***Original Research Article***

**Detection of Cytological Atypia in Sputum Samples among Sudanese Females Practicing *Dukhan* in Shendi Town**

**ABSTRACT**

**Background**: Sputum cytology is a diagnostic technique used to examine sputum samples under a microscope to detect abnormal cellular changes. Dukhan, a traditional smoke bath practice widely used by women in Sudan, particularly in Shendi town, involves exposure to wood smoke that may lead to cytological alterations in the respiratory system. **Materials and Methods:** This descriptive cross-sectional study was conducted between December 2024 to February 2025رamong women who practice Dukhan in Shendi town. A total of 40 sputum samples were collected and processed using conventional cytological techniques to assess potential cellular abnormalities. **Results**: The most common age group among participants was 30–40 years. The study revealed several cytological changes, including keratosis (100%), cellular degeneration (87.5%), cytoplasmic vacuolization (50%), inflammation (55%), nuclear atypia (20%), necrosis (7.5%) A statistically significant association was observed between Dukhan use and cytological alterations, especially squamous metaplasia and degenerative changes (P-value = 0.00). **Conclusion**: The use of Dukhan is associated with cytological changes in sputum, including cytoplasmic and nuclear atypia, inflammation, and cellular degeneration. These findings highlight the importance of more research and public awareness efforts on the health risks linked to Dukhan exposure.

**Keywords**: Dukhan, Smoke Bath, Sputum Cytology, Atypia, Sudanese Women, Shendi Town

1. **INTRODUCTION**

The lungs are two air-filled, spongy organs that are situated on either side of the thorax, or chest. The trachea (windpipe) conducts inhaled air into the lungs through tubular branches called bronchi, which progressively divide into smaller branches (bronchioles), eventually ending in microscopic clusters of air sacs known as alveoli [1]. The body naturally produces mucus—also known as phlegm or sputum—to protect the respiratory tract. This mucus aids in capturing and eliminating potentially harmful foreign particles. Changes in the color, consistency, or amount of sputum may indicate underlying health conditions [2]. Sputum cytology is a diagnostic technique that involves microscopic examination of sputum samples to detect abnormal or atypical cells. It is primarily used to investigate infections, chronic inflammatory conditions, or suspected malignancies of the respiratory system [3]. For generations, traditional medical methods have made use of medicinal herbs. In many cultures, their applications extend beyond therapeutic uses to include ceremonial and spiritual purposes [4–6]. Various methods exist for the preparation and administration of plant-based remedies, including inhalation of smoke, one of the oldest forms of application, especially in African traditional medicine [7,8]. In Sudanese tradition, *Acacia seyal* Delile (family: Leguminosae), known locally as "Talh", is widely used for its medicinal and cosmetic benefits. Historically, its bark and wood, along with species from the genus Combretum, are burned to produce smoke used in the traditional smoke bath, known as *Dukhan* [10,11]. This practice is prevalent among Sudanese women and is believed to relieve rheumatic pain, smooth the skin, treat wounds, and promote general relaxation. It is also considered a symbol of beauty and femininity [12]. In recent years, *Dukhan* smoke has been commercialized in the form of cosmetic creams that serve as emollients and skin softeners [13]. Despite its cultural significance, the inhalation of wood smoke raises health concerns due to its potential respiratory effects. The chronic exposure to smoke particulates may lead to cellular alterations in the respiratory tract. Such exposure may contribute to inflammation and irritation through mechanisms involving prostaglandins—compounds synthesized from arachidonic acid by cyclooxygenase enzymes. These molecules play key roles in inflammatory pathways and pain modulation [14]. Given the widespread and prolonged use of *Dukhan* among Sudanese women, especially in Shendi town, it is crucial to investigate its potential impact on respiratory health. This study aims to assess cytological changes in sputum among women who practice *Dukhan*, thereby contributing to a better understanding of its health implications.

**2. Materials and Methods**

**Study Design and Setting**

This was a community-based descriptive cross-sectional study conducted in Shendi Town, located approximately 172 km north of the capital Khartoum, in the southern part of the River Nile State, Sudan. The collected sputum samples were transferred to the Histopathology and Cytology Laboratory at Shendi University, where they were processed and examined.

**Study Duration**

The study was carried out over three months, from December 2024 to February 2025.

**Study Population and Eligibility Criteria**

The study population consisted of Sudanese women residing in Shendi Town who regularly use the traditional smoke bath (*Dukhan*).

**Inclusion criteria:** Women of various age groups who use *Dukhan* and were free from any known chronic pathological illnesses.

**Exclusion criteria:** Women with known chronic respiratory diseases or other systemic illnesses were excluded.

**Sample Size and Sampling Technique**

A total of forty (40) women who reported regular Dukhan use and agreed to participate were included in the study using a non-probability purposive sampling technique.

**Data Collection Tools and Procedures**

Data were collected using structured questionnaire sheets to document participants’ sociodemographic information and relevant clinical history. Sputum samples were collected from each participant for cytological examination to detect possible cytomorphological alterations.

**Method of Detection**

All sputum samples were processed and stained using conventional cytological techniques, and slides were examined microscopically to identify cellular atypia and other morphological changes.

**Sample Collection and Processing**

Sputum samples were collected from each participant early in the morning, right after waking up. The collection procedure involved these steps: Participants were instructed to remove dentures (if present), rinse their mouth thoroughly with water, then take about four deep breaths followed by a series of forceful coughs. The sputum sample—not saliva—was expectorated into a new sterile, disposable plastic container with a screw-on lid, pre-filled with 1 mL of 70% ethanol to preserve it. A small amount of each sample was selected, centrifuged at 2,500 revolutions per minute (rpm) for 5 minutes, and the deposit was transferred onto a frosted-end, labeled glass slide. A smear was prepared using a spreader slide to make a thin layer, slightly thicker than a blood smear. All slides were immediately fixed in 95% ethyl alcohol for 15 minutes to ready them for Papanicolaou (PAP) staining. The first step involved the use of hematoxylin, which stains cell nuclei [15]. Historically, Papanicolaou used Harris’s hematoxylin in all three versions of his stain formulation [16].

**Papanicolaou (PAP) Stain Procedure**

Each fixed slide was rehydrated using descending grades of ethanol (100%, 95%, 80%, 70%) for 2 minutes in each grade. The slides were then stained regressively in Harris's hematoxylin for 2 minutes, followed by differentiation in 1% acid alcohol for two rinses, with microscopic control. Slides were then blued in running tap water for 10 minutes, dehydrated again using ascending grades of ethanol (70%, 80%, 95%, and 100%), and subsequently stained with Orange G6 (OG6) for 3 minutes. After rinsing in 95% ethanol, EA50 (Eosin Azure 50) was applied for 7 minutes, followed by a second rinse in 95% ethanol. Finally, slides were cleared in absolute ethanol, dried at room temperature, cleared in xylene, and mounted using DPX (Distrene Plasticizer and Xylene). All smears were initially screened under a light microscope by the researcher and confirmed by the supervising cytopathologists [17].

**Quality Control for PAP Staining**

To ensure optimal stain quality and diagnostic accuracy, several quality control measures were implemented: the Use of fine filters and distilled water to eliminate impurities and prevent contamination. Thorough mixing of stains using magnetic stirrers or centrifugation. Drying stained slides in specialized ovens to ensure proper fixation and avoid external contamination. Use of high-quality, clean microscopes equipped with stable light sources (e.g., LED). Routine maintenance, including cleaning of filters, glassware, and microscope lenses, and timely replacement of consumables. These procedures ensure uniform stain distribution, defect-free slides, and enhanced diagnostic reliability.

**Interpretation of Results**

Cytological atypia observed in the sputum samples included the following features: Epithelial alterations such as squamous metaplasia, reactive cell hyperplasia, mucin cytoplasmic vacuolization, parakeratosis, hyperkeratosis, and cellular degeneration. Inflammatory changes, including background necrosis, infection, and inflammation, were also present. Cytoplasmic changes encompassed cytoplasmic granulation, vacuolization, and degeneration. Nuclear atypia involved enlargements of cells and nuclei, increased nuclear-to-cytoplasmic (N/C) ratio, coarse chromatin, irregular nuclear membranes, bi- or multinucleation, hyperchromasia, enlarged nucleoli, the presence of mitotic figures, and increased chromatin content. These findings were interpreted following established cytological criteria for atypia and premalignant changes [18,19].

 **Data Analysis**

After examination of the sections, the results of the laboratory investigation, as well as the demographic data from the patient’s records, were processed using the Statistical Packages for Social Sciences (SPSS) computer program. Frequency, mean, and chi-square test values were calculated at <0.05 and considered statistically significant.

**3. RESULTS**

This study included 40 Sudanese females from Shendi Town who reported using *Dukhan* (smoke bath). The majority of participants were between 30 and 40 years of age, and 77.5% used Talh (*Acacia seyal*) wood exclusively, while 22.5% used a combination of *Talh* and *Shaf* (*Terminalia brownii*). Regarding weekly exposure, 35% used *Dukhan* 2–3 times, 20% more than 3 times, and 40% irregularly. Macroscopic examination of the sputum showed visible changes in only 5% of samples, while 100% of the participants exhibited cytological atypia (Table 1). The most common cytological alterations included keratosis (100%), cellular degeneration (87.5%, *P-value* = 0.000), cytoplasmic granulation/vacuolization (50%), and inflammation (55%, *P-value* = 0.527). Nuclear atypia was observed in 20% of samples (*P-value* = 0.000), and necrosis in 7.5% (*P-value* = 0.000) (Table 2). A total of 7.5% of participants reported respiratory health issues such as chronic cough and allergies, all of whom also had cytological atypia (Table 3). No difference in the presence of atypia was observed across groups categorized by years of *Dukhan* use. Cellular changes were found in all participants regardless of exposure duration: <5 years (37.5%), 5–10 years (25%), and >10 years (37.5%) (Table 4). Similarly, cytological atypia was present across all categories of weekly exposure, with the highest frequency among irregular users (40%) (Table 5).

**Table 1. Presence of macroscopic and cytological changes in sputum**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Frequency** | **Percentage (%)** | ***P*-*value*** |
| Macroscopic sputum changes | 2 | 5.0% | 0.000 |
| Cytological atypia | 40 | 100% |

**Table 2. Subtypes of cytological atypia among participants**

|  |  |  |
| --- | --- | --- |
| **Cytological Feature** | **Frequency (%)** | ***P*-*value*** |
| Keratosis | 100% |  |
| Cellular degeneration | 87.5% | 0.000 |
| Cytoplasmic granulation/vacuole | 50% |  |
| Inflammation | 55% | 0.527 |
| Nuclear atypia | 20% | 0.000 |
| Necrosis | 7.5% | 0.000 |
| Perinuclear halo | 40% | 0.206 |

**Table 3. Association between cytological atypia and respiratory health symptoms**

|  |  |  |
| --- | --- | --- |
| **Respiratory Condition** | **Frequency** | **Percentage (%)** |
| Present | 3 | 7.5% |
| Absent | 37 | 92.5% |

**Table 4. Cytological atypia by duration of *Dukhan* use (years)**

|  |  |  |
| --- | --- | --- |
| **Duration** | **Frequency** | **Percentage (%)** |
| < 5 years | 15 | 37.5% |
| 5–10 years | 10 | 25.0% |
| > 10 years | 15 | 37.5% |

**Table 5. Cytological atypia by weekly frequency of *Dukhan* use**

|  |  |  |
| --- | --- | --- |
| **Frequency per Week** | **Frequency** | **Percentage (%)** |
| Once | 2 | 5.0% |
| 2–3 times | 14 | 35.0% |
| >3 times | 8 | 20.0% |
| Irregular | 16 | 40.0% |

**4. DISCUSSION**

This study investigated cytological alterations in the sputum of Sudanese women who regularly use *Dukhan*(smoke bath) in Shendi Town. Despite the absence of visible macroscopic changes in 95% of participants, all of them (100%) exhibited cytological atypia (Table 1), indicating the presence of subclinical cellular damage potentially caused by wood smoke inhalation. This underscores the value of sputum cytology as a sensitive diagnostic tool even in asymptomatic individuals. The most frequent cytological abnormalities were keratosis (100%), cellular degeneration (87.5%, p = 0.000), cytoplasmic vacuolization (50%), nuclear atypia (20%, p = 0.000), and necrosis (7.5%, p = 0.000), while inflammation (55%) was not statistically significant (p = 0.527) (Table 2). These findings are consistent with previous studies that demonstrated wood smoke-induced cellular damage and genotoxicity [20,21] (28, 30). In particular, Esam et al. (2022) [22] confirmed DNA damage associated with Acacia seyal smoke using bacterial and mammalian assays, which supports our detection of nuclear atypia and degeneration by cytomorphological analysis. Furthermore, all participants showed cytological atypia regardless of the duration or frequency of *Dukhan*use (Tables 4 and 5), suggesting that both short-term and irregular exposures may be sufficient to induce early cellular alterations. This contrasts with assumptions that only prolonged or high-frequency use is harmful. Irregular users accounted for the highest proportion of atypia (40%), indicating the need to explore the role of exposure intensity and individual susceptibility. Only 3 participants (7.5%) reported respiratory symptoms, such as asthma or chronic cough, yet all showed atypical cellular features (Table 3). This highlights the ability of sputum cytology to detect preclinical cellular damage before symptoms emerge—a finding supported by previous research on pollution-related atypia [23], and indoor smoke exposure [24]. Our results, however, showed a lower frequency of clinical symptoms compared to Amna et al. (2005) [24], likely due to differences in exposure duration and inhalation method (passive exposure in *Dukhan*vs. continuous indoor smoke). Our findings also partially reflect those of Ahmed and Rezgalla (2010) [23], who reported a strong association between traffic-related air pollution and lung epithelial atypia, particularly metaplasia and dysplasia. In contrast, our study detected no dysplastic or malignant transformations, possibly due to the limited duration of exposure or small sample size. Nonetheless, the presence of nuclear atypia and necrosis indicates a possible early stage of genotoxic stress. While Chun et al. (2008) [21] found no mutagenic effects from wood smoke flavors (WSF), our findings contradict this, likely due to differences in wood type (Acacia seyal vs. WSF), combustion temperature, and exposure route. Similarly, Ozturk et al. (2018) [18] identified airborne contact dermatitis linked to Aldukhan, which aligns with our observation of keratinization and epithelial irritation. Although our focus was on respiratory cells, the parallel in mucosal responses is noteworthy. Moreover, White and Sandler (2017) [25] suggested a potential association between prolonged wood smoke exposure and breast cancer risk. While our study did not assess carcinogenicity, the detection of nuclear atypia suggests the need for future molecular and longitudinal studies to explore this possible link. In conclusion, this study provides compelling evidence that *Dukhan*exposure induces significant cytomorphological changes in the respiratory tract. The universal presence of cellular atypia—regardless of exposure pattern—raises concern about the safety of this traditional practice and highlights the importance of public health education and further research.

## **Limitations**

Because of the small sample size (n = 40) and non-probability sampling method, the findings might not be applicable to all *Dukhan* users in Sudan.

## **5. CONCLUSION**

This study demonstrates a strong association between *Dukhan*use and cytological atypia in sputum samples of Sudanese women. Despite the lack of visible sputum changes or overt clinical symptoms in most participants, significant microscopic alterations were present in all cases. The most common changes included keratosis, cellular degeneration, cytoplasmic vacuolation, and nuclear atypia. Although no malignant or dysplastic features were observed, the presence of nuclear atypia and necrosis suggests a potential risk of long-term genotoxicity. These findings highlight the need for increased awareness about the possible respiratory health impacts of traditional *Dukhan* practices.

## **6. RECOMMENDATION**

Based on the findings of this study, it is recommended to raise awareness among Sudanese women about the potential respiratory health risks associated with *Dukhan* use, encourage the application of sputum cytology as a simple and non-invasive screening method for early detection of cellular alterations, promote safer practices such as ensuring adequate ventilation during *Dukhan* sessions to reduce exposure to harmful emissions, and conduct further large-scale and long-term studies to explore the possible genotoxic and carcinogenic effects of prolonged or frequent *Dukhan* exposure.

**CONSENT**

The patient’s written consent has been collected.

**ETHICAL APPROVAL**

The study was approved by the Department of Histopathology and Cytology in Medical Laboratory Sciences at Shendi University, and the study was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the area of study. The aims and the benefits of this study were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

As a result, the Author (s) declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

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