Submucosal Gland Hypertrophy in Trachea of Mice Exposed to Shisha Smoke

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Abstract

Objective: To determine the histological effects of Shisha smoke on submucosal glands in trachea of experimental animals.

Material & Methods: This analytical experimental randomized control trial was carried out in the department of anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), in the year 2013. Twenty-five adult male BALB/c mice were used and were randomly divided into 2 groups. Control group C was kept in a chamber exposed to fresh air. Shisha smoke group SS was exposed to Shisha smoke in a separate chamber. All mice were dissected after eight weeks and tissues of trachea were examined microscopically. The trachea was examined for the presence of submucosal gland hypertrophy by using Reid’s index. Results were entered on SPSS version 20 and compared in experimental groups.

Results: Reid’s index was used to assess submucosal gland hypertrophy. There was marked submucosal gland hypertrophy in trachea of group SS exposed to Shisha smoke as compared to control group C mice.

Conclusion: Shisha smoke contains high level of toxins in its smoke that cause disruption of mucociliary clearance and hypertrophy of submucosal glands in experimental animals.

Key Words: Reid’s Index, Shisha smoke, Submucosal glands, Trachea.

Introduction

Damage to the respiratory system by tobacco smoke leading to a high mortality rate remains a challenge to the modern society. Tobacco smoking is associated with a number of pulmonary diseases including airway obstruction, emphysema and bronchitis. Collectively these are referred to as chronic obstructive pulmonary disease (COPD). COPD is prevalent in 20 million men and women in the United States and is the fourth leading cause of death.1 The main pathology of COPD includes airway mucous gland hyperplasia, mucous hypersecretion, and an influx of inflammatory cells including neutrophils, macrophages, and lymphocytes.2 The genesis of this disease is thought to lie in the inflammatory process induced by tobacco smoke, leading to cell injury, cellular hyperplasia, and occasionally neoplasia. Therefore, it is important for us to understand the process by which tobacco smoke induces inflammation in the trachea and lungs. Tobacco is most commonly inhaled in form of a cigarette. Effects of cigarette smoking on human health have been extensively studied worldwide and it has been found that it is responsible for 90% of lung cancers in men and accounts for 30% deaths due to cancers.3 Another common form of tobacco intake that has rather re-emerged is through Shisha. This is also known as Water Pipe Tobacco Smoking i.e. smoking through any apparatus that involves passage of tobacco smoke through water before it is being inhaled. The traditional water pipe originated in India in the 15th century and then spread to the Near East countries.4 Hookahs spread first to Persia and underwent further changes to its original shape to the current known shape. Recently there has been an emergence of the practice among younger adults and adolescents and an estimated 100 million people worldwide smoke Shisha daily. The common perception about this type of smoking of being less harmful than cigarette smoking is leading to tolerance of this practice.5 A widespread perception among smokers is that the water, through which the smoke bubbles, filters the toxic components, rendering the smoke less harmful than cigarette smoke. On the contrary a single water pipe smoking session yields 20 times the amount of acetaldehyde, formaldehyde, acrolein and polycyclic aromatic hydrocarbons found in mainstream cigarettes; all of these are the prime cause of cancer.6 In addition to this it also contains an addictive drug nicotine. Moreover depending upon the frequency of puffing, depth of inhalation, and length of the smoking
session, hookah smokers may absorb higher concentrations of the toxins as compared to cigarette smoke. 

Cigarette smokers take 8 to 12 puffs over a period of 5-7 minutes, inhaling a total of 500-600 ml of smoke. In contrast, water pipe sessions typically last 30-60 minutes, during which the smoker may take 50-200 puffs inhaling 500ml of smoke in each puff. Thus a single session produces 50,000ml of smoke. 

There is less research done addressing the tobacco effects of water pipe smoking despite the fact that there are millions of current water pipe smokers especially the youth and that water pipe smoking is spreading globally. Given the potential for harm from waterpipe smoking, this research was conducted with the aim to see the histological effects of Shisha smoke inhalation on submucosal glands in trachea among study groups.

**Material and Methods**

This study was conducted at department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH) in 2013. It was an analytical experimental randomized control trial and was approved by the Institutional Review Committee of Riphah International University. Twenty-five adult male BALB/c mice having weight of 35-45g and age between 10-12 weeks were included in the study and were kept under standard laboratory conditions at NIH Islamabad. All mice were acclimatized for one week. They were given pelleted diet that was prepared in the animal house. They were kept at 12 hours light and dark cycle in a room at 22-24 °C and were given food and water ad libitum.

Animals were randomly divided into two groups. Whole body inhalation exposure was given using a locally made plastic chamber (Fig-1a) designed according to the specifications of World Health Organization. The control group C had ten mice that were kept in a chamber exposed to fresh air. Shisha smoke was given to experimental group SS that had 15 mice.

The water pipe apparatus used consisted of a head to hold 10-20g of tobacco. It was connected to a body below which was a bowl half filled with water. A tube was connected to the head that passed through the body and was submerged in water in the bowl. A hose and a mouthpiece were connected to the bowl above the level of the water. 10g of Shisha flavour was placed in the trough on top and covered with aluminium foil with small holes in it. A piece of hot coal biscuit was placed over the foil. The smoke was sucked by a manual vacuum pump through the mouthpiece, which drew air over the burning charcoal and through the tobacco creating an aerosol consisting of volatilized and pyrolized tobacco components (Fig-1a). The smoke then bubbled into water jar and a post bubbling mainstream smoke was carried via a connecting pipe into the smoke inhalation chamber of experimental group SS. Mice were exposed to Shisha smoke in morning and evening, one puff /2 sec, 5days/week for 8 weeks.

The mice in Shisha group were exposed to 5 min of smoke followed by 5 min of air until all Shisha flavour was consumed, which took 1-1½ hours. All the animals were sacrificed at the end of 8th week. They were dissected and

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**Figure 1**: (a) Photograph of whole body smoke exposure chamber showing pumping of Shisha smoke with a manual vacuum pump. (b) Dissected chest cavity of mouse showing trachea (a), right lung (b) and left lung(c)

**Figure 2**: Photomicrograph of trachea of mouse. The Reid’s index was calculated by taking ratio of ab (submucosal gland thickness) to cd (mucosal thickness) (H & E 10x10)
Trachea was removed and preserved in containers, containing 10% formalin. (Fig 1(b) Tissue processing and embedding was done in paraffin. Slides were prepared and stained with haematoxylin and eosin. Microscopic study was done under 40X objective of a CX 21 light microscope.

Figure 3: Photomicrographs of trachea of control group C showing normal trachea (a), and group SS showing marked submucosal gland hypertrophy (b & c). Note the serous acini (a) and mucous acini (b) in photograph b (Fig a & b-H&E 10x40)(Fig c-H&E 10x10)

Figure 4: Graph showing submucosal gland hypertrophy in group SS

Slides were studied for submucosal gland hypertrophy by calculating the Reid’s Index. All measurements were taken by using an ocular micrometre fitted into the eyepiece of the microscope. The Reid’s index to assess the submucosal gland hypertrophy was measured with linear eyepiece micrometer.\(^{11}\) In the sections of the trachea, the maximal gland thickness was measured on a line at right angles to the plane of the cartilage (ab), and the total mucosal thickness (inner aspect of perichondrium to inner aspect of basement membrane) was measured on exactly the same line (cd) (fig 2).

The mean Reid index for each case was calculated by taking the mean of the indices of the trachea, which, in turn, were the means of at least four individual glands: wall ratios measured at the 4 different sites of each slide. Statistical analysis was done in SPSS version 20.0. A \(p\)-value of <0.05 was considered as statistically significant.

**Results**

The Haematoxylin and Eosin stained slides were examined for submucosal gland hypertrophy. The submucosa in slides of control group C showed mixed seromucous glands (Fig 3-a). There were mucus acini with crescent shaped serous demilunes. Mucus cells were identified by their flattened basally placed nuclei as compared to the rounded nuclei of serous cells (Fig 3-b).

Marked hypertrophy of submucosal glands was observed in experimental groups SS (Fig 3-c) and it was confirmed by expressing the gland size as a ratio of the mucous gland thickness to the total width of the tracheal mucosa (Reid’s Index). Group SS showed marked submucosal gland hypertrophy as compared to control group with Reid’s index more than 0.4. In group SS, submucosal gland hypertrophy was present in 14 (93.3%) mice and absent in 1 (6.7%) mice (Fig 4).
Discussion

The deleterious effects of tobacco on various body organs are extensively researched. A lot of studies are being conducted worldwide to unveil these effects and to curb the tobacco epidemic. The growing awareness of the adverse effects of cigarette, which is the commonest form of tobacco intake, has unfortunately urged people to look for alternate ways to fulfill their craving for nicotine. This has resulted in the re-emergence of the centuries old practice of water pipe smoking commonly known as “Shisha”. The common belief that the water filters the smoke has led to a wide acceptance of this practice that has resulted in a mushroom growth of Shisha cafés all around the world.

Serous and mucus cells are the two predominant epithelial cell types of mammalian airway glands. According to Fahy, submucosal glands of larger airways are mixed seromucous glands with mucus cells constituting 60% of gland volume. Serous cells, located distally, make up the remaining 40% of the gland. In pathological states volume of submucosal glands can increase to several times the normal volume with disturbance in the normal mucus to serous cells ratio. This is the main pathology behind excessive mucus secretion in COPD causing congestion and obstruction of airways. Quantitative differences were seen in the trachea among experimental groups in terms of submucosal gland hypertrophy. This pathological tissue change is interconnected and occurs as a consequence of disruption of the normal mucociliary clearance (MCC). MCC is a self-cleaning mechanism of the airways to remove inhaled pathogens and particulates and forms an important component of lung innate immunity. Effective mucus clearance is essential for lung health, and airway disease is a consequence of poor clearance. Inhaled tobacco smoke contains toxic chemicals out of which nicotine, acetaldehyde and acrolein have been identified as the main culprits that negatively affect MCC by increasing mucus secretion and decreasing ciliated cell numbers. Another feature in abnormal MCC is the submucosal gland hypertrophy measured by the Reid’s Index. Reid in 1960 used this index as a tool to measure the severity of chronic bronchitis. It is a proportion of gland thickness to bronchial wall thickness and has the advantage that all glands of a section of trachea or bronchus are covered and results are not influenced by wrinkling of the bronchial mucosa. The results of submucosal hypertrophy in experimental groups were significantly different from the control. The number of acidic glycoprotein containing secretory cells was increased while neutral glycoprotein containing secretory cells were decreased. This finding is consistent with finding by Dye and Shraideh who studied the histology of airway epithelial cells of mice exposed to tobacco smoke for 90 day. Similar results have been reported by Carter, Ying Le et al, Stefano et al and Soltani et al. Ning et al in their study explains that tobacco smoke generates free radicals which induce inflammation and mucus gland hyperplasia.

According to Chung, submucosal gland hypertrophy is seen in large airways with a disproportionate increase in mucous acini and reduction in serous acini in COPD. Presence of glandular hypertrophy in this research in group SS is consistent with findings of a study by Shraideh. High degree of hypertrophy in group SS can be explained on account of greater oxidative stress induced by Shisha smoke as indicated by Chaouachi. According to him, prolonged submucosal inflammation induced by toxicant injury by Shisha smoke causes hypertrophy of submucosal glands with a shift to acidic, sialidase resistant intracellular glycoproteins.

Conclusion

Shisha smoke induces inflammation and disruption of mucociliary clearance. The main culprit is the nicotine in Shisha smoke that is a powerful mucus secretagogue that leads to production of excess amount of mucus due to the hypertrophy of submucosal glands as well as the mucus cells.

Conflict of interest

This study has no conflict of interest to declare by any another.

References

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