Cytomorphological Feature of the Sputum among Hookah Smokers in *Elmangail*, Gezira State, Sudan (2016)

Ammar Ali Tagaldeen Gasmallah

BSc. in medical laboratory sciences
Department of histopathology and cytology
Omdurman Islamic university – 2010

A dissertation
Submitted to University of Gezira in
Partial fulfillment for the Requirement for the award of Degree of
Master of Science in
Histopathology and Cytology
Department of Histopathology and Cytology
Faculty of Medical Laboratory sciences
University of Gezira

2016
Cytomorphological Feature of the Sputum among Hookah Smokers in Elmangail, Gezira State, Sudan (2016)

Ammar Ali Tagaldeen Gasmallah

Supervision Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Albadawi Abdelbagi Talha</td>
<td>Main supervisor</td>
<td>.............</td>
</tr>
<tr>
<td>Prof. Adam Dawood Abakar</td>
<td>Co-supervisor</td>
<td>.............</td>
</tr>
</tbody>
</table>

Date:
Cytomorphological Feature of the Sputum among Hookah Smokers in *Elmangail*, Gezira State, Sudan (2016)

Ammar Ali Tagaldeen Gasmallah

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr.</td>
<td>Chairman Person</td>
<td>…………………</td>
</tr>
<tr>
<td>Dr.</td>
<td>External Examiner</td>
<td>…………………</td>
</tr>
<tr>
<td>Dr.</td>
<td>Internal Examiner</td>
<td>…………………</td>
</tr>
</tbody>
</table>

Date of Examination:
DECLARATION

This thesis is a presentation of my original research work, wherever contributions of others are involved, and every effort is made to indicate this clearly, with due to reference to the literature, and acknowledgment of collaborative research and discussion.

The work was done under the guidance of Dr. AlbadawiAbdelbagiTalha, at University of Gezira, Faculty of Medical Laboratory sciences.

Ammar Ali TagaldeenGasmallah
Dedication

This work is dedicated to my parents.

For their endless love, support and encouragement
Acknowledgments

Thanks to Allah. First and foremost

I would like to sincerely thank my supervisor, Dr. AlbadawiAbdelbagiTalha for his guidance and support throughout this study.

I would also thanks my colleague Dr. muhammedAbdallah for his grateful efforts.

To all my friends, thank you for your support and understanding in many moments of crisis.
Cytomorphological Feature of the Sputum among Hookah Smokers in Elmanagail, Gezira State, Sudan (2016)

Ammar Ali Tagaldeen Gasmallah

Abstract

Hookah smoking has been demonstrated to have adverse effects on human health, particularly increasing the risk of lung cancer because of ammonia, nicotine and tar. This study was aimed to detect pathological cellular changes of the sputum due to the effect of hookah smoke, in Shisha cafes in Almanagil city, Gezira state, Sudan, in 2016. This is a cross-sectional study. Fifty sputum specimens and anonymous survey in hookah coffee shops in Almanagil market had been conducted among cafe attendant individuals. All smokers in a randomly selected chairs agreed to participate. According to their age, smokers were divided into four groups, the first group was (20-30), the second group was (31-40), the third group was (41-50) and the fourth group was more than 50 years. Based on the duration of smoking per year, the smokers were divided into four groups, the first group was (0-5), the second group was (6-10), the third group was (11-15), and the last group was (16-20 years). Cytological smears from each sample were prepared, stained by H&E stain, and examined microscopically. Significant abnormal cytological features were found among different age groups include 8/50 showed acute inflammation cells, 10/50 showed chronic inflammation cells, 8/50 showed doubtful cells, 24/50 was normal, and no cancer cells. The study conclude that there is pathological cellular changes in the sputum associated with hookah smoking, this changes are: acute inflammatory cells, chronic inflammatory cells, and doubtful cells. There is association between Mua`ssel type and the severity of the cytological abnormalities.
التبديلات الخلوية في القشع بسبب تدخين "النرجيلة" في مدينة المناقل، ولاية الجزيرة - السودان (2016)

عمار علي تاج الدين بسم الله

ملخص الدراسة

وضوح أن تدخين النرجيلة له آثار ضارة على صحة الإنسان، على وجه الخصوص زيادة احتمالية الإصابة بسرطان الرئة، بسبب وجود مواد، مثل: الأمونياء، النيوكتون، والقطرات في مكونات حجر المتع. وقد هدفت هذه الدراسة إلى الكشف عن التغييرات الخلوية في القشع عند مدخني النرجيلة في مدينة المناقل. نوع هذه الدراسة مقطعية. تم جمع عدد (50) عينة قشع، وتعينة (50) استبانة، في مقهى نرجيلة في سوق مدينة المناقل، جميع رواد المقهى الذين احترمو مقاعدهم بصورة عشوائية وافقوا على المشاركة في الدراسة. وفقاً لأعمارهم، تم تقسيم المدخنين إلى أربعة مجموعات، وكانت الأولى: المجموعة الأولية (20-30)، المجموعة الثانية (31 - 40)، المجموعة الثالثة (41 - 50)، والمجموعة الرابعة (51-60) عاماً. كذلك تم تقسيم المدخنين حسب مدة التدخين بالسنوات، فكانت المجموعة الأولى (0-5)، المجموعة الثانية (6-10)، والمجموعات الرابعة هي (16-20 سنة). تم إعداد مسحات خلوية من عينات القشع، وتم صبها بصبغ (H&E) وقد تم اختباره عن طريقه معينه في الصبغ لكي نحصل على النتيجة المطلوبة، ثم فحصناها مجهرياً. تم العثور على تغيرات خلوية بين مختلف الفئات العمرية تشمل: 0.8 عينة التهاب حاد، 10.01 عينة التهاب مزمن، وكانت العينات المشكوك فيها بالإصابة بالسرطان تجاوزت 50/01، وكانت العينات الطبيعية تجاوزت 24/50، بينما لم ترد أي عينة فيها خلايا سرطانية. خلصت هذه الدراسة إلى أن هناك تغيرات مرضية في عينات قشع مدخنو النرجيلة، هذه التغيرات تراوحت بين الالتهاب الحاد، الالتهاب المزمن، والخلايا المشكوك فيها، وأن هناك علاقة بين نوع العسل ومقدار التغييرات المرضية.
# Table of Contents

**Supervision Committee:** .................................................................................................................. I

**Examination Committee:** ................................................................................................................ II

**DECLARATION** .................................................................................................................................... III

**Acknowledgments** ............................................................................................................................ V

**Abstract** ............................................................................................................................................... VI

**Abstract (in arabic language)** ................................................................................................................ VII

**Chapter One** ........................................................................................................................................ 1

1.1. General introduction......................................................................................................................... 1

1.2. Problem identification: ...................................................................................................................... 1

1.3. Problem justifications: ...................................................................................................................... 1

1.4. Research objectives: ......................................................................................................................... 3

   1.4.1. General objective ....................................................................................................................... 3

   1.4.2. Specific objectives: .................................................................................................................... 3

**Chapter Two** ....................................................................................................................................... 4

**Literature Review** .................................................................................................................................. 4

2.1. Cytology: .......................................................................................................................................... 4

   2.1.1. Definition: ................................................................................................................................. 4

   2.1.2. Role in diagnosis: ...................................................................................................................... 4

2.2. The respiratory system ...................................................................................................................... 4

   2.2.1. Air passage: .............................................................................................................................. 4

   2.2.2. Bronchial tree: ........................................................................................................................ 5

   2.2.3. Histology of the respiratory system: ......................................................................................... 6

2.3. Sputum .............................................................................................................................................. 6

   2.3.1. Definition: ............................................................................................................................... 6

   2.3.2. Components:............................................................................................................................ 6

   2.3.3. Pathological changes: ............................................................................................................. 7

2.4. The hookah ...................................................................................................................................... 7

   2.4.1. Definition: ............................................................................................................................... 7

   2.4.2. Chemical compositions: .......................................................................................................... 8

   2.4.3. Its harm effects: ....................................................................................................................... 8
List of Tables

<table>
<thead>
<tr>
<th>Table NO</th>
<th>Title</th>
<th>Page NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Distribution of study cases according to age group</td>
<td>10</td>
</tr>
<tr>
<td>4.2</td>
<td>Total number of smoking per day among study cases</td>
<td>10</td>
</tr>
<tr>
<td>4.3</td>
<td>duration of smoking per years among study cases</td>
<td>11</td>
</tr>
<tr>
<td>4.4</td>
<td>Type of Mo’assel among study cases</td>
<td>11</td>
</tr>
<tr>
<td>4.5</td>
<td>Diagnosis of smeared sputum</td>
<td>12</td>
</tr>
<tr>
<td>4.6</td>
<td>Diagnosis of abnormal smears</td>
<td>12</td>
</tr>
<tr>
<td>4.7</td>
<td>Nuclear changes of abnormal smears among study cases</td>
<td>13</td>
</tr>
<tr>
<td>4.8</td>
<td>Cytoplasmic changes of abnormal smears among study cases</td>
<td>13</td>
</tr>
<tr>
<td>4.9</td>
<td>Correlation between the cytology results and duration of smoking per years</td>
<td>14</td>
</tr>
<tr>
<td>4.10</td>
<td>Correlation between the cytology results and mua`ssel type</td>
<td>14</td>
</tr>
</tbody>
</table>
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>BHF</td>
<td>British Heart Foundation</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>PAHs</td>
<td>Polycystic Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>ALA</td>
<td>American Lung Association</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxy Ribonucleic Acid</td>
</tr>
<tr>
<td>RT</td>
<td>Respiratory Tract</td>
</tr>
<tr>
<td>CO</td>
<td>Carbon Monoxide</td>
</tr>
<tr>
<td>RZ</td>
<td>Respiratory Zone</td>
</tr>
<tr>
<td>RB</td>
<td>Respiratory Bronchial</td>
</tr>
<tr>
<td>KSS</td>
<td>Keratinized Squamous Stratified</td>
</tr>
</tbody>
</table>
Chapter One

Introduction

1.1. General introduction

Water pipe tobacco smoking is a general term given to an apparatus where tobacco is inhaled after passing through water. (Maziak, 2005). In Sudan, Water pipe tobacco smoking is commonly known as shisha, however certain ethnicities may continue to use other cultural names ascribed to it. In Sudan the type of tobacco used is known as Mo’assel, a mixture thought to contain 30% tobacco and 70% honey, humectants and fruit-flavors. (Nakkash, 2011). There are also forms of non-tobacco Mo’assel, known as ‘herbal’. Many suggest that only tobacco and non-tobacco (‘herbal’) Mo’assel types are consumed in Sudan, where there has been a witnessed increase in use.

1.2. Problem identification:

The spontaneous production of significant amounts of sputum often indicate pulmonary disease. Sputum is composed predominantly of mucoid substances, as well as variable numbers of inflammatory and epithelial cells. (Greenberg, 1976). Variations in the numbers of macrophages, neutrophil, and epithelial cells and morphologic alterations in the latter elements can yield significant insight into the underlying pathologic process. Similarly, the level of pigmentation within macrophages and the presence or absence of Curschmann’s spirals indicate much about the underlying pulmonary pathophysiology. (DeMello D, 1994).

1.3. Problem justifications:

In our local community there is a lot of bad habits, one of them is smoking hookah which is rising up in a pattern that let me think about it, read about its harm effects, and choose it as a problem for discussion in this research. Hookah smoking has dangerous effects, and highly popular among Sudanese. Many hookah smokers consider the practice less harmful than smoking cigarettes. This is troubling from a public health perspective since evidence shows that hookah smoking carries many of the same health risks and has been linked to many of the same diseases caused by cigarette smoking. No reports about hookah smoking in Sudan but there is a recent report by the British Heart Foundation (BHF) identified a 210% rise in the number of UK. Other countries have also witnessed a
rise in shisha smoking. The Global Youth Tobacco Survey concluded that, whilst cigarette smoking prevalence was decreasing among 13-15 year olds, 33 out of 97 global regions showed an increase in other tobacco use which was mostly attributed to shisha, (Warren CW, Lea V, Lee J, 1999). Several other longitudinal studies confirm these findings. Hookah’s public health status has been described by the American Lung Association as “a growing deadly trend”, (ALA. 2007). Several factors can explain this popularity. Shisha emanates a cool, aromatic smoke that provides a sensory appeal to its users contributing to a falsely reduced harm perception. Hookah smoking increases the risk of lung cancer because of ammonia, nicotine and tar. Ammonia that is present in additives triggers the release of nicotine in the tobacco. Pesticides that are used in fruits and other organic materials may also surface if not properly cleaned. Furthermore, the smoke in shisha is cooler than that of cigarettes. What this means is that its smoke is heavier thus sinking deeper into the lungs along with the tar. According to research conducted by the World Health Organization, a smoking session with a hookah that usually takes around 40-80 minutes or over an hour is equivalent to smoking around 200 sticks of regular cigarettes. Hookah smoking increases the chances of organ failure. Though organ failure with the use of hookah is not aggressive, it does sure leave damage in the human body most especially in the vital organs such as the brain, liver, kidneys, heart and lungs. This is due to the massive amount of carbon monoxide present when a person smokes a hookah. The body needs oxygen to function properly, but as it is overshadowed by carbon, it makes this simple fact complex. The human's vital organs need more amounts of oxygen than other parts of the body, and as it is deprived of the proper amount of oxygen it also functions less than its capability, thus resulting to organ failure in the long run. Hookah is apparently spread in Sudan “especially Gezira state, and Almanagil area”, with no governing roles, and no previous researches done here. So this research importance comes from its aim to explain the relation between hookah smoking and pathological cellular changes of the sputum.
1.4. Research objectives:

1.4.1. General objective

This study aims to find out cellular changes of the sputum due to the effect of hookah smoke.

1.4.2. Specific objectives:

- Find out nuclear changes.
- Find out cytoplasmic changes.
- Find out inflammatory cells.
- Find out precancerous cells.
- Find out malignant cells.
Chapter Two

Literature Review

2.1. Cytology:

2.1.1. Definition:

Cytology (from Greek κύτος, kytos, "a hollow"; and -λογία, -logia) is the study of cells. Cytology is that branch of life science that deals with the study of cells in terms of structure, function and chemistry. Based on usage it can refer to: Cytopathology: the study of cellular disease and the use of cellular changes for the diagnosis of disease. Cell biology: the study of (normal) cellular anatomy, function and chemistry. (Merriam, 2016)

2.1.2. Role in diagnosis:

Cytopathologic test (also called smear tests) used to show cell organelles under the microscope, which are used in diagnosis; In cancerous cells, the nucleus may show altered DNA activity which can be seen as a physical change in the nuclear qualities. Since more DNA is unfolded and being expressed, the nucleus will be darker and less uniform, larger than in normal cells, and often show a bright-red nucleolus. Other than cancerous or precancerous pathology, the cellular change may be: microbial infections: parasitic, viral, and/or bacterial – reactive changes - immune reactions - cell aging - amyloidosis – autoimmune diseases. (Wikipedia, 2016)

2.2 The respiratory system

2.2.1 Air passage:

After the inspired air passing through the nasal passages and pharynx (to be warmed and takes up water vapor) it passes throw the trachea, the bronchioles, respiratory bronchioles, and alveolar ducts to the alveoli. Between the trachea and the alveolar sacs, the airways divide 23 times. The first 16 generations of passages form the conducting zone of the airways that transports gas from and to the exterior. They are made up of bronchi, bronchioles, and terminal bronchioles. The remaining seven generations form the transitional and respiratory zones where gas exchange occurs; they are made up of
respiratory bronchioles, alveolar ducts, and alveoli. These multiple divisions greatly increase the total cross-sectional area of the airways, from 2.5 cm$^2$ in the trachea to 11,800 cm$^2$ in the alveoli. (Lippincott, 2014)

2.2.2. Bronchial tree:

- **Conducting zone:**
  - **Primary bronchi:** (c) shaped incomplete rings of hyaline cartilage support the walls to ensure that they remain open. Primary bronchi enter the hilum of each lung together with the pulmonary vessels, lymphatic vessels, and nerves. Intrapulmonary the cartilages are irregular. As smaller, bands of muscle become prominent, lamina propria has abundant elastic fibers, each primary bronchus then branches into secondary bronchi.
  - **Secondary bronchi:** or lobar bronchi: The left lung has two, and the right lung has three, they are smaller in diameter than primary. They are divide into tertiary bronchi.
  - **Tertiary bronchi "segment":** the right lung has 10, and the left has 8 or 10. They Supply bronchopulmonary segment. (Lippincott, 2014)
  - **Further divisions:** There are approximately 9 to 12 {of 23 division of air way} different levels of divisions exhibit some common characteristics:
    - Incomplete rings of cartilage in the walls become less numerous and smaller
    - When become smaller lined by ciliated columnar instead of respiratory.
    - Complete ring of smooth muscle is found between the mucosa of the airways and the cartilaginous support in the wall.
  - **Terminal bronchioles:** final segment of conducting. Less than 1 millimeter in diameter. Have ciliated simple columnar or simple cuboidal epithelium. Have no glands. Have Clara cells. Have no cartilages, as their small diameter alone prevents collapse. Instead, they have a thicker layer of smooth muscle regulates the amount of air.
  - **Respiratory bronchioles:** regards as area of transition. They are 0.5 mm in diameter. Have No cilia, No glands, and No cartilages.

- **Respiratory zone:**
  Functional unit of the lung (primary lobule) includes the: Alveolar ducts, Alveolar sacs, Alveoli.
These form sponge-like, elastic, thin-walled air-filled structures with extensive capillary beds, where respiratory gas exchange occurs.

- **Alveoli:** Humans have 300 million alveoli, and the total area of the alveolar walls in contact with capillaries in both lungs is about 70 m².
- **Capillaries:** surrounding alveoli. In most areas, air and blood are separated only by the alveolar epithelium and the capillary endothelium, so they are about 0.5 m apart.

### 2.2.3. Histology of the respiratory system:(Lippincott, 2014)

- The epithelium of the Nose skin is keratinized squamous stratified.
- The epithelium of the Nostrils are non-keratinized squamous stratified.
- The epithelium of the Nasopharynx is pseudostratified ciliated columnar epithelium.
- The epithelium of the Oropharynx is squamous stratified.
- The epithelium of the bronchial tree is respiratory epithelium
  - (The respiratory epithelium is pseudostratified ciliated columnar epithelium). Except: smaller branches and bronchi
- Bronchioles; simple cuboid or columnar.
- Terminal bronchioles; columnar at the beginning then changed to cuboid.
- The Alveoli; is squamous simple epithelium.

### 2.3. Sputum

#### 2.3.1. Definition:

Sputum is a mucous substance (consisting of cells and other matter) that is secreted into the airways of the respiratory tract (lungs, bronchi, and trachea) and can be coughed up spit out by a process called spetulization or swallowed. It is produced by surface epithelial cells and submucous glands, which are lie in a 5 µm thick mucus layer. It works to cover the airways and protect bronchial epithelium against inhaled noxious substances. In medicine, sputum samples are usually used for microbiological investigations of respiratory infections and cytological investigations of respiratory systems.(Merriam, 2016)

#### 2.3.2. Components:

- 95% water
• 5% organic components:
  • Carbohydrates (such as sialic acid which contribute to its high viscosity)
  • Proteins (enzymes, immunoglobulins), glycoproteins which also contributes to the viscoelastic property of sputum.
• Lipids
• Cellular components (J. bras, 2011): The mean total cell count was \(4.8 \pm 4.2 \times 10^6\) cells/g. There was a predominance of macrophages (mean, 77.5 ± 14.7%) and neutrophils (mean, 23.4 ± 14.3%). Eosinophils were virtually absent (mean, 0.1 ± 0.3%). Lymphocytes and bronchial epithelial cells were scarce. Neither age nor atopy had any effect on the total or differential cell counts.

2.3.3. Pathological changes: (Huether, S. E. &McCance, K. L. 2008)

2.3.3.1. Macroscopically:
  o Normal sputum is either white or colorless.
  o Yellow to green sputum can be an indication of pus, infection such as pneumonia.
  o Blood in sputum is called hemoptysis which could be due to e.g. lung cancer, tuberculosis, lung abscess, hemorrhage…
  o Rust color is due to decomposed Hemoglobin.
  o Very thick (viscose) sputum is a characteristic of cystic fibrosis.
  o Parasites in sputum can occur as in Ascaris.

2.3.3.2. Microscopically:
  o The examination under microscope may show normal cellular components “macrophages, neutrophils, Lymphocytes and bronchial epithelial cells” or pathological components “Inflammatotary, premalignant, or malignant cells”.

2.4. The hookah

2.4.1. Definition:

Also called: Persian, waterpipe, narghile, arghila, galyan, or other names) is a single or multi stemmed instrument for vaporizing and smoking flavored tobacco called shisha.
in which the vapor or smoke is passed throw a water basin- of glass – based – before inhalation. (Bernard quaritch, 2005)

2.4.2. Chemical compositions:

Include tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons (PAHs; e.g., benzopyrene and anthracene), volatile aldehydes (e.g. formaldehyde, acetaldehyde, acrolein), benzene, nitric oxide, heavy metals (arsenic, chromium, lead), and carbon monoxide (CO).

2.4.3. Its harm effects: (WHO, 2003)

Hookah smoking appears to increase the risk of several cancers (lung, esophageal, and gastric), pulmonary diseases (impaired pulmonary function, chronic bronchitis, and emphysema), coronary artery disease, periodontal disease, obstetrical and perinatal problems (low birth weight and pulmonary problems at birth), larynx and voice changes, and osteoporosis.

2.5. Previous studies

✓ A study titled “Hookah smoking: a growing threat to public health”, American lung association, 2011, concluded that A comprehensive approach to limiting access to hookah use is critical to averting a potentially deadly trend. Hookah smoking is a growing public health threat that may lead to a resurgence in tobacco use among vulnerable populations. Both the American Lung Association and the World Health Organization recommend that laws or regulations prohibiting cigarette or other tobacco use in public places apply to hookah smoking. Efforts should be made to restrict hookah use, especially among teens and young adults. (ALA, 2007)

✓ A study titled “Health effects associated with waterpipe smoking” Ziad M El-Zaatari1, Hassan A Chami, Ghazi S Zaatari, 2014, concluded than Chronic bronchitis, emphysema and coronary artery disease are serious complications of long-term use. Lung, gastric and oesophageal cancer are associated with WPS as well as periodontal disease, obstetrical complications, osteoporosis and mental health problems. (Ghazi S Zaatari, 2014)
Chapter Three

Material and Method

3.1. Study design:
Case control, laboratory based study. There was two manners used in this study:

- **The Theoretical manner:** in reading references, previous studies, and collecting data from different references about sputum and its role in diagnosis, the respiratory system, the hookah and its effects.
- **The applicable manner:** collecting data from the study field by using questionnaires (quantitative data) and sputum samples (qualitative data), examine it, writing the results, analyzing the results and figure out the conclusions.

3.2. Study area:
This study had been applied in Almanagil city, in Gezira state, Sudan.

3.3. Criteria of selection of study cases:
Sputum sample and anonymous survey in Hookah coffee shops in Almanagil market had been conducted among cafe attendant individuals. All smokers in a randomly selected chairs agreed to participate.

3.4. Materials:
- Frosted glass slides.
- Harries haematoxylin and eosin.
- 95% and 70% Alcohol.
- Wide, dry, clean and sterile sputum containers.
- Printed (50) copies of the questionnaire
- pen

3.5. Data collection:
A total of 18 questions were asked: 8 on prevalence, 5 on attitudes, and 5 on socioeconomic data. The definition of a current smoker was somebody who had smoked at least once in the past 30 days. Sputum sample had been taken from every volunteer who fill a questionnaire. A sputum sample was obtained by deep down cough early morning before brushing (avoiding food matter) and expelling the material that comes
from the lungs into a sterile cup. The sample was taken to the laboratory and placed in an appropriate medium.

3.6. Smear preparation and fixation:

   Pull part and immediate fixation in 95% ethanol for 15 minutes to prevent degenerative effect.

3.7. H&E staining:

   - Rinse in 95% ethanol for 2 minutes.
   - Rinse in 80% ethanol for 2 minutes.
   - Rinse in 70% ethanol for 2 minutes.
   - Rinse in D.W for 1 minute.
   - Stain with HARRIS for 40 second.
   - Wash in D.W.
   - Bluing in running tap water for 10 minutes.
   - Stain with EOSIN for 1 minutes.
   - Rinse in D.W.
   - Rinse in 70% ethanol for 10 second
   - Rinse in 80% ethanol for 10 second
   - Rinse in 95% ethanol for 10 second
   - Clear xylene and mount DPX

3.8. Criteria for diagnosis of sputum:

   - Duration required to perform cytological method.
   - Cost of the reagent.
   - Quality of the stain.
   - Type of cell.
   - Inflammatory and malignant cell.

3.9. Microscopic examination:

   The sputum samples had been examined under the microscope searching for any pathological change.
3.10. **Data analysis:**

Data collected was analyzed using the Statistical Package for the Social Science software (SPSS version 18).

3.11. **Ethical consideration:**

Ethical clearance of the study was taken from ministry of health Gezira state and verbal consent from the participants.
Chapter Four

RESULTS

(Table 4.1) Distribution of study cases according to age group:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(20 - 30)</td>
<td>27</td>
<td>54.0%</td>
</tr>
<tr>
<td>(31 - 40)</td>
<td>17</td>
<td>34.0%</td>
</tr>
<tr>
<td>(41 - 50)</td>
<td>5</td>
<td>10.0%</td>
</tr>
<tr>
<td>more than 50</td>
<td>1</td>
<td>2.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Result: The most frequent age group whom smoking hookah are less than (30) years old, and those consist more than (50)% of the study population.

(Table 4.2) Total number of smoking per day:

<table>
<thead>
<tr>
<th>Total number of smoking / day</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once</td>
<td>9</td>
<td>18.0%</td>
</tr>
<tr>
<td>Twice</td>
<td>14</td>
<td>28.0%</td>
</tr>
<tr>
<td>(3) times</td>
<td>13</td>
<td>26.0%</td>
</tr>
<tr>
<td>(4) times</td>
<td>5</td>
<td>10.0%</td>
</tr>
<tr>
<td>(5) times</td>
<td>4</td>
<td>8.0%</td>
</tr>
<tr>
<td>(6) times</td>
<td>2</td>
<td>4.0%</td>
</tr>
<tr>
<td>(7) times</td>
<td>3</td>
<td>6.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Result: 14 (28%) are smoking twice daily.
(Table 4.3) Duration of smoking per years:

<table>
<thead>
<tr>
<th>Duration/year</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0-5)</td>
<td>22</td>
<td>44.0%</td>
</tr>
<tr>
<td>(6-10)</td>
<td>22</td>
<td>44.0%</td>
</tr>
<tr>
<td>(11-15)</td>
<td>4</td>
<td>8.0%</td>
</tr>
<tr>
<td>(16-20)</td>
<td>2</td>
<td>4.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Result: More than (50)% of study sample are smokers for more than 5 year.

(Table 4.4) Type of Mo’assel:

<table>
<thead>
<tr>
<th>Mo’assel type</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerac</td>
<td>11</td>
<td>22.0%</td>
</tr>
<tr>
<td>Tofahteen</td>
<td>24</td>
<td>48.0%</td>
</tr>
<tr>
<td>Gerac + Mint</td>
<td>10</td>
<td>20.0%</td>
</tr>
<tr>
<td>Mint</td>
<td>5</td>
<td>10.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Result: The most frequent Mo’assel is “Tofahteen”.

13
(Table 4.5) Diagnosis of smeared sputum:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>24</td>
<td>48.0%</td>
</tr>
<tr>
<td>Abnormal</td>
<td>26</td>
<td>52.0%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

(Table 4.6) Diagnosis of abnormal smears:

<table>
<thead>
<tr>
<th>diagnosis</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute inflammation</td>
<td>8</td>
<td>30.77%</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>10</td>
<td>38.46%</td>
</tr>
<tr>
<td>Doubtful</td>
<td>8</td>
<td>30.77%</td>
</tr>
<tr>
<td>undifferentiated cells</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>100%</td>
</tr>
</tbody>
</table>

Result: As the number of abnormal smears are (26), Evidence of acute inflammation found in (8) of them, chronic inflammation in (10) stained smears, doubtful cells are in (8) stained smears, and no undifferentiated cells in any stained smear.
(Table 4.7) Nuclear changes of abnormal smears:

<table>
<thead>
<tr>
<th>Nuclear change</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granular change</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Finely deleted</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>karyorrhexis</td>
<td>1</td>
<td>50%</td>
</tr>
<tr>
<td>degeneration</td>
<td>1</td>
<td>50%</td>
</tr>
<tr>
<td>Multi-nucleation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Result: nuclear changes found in (2) stained smears, one of them showed Karyorrhexis, and the other showed degeneration.

(Table 4.8) Cytoplasmic changes of abnormal smears:

<table>
<thead>
<tr>
<th>cytoplasmic change</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytolysis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unequal size</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>dyskaryosis</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>100%</td>
</tr>
</tbody>
</table>

Result: cytoplasmic changes found in (7) stained smears, all of it is dyskaryosis.
(Table 4.9) Correlation between the cytology results and duration of smoking per years:

<table>
<thead>
<tr>
<th>Cytology result</th>
<th>duration of smoking per years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5</td>
<td>6-10</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Acute inflammation</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Doubtful</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>undifferentiated cells</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

Result: In correlation between cytology result and duration of smoking; (3) of the (8) acute inflammation smears are (0-5) years, and (3) are (6-10). In chronic inflammation (5) cases are (0-5). In doubtful cells (5) are smokers for less than (5) years, and the rest between (6-10).

(Table 4.10) Correlation between the cytology results and mua`ssel type:

<table>
<thead>
<tr>
<th>Cytology result</th>
<th>Gerac</th>
<th>Tofahteen</th>
<th>Gerac + Mint</th>
<th>Mint</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6</td>
<td>10</td>
<td>6</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>Acute inflammation</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Doubtful</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>undifferentiated cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>24</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
</tbody>
</table>

Comment: Tofahteen associated with (4) doubtful smears, while mint associated with zero.
Chapter Five

DISCUSSION

Cytological changes that involve respiratory tract can result from different causes. Hookah smoking is frequently reported with different changes. And the sputum represents a mirror reflecting these alterations.

The aim of the present study was to detect cytological changes of the sputum in (50) Smokers of Hookah, in Almanagil city, Gezira state, Sudan.

In this study the pathological cytological changes among study sample was found in (26) cases from (50).

Turbid smear and presence of neutrophils indicate acute inflammation. In this study, acute inflammation was found in (8) smears, represents (30.8%) of abnormal smears, this result agree with a study done by Ali Yousif, Concluded that shisha users were found more susceptible for bacterial infections 34% compared to controls 3%. (Ali Yousif, 2014)

Chronic inflammation diagnosed by the presence of chronic inflammatory cells, likely lymphocytes. In this study, chronic inflammation diagnosed in (10) smears out of (50), (5) of them are smoker for less than (5) years, (3) of them are smoker between (6 – 10) years, and the other (2) are smokers for more than (10) years.

Doubtful cells are found in (8) smears out of (50). This doubtful cells are: Dyskaryotic cells, squamous ca cells, adenocarcinoma cells, and squamous metaplastic cells. In correlation with the duration of smoking, (5) of the doubtful are belong to smokers for less than (5) years, and the other (3) are belong to smokers for (6-10) years. In correlation with mua`ssel type, (4) of the doubtful are belong to smokers who use “Tofahteen”, (3) of the doubtful are belong to smokers who use mixed mint and geracmua`ssel, (1) of the doubtful are belongs to smoker who use gerac, and no doubtful cells in the group of smokers who use mint. From this we can recommend smokers to use mint mua`ssel if they couldn`t stop smoking, as mint is less harmful compared with others in this study.

There is no undifferentiated cells in this study.
Chapter Six

CONCLUSION AND RECOMMENDATION

6.1 Conclusion:
This study concludes that there is a pathological cellular change in the sputum associated with hookah smoking, these changes are: acute inflammatory cells, chronic inflammatory cells, and doubtful cells. There is association between Muà’ssel type and the severity of the cytological abnormalities.

6.2 Recommendations:
- Health education of smokers, using mass media, or reaching them in shisha cafes, telling them about its harm effects in the respiratory system.
- Health system designers in need to organize regular periodic cytological sputum examination for the customers of hookah smoking cafes, particularly for early detection and treatment of suspected cases.
- Government should initiate deliberated policy to restrict or eliminate the smoking of hookah, and making it difficult, and so reducing the number of population who are predisposed to pathological changes.
- More studies are needed in order to provide a more precise assessment of the effect of long-term exposure of hookah smoke on morbidity and mortality, including a more precise characterization of specific dose-response and muà’ssel type relationships for the various adverse health effects.
References


Assessment of lung cytological atypia among shisha smokers


Ghazi S Zaatari, 2014, Health effects associated with waterpipe smoking.


J. bras. pneumol. vol.37 no.3 São Paulo May/June 2011


Se’bastienCouraud, Ge´lardZalcman, Bernard Milleronbd,(2012). Franck Morin, Pierre-Jean Souquet, European Journal of Study the lunge Cancer.1300


The cyclopedia of india, volume 2, Bernard quaritch, retrieved November 2015


WHO Study Group on Tobacco Product Regulation (TobReg) an advisory note Waterpipe tobacco smoking: dangerous health effects include risk to public safety if used by multiple users, research needs and recommended actions by regulators, 2005” (PDF). World Health Organization. Retrieved 2013-09-03.


Appendix

Questionnaire

Serial No: _____________________________________________________________

Name: ___________________________________________________________________

Age: ___________________________ 4. Sex: _____________________________

5. Place: ___________________________ 6. Tel No: __________________________

7. Duration of hookah smoking (in years) __________________________________

8. Numbers of smoking per day: ___________________________________________

9. Type of mu`assel: _____________________________________________________

Lab finding:

Normal: ( ____)

Imprint cytology:

___________________________________________________________________________

___________________________________________________________________________

___________________________________________________________________________

____________________

Report:
# Bronchial Tree

<table>
<thead>
<tr>
<th>Name of branches</th>
<th>Number of tubes in branch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>1</td>
</tr>
<tr>
<td>Bronchi</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Bronchioles</td>
<td>16</td>
</tr>
<tr>
<td>Terminal bronchioles</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>$6 \times 10^4$</td>
</tr>
<tr>
<td>Respiratory bronchioles</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^5$</td>
</tr>
<tr>
<td>Alveolar ducts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$8 \times 10^6$</td>
</tr>
<tr>
<td>Alveolar sacs</td>
<td></td>
</tr>
</tbody>
</table>
Histology of the respiratory system