INTRODUCTION

Tobacco is consumed all around the world in several ways. Numerous epidemiological studies in Western countries and certain parts of Asia and Africa have identified tobacco consumption as the main risk factors for several types of cancers; out of which, lung cancer is the leading cause of death, and cigarette smoking causes most of the cases.\(^1\) A World Health Organization survey states that tobacco alone is expected to cause high mortality than caused by tuberculosis, HIV/AIDS and malaria combined.\(^1\) Tobacco smoking is the culprit causing a wide range of pulmonary pathologies like airway obstruction, emphysema and bronchitis, which are collectively referred to as chronic obstructive pulmonary disease (COPD).\(^1\)

Long-term exposure to toxic gases and particles leads to COPD, and is most often related to cigarette smoking.\(^2\) This chronic disease is characterized by exacerbations, which accelerate the decline in lung functions, resulting in reduced physical activity, poor quality of life, and an increased risk of death. According to the Global Burden of Disease study, COPD will become the third leading cause of death by the year 2020.\(^3\) World Health Organization has also classified it as the fourth leading cause of mortality in the United States.\(^3\)

Another form of tobacco smoking that has reemerged recently as a very common tool of tobacco intake is waterpipe smoking, commonly known as Shisha smoking. The traditional waterpipe, in common use, originated in India in the 15th century from where it spread to the Near-East countries.\(^4\) In the last few years, there has been a revival of waterpipe smoking, especially among the youth. This recent trend has posed challenges to the healthcare professionals, as they are not fully aware of the consequences of this type of tobacco intake. The composition of the tobacco used in waterpipe smoking is variable and unstandardised. Some researches on waterpipe smoke have shown high

ABSTRACT

Objective: To observe the effects of Shisha smoke on submucosal glands of trachea of mice; and compare it with tracheal glands of mice exposed to cigarette smoke.

Study Design: Randomised controlled trial.

Place and Duration of Study: Department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad from October 2013 till April 2014.

Methodology: Sample comprised of 40 adult male mice of strain BALB/c. They were randomly divided into three groups. Control group was labelled as Group 'C'. The mice in this group were kept in a whole body smoke exposure chamber and were exposed to fresh air. Shisha group was labelled as Group 'SS', and the mice in this group were exposed to Shisha smoke. Mice in the third group labelled as Group CS were exposed to cigarette smoke. All the mice were dissected after an exposure period of eight weeks. Tracheal tissue was stained and examined microscopically for submucosal gland hypertrophy and compared with the control group, using Reid's Index. An Index of more than 0.4 is termed as hypertrophy.

Results: There was significant submucosal gland hypertrophy in groups CS and SS as compared to group C. There was also significant difference in the frequency of mucosal hypertrophy between SS (93.7%) and CS groups (53.3%), which was found statistically significant (p<0.001).

Conclusion: Shisha smoking was significantly associated with mucosal hypertrophy when compared with cigarette smoking and controls. Shisha smoke contains higher level of toxicants as compared to cigarette smoke, and it causes more oxidative damage of tissues.

concentrations of carbon monoxide, tar, nicotine and heavy metals as compared to cigarette smoke.\(^5\)

The prime objective of this research was to compare the effects of Shisha and cigarette smoke inhalation on histology of trachea in mice.

### METHODOLOGY

This research was conducted in the Department of Anatomy, Islamic International Medical College in collaboration with National Institute of Health (NIH), Islamabad, from October 2013 till April 2014. It was a randomised control trial. The Institutional Review Committee of Riphah International University approved the study. Forty adult male BALAB/c mice of 10-12 weeks of age and weighing 35-45 grams were randomly selected and divided into 3 study groups. They were given pelleted diet and kept under standard laboratory conditions at NIH.

The mice were divided into three groups as control group C (10 mice), group SS (15 mice), and group CS (15 mice). Group C mice were kept in fresh air; and groups SS and CS received Shisha smoke and cigarettes smoke, respectively. Whole body inhalation exposure was given in a plastic chamber that was designed locally according to the specifications of World Health Organization.\(^6\)

Group SS was exposed to Shisha smoke by burning 10g of Shisha flavour that was placed on top of the apparatus used and was covered with aluminum foil. Hot coal biscuit was placed over it. The head was then connected below a body, which was half filled with water. A tube, connected to the head, passed through the body and was submerged in the water. A hose with a mouthpiece were connected to the bowl above the level of the water. Shisha smoke was sucked by a manual vacuum pump connected to mouthpiece. An aerosol was formed that consisted of volatilised and pyrolysed tobacco components. It passed through water and was distributed into the chamber (Figure 1a).\(^7\) The flavour used in Shisha contains approximately 2.5 mg of nicotine.\(^8\) The nicotine content in side stream smoke of one cigarette is 0.12 mg.\(^9\) So, nicotine content in 20 cigarettes is 2.4 mg.

Group CS mice were exposed to cigarette smoke by burning 20 commercial nonfiltered cigarettes that were placed vertically in a plastic stand (Figure 1b). Both groups were exposed to equal quantities of nicotine twice a day. The mice were exposed to the smoke for five minutes followed by a five minutes exposure to fresh air and this continued until 20 cigarettes and all Shisha flavour were consumed. The entire cycle took 1-1½ hours.\(^7\) The exposure continued for eight weeks.

All the mice were sacrificed at the end of eighth week. Their trachea was dissected out and preserved in small plastic containers containing 10% formalin. Slides were prepared after tissue processing and paraffin embedding. They were stained with haematoxylin and eosin. The histological features were studied under 40X objective of a light microscope and Reid’s Index was calculated.\(^10\) Reid’s Index is a ratio of submucosal gland to wall thickness, and is used to assess submucosal gland hypertrophy. In the slides of the trachea, the maximal gland thickness was measured on a line at right angles to the plane of the cartilage. The total mucosal thickness was measured from inner aspect of perichondrium to inner aspect of basement membrane on exactly the same line. This was done with a linear eyepiece micrometer fitted in the eyepiece of the light microscope (Figure 2).\(^8\) The mean indices were calculated, which, in turn, were the mean value of four individual gland-to-wall ratios measured at four different sites of each slide.\(^7\) The mean Reid Index for each slide was thus calculated.

SPSS version 20.0 was used for statistical analysis. As per study objective, the frequencies of hypertrophy were compared among SS, CS and Controls, using Chi-square test. Frequencies with percentages were given to report categorical variables. The mean Reid’s Index ± SD of all the groups was compared using ANOVA. A p-value of <0.05 was considered statistically significant.

![Figure 1: Photographs showing generation of Shisha smoke with a manual vacuum pump (a), and cigarettes burning in a whole body cigarette smoke exposure chamber (b)].

![Figure 2: Photomicrograph of slide of trachea of mice showing Reid’s index calculated by taking ratio of ab (submucosal gland thickness) to cd (mucosal thickness)].\(^8\) H&E stain 40X.
RESULTS

There were normal seromucous glands in submucosa of trachea of mice in control group (Figure 3). Both smoke exposure groups SS and CS showed hypertrophy of these glands when results were compared with the control group. In experimental group CS, submucosal gland hypertrophy was present in 8 (53.3%) mice compared to 14 (93.7%) in SS group and this difference was statistically significant (p-value <0.001, Table I). The mean values ± SD of Reid's Index of the groups were compared (Table I). It was markedly raised in Group SS as compared to Group CS and control group. Group SS mice showed significantly marked hypertrophy of submucosal glands as compared to group C and group CS (Figure 3).

Table I: Comparison of frequency of submucosal gland hypertrophy among three study groups.

<table>
<thead>
<tr>
<th>Gland hypertrophy</th>
<th>SS group (n=15)</th>
<th>CS group (n=15)</th>
<th>Control (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>14 (93.7%)</td>
<td>8 (53.3%)</td>
<td>0 (0.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absent</td>
<td>1 (6.3%)</td>
<td>7 (46.6%)</td>
<td>10 (100.0%)</td>
<td></td>
</tr>
<tr>
<td>Mean Reid index</td>
<td>0.67 ±0.15</td>
<td>0.58 ±0.16</td>
<td>0.36 ±0.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DISCUSSION

The increasing awareness of adverse effects of tobacco has led some people to look for alternate ways to satisfy their nicotine craving. There has been a wide acceptance of Shisha, especially among the youth, due to a common belief that it is safer than cigarette. It is believed that the water, through which the smoke passes, filters the toxic components, rendering the smoke less harmful than cigarette smoke. On the other hand many studies have shown that Shisha smoke contains much higher concentrations of those toxic materials that make cigarette smoke harmful.

The mammalian airway is lined by pseudostatified ciliated columnar epithelium, underneath which lies seromucous glands, which pour their secretions through excretory ducts into the airway lumen. The submucosal glands in larger airways are of mixed variety. The mucus cells constitute 60% of the gland volume, while serous cells form remaining 40%. These cells play an important role in mucociliary clearance (MCC), which is a self-clearing mechanism of respiratory mucosa. Airway irritation leads to an increased production of mucus, which is then cleared by the sweeping action of cilia. This is an important component of airway's innate immunity.

Constant irritation of the mucosa, by the inhaled particles, leads to an increase in volume of submucosal glands with disturbances in normal mucus to serous cells ratio. The irritants known to be most damaging are: nicotine, acetaldehyde, and acrolein. These irritants affect the MCC adversely by damaging the ciliated cells and increasing submucosal gland volume. This is the main pathology that leads to chest congestion and airway obstruction in COPD.

There is submucosal gland hypertrophy and disproportionate increase in mucus acini and reduction in serous acini in larger airways in COPD. Reid in 1960 used a method to assess submucosal gland hypertrophy by taking proportion of gland thickness to bronchial wall thickness. This ratio is referred to as the Reid's Index. This method has the advantage that the measurements include all the submucosal glands and it is not affected by the wrinkling of the mucosa. Several harmful chemicals in tobacco smoke cause the formation of free radicals that lead to inflammation and mucus gland hypertrophy. Many studies have shown that tobacco smoke causes epithelial damage by mainly damaging the cilia. This makes the epithelium vulnerable to the harmful foreign dust particles and bacteria, which remain in contact with the respiratory mucosa for a longer period. In chronic inhalation, these harmful substances breach the basal lamina and reach the underlying lamina propria. In lamina propria, they invade the blood capillaries and lymphatic vessels, further increasing the risk of toxic damage.

The Shisha smoke causes more inflammation and a higher load of oxidative stress. This leads to much more destruction of MCC as compared to the cigarette smoke. While smoking, a cigarette smoker takes 8 to 12 puffs over 5-6 minutes, inhaling a total of 500-600 ml of smoke. On the other hand, a waterpipe session typically lasts for 30-60 minutes. The smoker takes 50-200 puffs inhaling 500 ml of smoke in each puff. Thus about 50,000 ml of smoke is produced by a single session of Shisha smoking.

The results of this research showed that submucosal gland hypertrophy is more marked in trachea of mice of group SS as compared to group CS and control group. These findings are consistent with findings of research by Chaouachi and Shraideh. Their researches have
also showed that prolonged exposure to Shisha smoke induces inflammatory changes in submucosa, leading to marked production of mucus and hypertrophy of submucosal glands.

**CONCLUSION**

Waterpipe/Shisha smoke contains higher levels of respiratory irritants that cause inflammation and disruption of mucociliary clearance in airways of mammals. The damage is more as compared to cigarette smoke given in same quantity and same duration. Thus Shisha smoking is not a safe alternative to cigarette smoking. Marked submucosal gland hypertrophy, assessed by Reid's Index, has shown that Shisha smoke is more damaging than cigarette smoke.

**REFERENCES**