. The included studies assessed as of low to very low quality.
4. If meta-analysis was done, how were the study results combined (pooled)? Were the meta-analysis methods appropriate? What statistical model was used for meta-analysis?	The statistical model used for meta-analysis was appropriately written in data analysis section. They conducted meta-analyses for the outcomes for which at least two studies reported effect estimates of their association with waterpipe tobacco smoking. They then pooled, for each outcome, the $\ln(ORs)$ of eligible studies using the generic inverse variance and the random effects model in Review Manager Version 5.0.20.
5. Did the reviewers check for heterogeneity? What method was used?	They measured homogeneity across study results using the I square statistic.
6. Did the reviewers check for publication bias? What method was used? Is it likely that relevant studies were missed?	They checked for possible publication bias using inverted funnel plots
7. Did the reviewers assess the potential impact of study quality on the results of the SR?	The reviewers assessed the potential impact of study quality on the results of the SR. In the discussion session they mentioned that it is part of the limitation methodology that the included studies were of low quality.

Sheesha caught the attention of many researchers. From 2008 to the present more than 50 articles have been published concerning the adverse health effects of sheesha. We are conducting a systematic review with meta-analysis to reassess the impact of sheesha on

human cancers. We modified the inclusion and exclusion criteria used by *Akl et al.* {{3457}} aiming to improve the generalizability of the results.

In a separate appendix, you can find a critical appraisal of the systematic review and meta-analysis conducted by *Akl et al.* {{3457}}

SR Critical Appraisal Worksheet

Title: The effects of waterpipe tobacco smoking on health outcomes: a systematic review

Citation: International journal of epidemiology

Authors: Akl,E.A.; Gaddam,S.; Gunukula,S.K.; Honeine,R.; Jaoude,P.A.; Irani,J.

Pub Year 2010 Pub Date Free Form Jun Volume 39 Issue 3 Page 834 -857

They should have been more rigour while appraising the included studies. The authors were not explicit in the terminology used where they used case-control study instead of matched case control study ex in Gupta et al article. In the same article they stated that Gupta et al matched for age and education, however No adjustment for any confounding factor has been done including other forms of tobacco consumption. In a more focused reading we found that on page 144 (table) they said that they adjusted for age, education and PY. However in the method section on page 143 they said the ORs were adjusted for age, smoking, education, religion and other variables.

What are the results of the systematic review?

Akl et al, 2010 identified 24 studies and included them in the review. They assessed the effect of waterpipe tobacco smoking on lung cancer, bladder cancer, oesophageal cancer, nasopharyngeal cancer, oral dysplasia, pregnancy outcomes, periodontal disease, infectious diseases, respiratory illness and infertility.

- Six included studies assessed the link between waterpipe smoking and lung cancer were included in the review;

The meta-analysis was run on four of the six included studies. Based on 909 cases and 1753 controls from four studies, sheesha smoking was positively associated with lung cancer OR 2.12 (95% CI 1.32-3.42), with little heterogeneity between the studies. Unlike the pattern of use in Canada and other developed countries, the tobacco in these studies was unprocessed and burned directly by charcoal, rather than indirectly heated. Most of sheesha smokers were minor's worker.

- One study assessed the link between waterpipe tobacco smoking and oesophageal cancer; In a single study included in the systematic review, which was assessed as providing a low quality of evidence, there was a positive association between sheesha smoking and oesophageal cancer OR =1.85 (95% CI 0.95-3.58).

- One study assessed the effect of waterpipe tobacco smoking and nasopharyngeal cancer; Akl et al, found an inverse associations between waterpipe tobacco smoking and nasopharyngeal

cancer OR= 0.49 (95% CI 0.2-1.23).

- One study assessed the effect of waterpipe tobacco smoking and bladder cancer; Akl et al, also found an inverse associations between waterpipe tobacco smoking and bladder cancer OR= 0.8 (95% CI 0.2-4.0).

- Five studies assessed the effect of waterpipe tobacco smoking on periodontal disease and dry socket;

In four studies conducted in the same group of participants, or a subgroup of these, sheesha smoking was positively associated with periodontal disease (OR in the range of 3 to 5 for different outcomes). However the fifth study assessed alveolar osteitis as a single outcome (also called dry socket, it is defined as failure to resorb the blood clot after tooth extraction. This blood clot is essential for the process of normal healing) OR of 3.7 was observed for alveolar osteitis after tooth extraction. These studies were assessed as of low quality.

- Three studies assessed the effect of waterpipe tobacco smoking on low birth-weight; In three studies of low birth-weight, assessed as of low quality, a positive association was observed OR =2.12, (95% CI 1.08-4.18).

- Three studies assessed the effect of waterpipe tobacco smoking on hepatitis C; Three cross-sectional studies were included. The pooled OR for the association between waterpipe tobacco smoking and hepatitis C was 0.98 with (95 % CI 0.8-1.21). These studies were assessed as providing very low quality of evidence.

- One study assessed the effect of waterpipe tobacco smoking on respiratory illness; The estimated OR was 2.3 with (95% CI 1.1-5.1). The included study was assessed as providing a very low quality of evidence.

- One study assessed the effect of waterpipe tobacco smoking on infertility; With very low quality of evidence the OR inferred from one study was 2.5 with (95% CI 1.0-6.3).

- One study assessed the effect of waterpipe tobacco smoking and oral dysplasia; Based on single study which was assessed as providing a low quality of evidence, Akl et al, found that sheesha smoking was positively associated with oral dysplasia OR= 8.33 (95% CI 0.78-9.47), but this finding is difficult to interpret because participants chewed tobacco and smoked sheesha simultaneously.

1. Were the results similar from study to study, both clinically and statistically? ✓ Were the populations of the different studies similar? ✓ Was the same exposure/intervention evaluated by the individual studies? ✓ Was the same outcome evaluated by the individual studies? ✓ If heterogeneity was found, did the reviewers seek to explore reasons for heterogeneity? ✓ What methods (e.g. subgroup analysis or meta-regression) were used to ascertain reasons for heterogeneity?	For most of the outcomes of interest the evidence was either lacking, indirect or of lower quality. In terms of population, exposure, and outcome: Lung cancer studies were conducted mostly in China and India where tobacco (exposure) is typically unprocessed and burned directly by charcoal. The practice of waterpipe smoking that is involved in the recent global epidemic involves tobacco that is processed and flavoured and indirectly heated by the charcoal. In terms of quality, the one methodological study limitation that affected our rating of the quality of evidence for most outcomes was measurement bias, which has been reported in similar systematic reviews. In fact, only one study used a standardized exposure measurement tool in spite of the fact that the practice of waterpipe smoking can vary widely. Variables include the quantity of tobacco used, the type of tobacco used, the concomitant use of other substances, the frequency of smoking sessions, and the length of sessions, the number of years of smoking. In addition, no study reported using a standardized measurement tool for other forms of tobacco smoking in spite of the variety of these forms and the need to account for passive smoking and past smoking history. The other methodological study limitation that affected our rating of the quality of evidence for many outcomes was the inappropriate handling of confounding, particularly for other forms of tobacco smoking and for factors such as radon exposure among miners as a risk factor for lung cancer. They measured homogeneity across study results using the I² statistic. the reviewers seek to explore reasons for heterogeneity in the discussion section. In spite of the many methodological study limitations for lung cancer and pregnancy outcomes, the findings showed consistency (i.e. the low heterogeneity) across studies. Most of the reasons that infer heterogeneity are
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	coming from the methodology section making it hard to overcome heterogeneity using subgroups analysis or even a logistic regression method.
2. What are the overall results of the review?	Based on the available insufficient evidence, where all included studies were assessed to be as of low to very low quality. They concluded that waterpipe tobacco smoking was significantly associated with lung cancer, respiratory illness, low birth-weight and periodontal disease. It was not significantly associated with bladder cancer, nasopharyngeal cancer, oesophageal cancer, oral dysplasia or infertility.

3. How precise were the results?	Twenty-four studies were eligible for this review. Based on the available evidence, waterpipe tobacco smoking was significantly associated with lung cancer OR 2.12 (95% CI 1.32-3.42], respiratory illness OR= 2.3 with 95% CI 1.1-5.1, low birth-weight (OR 2.12, 95% CI 1.08-4.18). and periodontal disease OR in the range of 3 to 5 for different outcomes It was not significantly associated with bladder cancer (OR 0.8, 95% CI 0.2-4.0). nasopharyngeal cancer (OR 0.49, 95% CI 0.2-1.23), oesophageal cancer (OR 1.85, 95% CI 0.95-3.58)., oral (OR 8.33, 95% CI 0.78-9.47), or infertility OR = 2.5 ; with 95% CI 1.0-6.3, but the CIs did not exclude important associations. Smoking waterpipe in groups was not significantly associated with hepatitis C infection 0.98 with 95 % CI (0.8-1.21), The quality of evidence for the different outcomes varied from very low to low.
4. Were conclusions made by the review authors supported by the data presented in the review?	Yes, the conclusions made by the review authors were supported by the data presented in the review. Actually the review shows high consistency between the conclusions and data inferred in the result section. In a harmony way the discussion section links all the parts together.

In summary:

What are the major strengths of this systematic review? The main strength of this study is the use of the Cochrane Collaboration methodology for conducting systematic reviews, i.e. using a very sensitive and comprehensive search strategy, a duplicate and independent selection process, a duplicate and independent data abstraction process, and a rigorous appraisal of the methodological quality of included studies. They also used the GRADE approach to rate the overall quality of evidence for each outcome.
What are the major limitations of this systematic review? The major limitations are: As acknowledged by authors, first the heterogeneities among studies, regarding methodologies, exposures are not standardised, in terms of material used, the frequency of smoking sessions, and the length of sessions, the number of years of smoking. Second is the inappropriate

handling of confounding, particularly for other forms of tobacco smoking and for factors such as radon exposure among miners as a risk factor for lung cancer.

They could have been more selective while adopting inclusion and exclusion criteria that might overcome some limitations.

Based on your assessment of this systematic review, would you accept the conclusions of the review?

The limitations in the review act as barriers, yet the conclusions act as corner stone in public health implications.

AMSTAR CHECKLIST

1. Was an 'a priori' design provided?

The research question and inclusion criteria should be established before the conduct of the review.

■ Yes
r No
r Can't answer
r Not applicable

2. Was there duplicate study selection and data extraction?

There should be at least two independent data extractors and a consensus procedure for disagreements should be in place.

■ Yes
r No
r Can't answer
r Not applicable

3. Was a comprehensive literature search performed?

At least two electronic sources should be searched. The report must include years and databases used (e.g. Central, EMBASE, and MEDLINE). Key words and/or MESH terms must be stated and where feasible the search strategy should be provided. All searches should be supplemented by consulting current contents, reviews, textbooks, specialized registers, or experts in the particular field of study, and by reviewing the references in the studies found.

■ Yes
r No
r Can't answer
r Not applicable

4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?

The authors should state that they searched for reports regardless of their publication type. The authors should state whether or not they excluded any reports (from the systematic review), based on their publication status, language etc.

r Yes
r No
■ Can't answer
r Not applicable

5. Was a list of studies (included and excluded) provided?

A list of included and excluded studies should be provided.

■ Yes
r No
r Can't answer
r Not applicable

6. Were the characteristics of the included studies provided?

In an aggregated form such as a table, data from the original studies should be provided on the participants, interventions and outcomes. The ranges of characteristics in all the studies analyzed e.g. age, race, sex, relevant socioeconomic data, disease status, duration, severity, or other diseases should be reported.

■ Yes
r No
r Can't answer
r Not applicable

7. Was the scientific quality of the included studies assessed and documented?

'A priori' methods of assessment should be provided (e.g., for effectiveness studies if the author(s) chose to include only randomized, double-blind, placebo controlled studies, or allocation concealment as inclusion criteria); for other types of studies alternative items will be relevant.

■ Yes
r No
r Can't answer
r Not applicable

8. Was the scientific quality of the included studies used appropriately in formulating conclusions?

The results of the methodological rigor and scientific quality should be considered in the analysis and the conclusions of the review, and explicitly stated in formulating recommendations.

■ Yes
r No
r Can't answer
r Not applicable

9. Were the methods used to combine the findings of studies appropriate?

For the pooled results, a test should be done to ensure the studies were combinable, to assess their homogeneity (i.e. Chi-squared test for homogeneity, I^2). If heterogeneity exists a random effects model should be used and/or the clinical appropriateness of combining should be taken into consideration (i.e. is it sensible to combine?).

■ Yes
r No
r Can't answer
r Not applicable

10. Was the likelihood of publication bias assessed?

An assessment of publication bias should include a combination of graphical aids (e.g., funnel plot, other available tests) and/or statistical tests (e.g., Egger regression test).

- Yes
- r No
- r Can't answer
- r Not applicable

11. Was the conflict of interest stated?

Potential sources of support should be clearly acknowledged in both the systematic review and the included studies.

- Yes
- r No
- r Can't answer
- r Not applicable

Appendix E: Details of search strategy for systematic review

Search strategy

In October, 2011, we ran the list of keywords for “waterpipe” prepared by Akel et al article in the four databases “Medline, Embase, Pubmed, and Global Health”, and then we looked at references to track other synonyms for waterpipe. One more keyword term (Argyle) was identified and added to our list to be used in our strategy.

We electronically searched the four databases: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1948 to Present>, by typing our list of keywords with no language restrictions. Then we used the same strategy to look through Embase classic and Embase from 1947 until present, Pubmed, and Global Health current on cabdirect. We searched for keywords in titles and abstracts, We ended up with 85 articles from Medline, 108 articles from Embase and 28 articles from Global Health one article from Ovid and 5 articles from CINAHL. In total there were 227 articles we exported them into reworks under the sheesha oct 2011 file we removed all the duplicate and deleted all the articles that are not related to sheesha like environmental, or electrical or even viral and some articles related to problem of inducing anaesthesia in children from 227 we saved only 36 potential articles to be included in our systematic review.

Global Health:

[cancer*](#) OR [neoplasm*](#) OR [tumor*](#) OR [tumour*](#) OR [carcinoma*](#) OR [Cancer](#) AND title: "[water pipe](#)*" OR title: [waterpipe*](#) OR title: [shisha*](#) OR title: [sheesha*](#) OR title: [hooka*](#) OR title: [huqqa*](#) OR title: [guza*](#) OR title: [narghil*](#) OR title: [nargil*](#) OR title: [arghil*](#) OR title: [argil*](#) OR title: [argyle*](#) OR title: [goza*](#) OR title: [hubbl*](#) AND title: [bubbl*](#) OR Abstract: "[water pipe](#)*" OR Abstract: [waterpipe*](#) OR Abstract: [shisha*](#) OR Abstract: [sheesha*](#) OR Abstract: [hooka*](#) OR Abstract: [huqqa*](#) OR Abstract: [guza*](#) OR Abstract: [narghil*](#) OR Abstract: [nargil*](#) OR Abstract: [arghil*](#) OR Abstract: [argil*](#) OR Abstract: [argyle*](#) OR Abstract: [goza*](#) OR Abstract: [hubbl*](#) AND Abstract: [bubbl*](#)

Dissertations and thesis:

[cabs\(smok*\)](#) AND (([cabs\(waterpipe*\)](#) or [cabs\(shisha*\)](#) or [cabs\(sheesha*\)](#) or [cabs\(hooka*\)](#) or [cabs\(huqqa*\)](#) or [cabs\(guza*\)](#))) OR ([cabs\(goza*\)](#) or [cabs\(narghil*\)](#) or [cabs\(nargil\)](#) or [cabs\(arghil*\)](#) or [cabs\(argil*\)](#) or [cabs\(argyle*\)](#))) OR ((([cabs\(hubbl* near/3 bubbl*\)](#)) or ([cabs\(water near/1 pipe*\)](#)))))

Ovid Technologies, Inc. Email Service

Search for: 15 and 22

Results: 1

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1948 to Present>

Search Strategy:

1 "water pipe\$.ab,ti. (348)
2 waterpipe\$.ab,ti. (129)
3 shisha\$.ab,ti. (78)
4 sheesha\$.ab,ti. (11)

5 hooka\$.ab,ti. (103)
6 huqqa\$.ab,ti. (3)
7 guza\$.ab,ti. (3)
8 goza\$.ab,ti. (23)
9 narghil\$.ab,ti. (90)
10 nargil\$.ab,ti. (8)
11 arghil\$.ab,ti. (2)
12 argil\$.ab,ti. (136)
13 argyle\$.ab,ti. (86)
14 (hubbl\$ adj3 bubbl\$).ab,ti. (23)
15 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 (848)
16 limit 15 to yr="2011 -Current" (87)
17 exp Neoplasms/ (2313465)
18 neoplasm\$.ab,ti. (88014)
19 cancer\$.ab,ti. (895340)
20 tumo?r\$.ab,ti. (1009508)
21 carcinoma\$.ab,ti. (419832)
22 17 or 18 or 19 or 20 or 21 (2679589)
23 15 and 22 (85)

Appendix F: Data extraction form developed for systematic review

Data extraction sheet:

Refwork number:	
Title:	
Authors:	
Contact info:	
Journal:	
Souce:	
Year of publication :	
Place of Publication:	
Study sponsor:	
Study characteristics:	
Study design:	☐ RCT ☐ Case-Control ☐ Cohort ☐ Cross sectional study
Study ranked by Downs and Black checklist	
Population:	
Place:	
City:	
Country:	
Year:	
Inclusion criteria :	
% of enrolments:	
Gender of cases:	

Age of cases Inclusion criteria for control : Gender of control: Age of control: Ratio of case over control:	
Exposure: Measurement tools: Definition of ever-smokers: Definition of ex-smokers: self-developed questionnaire Exposure level of included subject Type of tobacco used: Method of application of charcoal:	☐ Sheesha ☐ Sheesha + cigarettes ☐ Sheesha +smokeless tobacco Was standardization reported ☐ Yes ☒ No ☐ Ever ☐ Never smoked ☐ Did they calculate the cumulative exposure and then categorized into quartiles of Liang/month year

Confounding factor:	☐ M'assel ☐ Herbal ☐ Ajami ☐ not specified Direct Indirect ☐ not specified
Cancer:	☐ Clinically diagnosed ☐ Xray ☐ MRI ☐ Biopsy confirmed histologically
Outcome measurements:	

***Appendix G: Reasons for exclusion of papers from systematic review,
and list of excluded papers***

Reasons for exclusion

We excluded case reports, case series or letter to editor such as (1, 2).

Other articles did not distinguish sheesha from other forms of smoking, or exposure, and so we excluded them (3-13).

In some articles the outcome was a mediator (14-17). Other articles did not report any measure of association (18-24).

We also excluded studies done in animals (21) articles that assessed effects of exposure to long stem pipes or pipes but not sheesha (22) letters to editors (23) and articles published in a language other than Arabic, English or French (24).

List of excluded articles

1. Roohullah, Nusrat J, Hamdani SR, et al. Cancer urinary bladder--5 year experience at Cenar, Quetta. J Ayub Med Coll Abbottabad. 2001;13(2):14-6.
2. El-Hakim I, Uthman MAE. Squamous cell carcinoma and keratoacanthoma of the lower lip associated with "Goza" and "Shisha" smoking. Int J Dermatol. 1999;38(2):108-10. (doi: 10.1046/j.1365-4362.1999.00448.x).
3. Gupta PC, Murti PR, Bhonsle RB. Epidemiology of cancer by tobacco products and the significance of TSNA. Crit Rev Toxicol. 1996;26(2):183-98.
4. Halboub E, Abdulhaq M, Al-Mandili A, et al. Oral and pharyngeal cancer in Yemen: A retrospective registry-based study. Oral Oncology.Conference: 3rd World Congress of the International Academy of Oral Oncology 2011, IAOO Singapore Singapore.Conference Start: 20110714 Conference End: 20110717.Conference Publication: (var.pagings). 2011;47:S102-3. (doi: <http://dx.doi.org/10.1016/j.oraloncology.2011.06.334>).
5. Hazelton WD, Luebeck EG, Heidenreich WF, et al. Analysis of a historical cohort of Chinese tin miners with arsenic, radon, cigarette smoke, and pipe smoke exposures using the biologically based two-stage clonal expansion model. Radiat Res. 2001;156(1):78-94.
6. Khan NA, Teli MA, Katoch S, et al. The effect of smoking habits on different histological types of lung cancer in Kashmir. JK Science. 2008;10(1):23-5.
7. Lo AC, Soliman AS, El-Ghawalby N, et al. Lifestyle, occupational, and reproductive factors in relation to pancreatic cancer risk. Pancreas. 2007;35(2):120-9.
8. Lubin JH, Li J-, Xuan X-, et al. Risk of lung cancer among cigarette and pipe smokers in Southern China. International Journal of Cancer. 1992;51(3):390-5.
9. Lubin JH, Qiao YL, Taylor PR, et al. Quantitative evaluation of the radon and lung cancer association in a case control study of Chinese tin miners. Cancer Res. 1990;50(1):174-80.
10. Taylor PR, Qiao YL, Schatzkin A, et al. Relation of arsenic exposure to lung cancer among tin miners in Yunnan Province, China. Br J Ind Med. 1989;46(12):881-6.

11. Tran GD, XiuDi S, Abnet CC, et al. Prospective study of risk factors for esophageal and gastric cancers in the Linxian general population trial cohort in China. *International Journal of Cancer*. 2005;113(3):456-63. (doi: 10.1002/ijc.20616).
12. Znaor A, Virag M, Znaor T, et al. Lifestyle habits and the risk of head and neck cancer in Zagreb region. *Coll Antropol*. 2011;35(4):1125-8.
13. Hosseini M, Alinaghi SAS, Mahmoudi M, et al. A case-control study of risk factors for prostate cancer in Iran. *Acta Med Iran*. 2010;48(1):61-6.
14. Gumus ZH, Du B, Kacker A, et al. Effects of tobacco smoke on gene expression and cellular pathways in a cellular model of oral leukoplakia. *Cancer Prev Res (Phila)*. 2008;1(2):100-11. (doi: 10.1158/1940-6207.CAPR-08-0007).
15. Guza RC. Effect of sequence context on the formation and repair of DNA adducts. *Biochemistry, Molecular Bio, and Biophysics* ed. Tretyakova and Y. N, editors. United States -- Minnesota: Biochemistry, Molecular Bio, and Biophysics, 2009; 2009.
16. Sajid KM, Chaouachi K, Mahmood R. Hookah smoking and cancer: carcinoembryonic antigen (CEA) levels in exclusive/ever hookah smokers. *Harm Reduct J*. 2008;5:19.
17. El-Setouhy M, Loffredo CA, Radwan G, et al. Genotoxic effects of waterpipe smoking on the buccal mucosa cells. *Mutation Research, Genetic Toxicology and Environmental Mutagenesis*. 2008;655(1):36-40.
18. Rastam S, Li F-, Fouad FM, et al. Water pipe smoking and human oral cancers. *Med Hypotheses*. 2010;74(3):457-9. (doi: <http://dx.doi.org/10.1016/j.mehy.2009.10.013>).
19. Salehi A, Khezri AA, Malekmakan L, et al. Epidemiologic status of bladder cancer in shiraz, southern iran. *Asian Pac J Cancer Prev*. 2011;12(5):1323-7.
20. Fossati C. The incidence of bronchopulmonary carcinoma in the woman. *Rass.Clin.Sci.Ist.Bio.chim.Ital*. 1969;45(2):49-53.
21. Druckrey H, Preussmann R, Schmaehl D. [Induction of cancer in rats by implantation of tar pitch from water pipes]. *Z Krebsforsch*. 1960;63:219-21.
22. Castellsague X, Munoz N, De Stefani E, et al. Independent and joint effects of tobacco smoking and alcohol drinking on the risk of esophageal cancer in men and women. . 1999;82(5):657-64.
23. Fouad FM, Rastam S, Al Moustafa A-. Involvement of water pipe smoking in the development of human pancreatic cancer. *International Journal of Cancer*. 2010;127(2):497-8. (doi: <http://dx.doi.org/10.1002/ijc.25061>).
24. Rakower J. Smoking habits and lung cancer in Israel (Hebrew). *Harefuah*. 1965;68(4):115-9.