Patterns of oral inflammatory cells infiltrate associated with cigarette smoking

Hussain Gadelkarim Ahmed\textsuperscript{1,2}, Muanera Ali Alqufeye\textsuperscript{1}, Ghaida Ali Alsulaiman\textsuperscript{1}, Latifah Lafi Alenezi\textsuperscript{1}, Reem Khaled Almuslumani\textsuperscript{1}, Ghofran Mohammed Alhussain\textsuperscript{1}, Ahad Khaled Alenezi\textsuperscript{1}, Adwaa Fahad Algharbi\textsuperscript{1}, Amjad Eid Alanazi\textsuperscript{1}, Noeer Khleef Alshammari\textsuperscript{1}

\textsuperscript{1}Department of Pathology, College of Medicine, University of Ha’il, Saudi Arabia
\textsuperscript{2}Department of Histopathology and Cytology, FMLS, University of Khartoum, Sudan

\textsuperscript{Correspondence to:}
Prof. Hussain Gadelkarim Ahmed, College of Medicine, University of Ha’il, Saudi Arabia, Email: hussaingad5@gmail.com

\textbf{Article History}
Received: 02 June 2020
Reviewed: 03/June/2020 to 10/July/2020
Accepted: 11 July 2020
E-publication: 18 July 2020
P-publication: September - October 2020

\textbf{Citation}

\textbf{Publication License}
This work is licensed under a Creative Commons Attribution 4.0 International License.

\textbf{General Note}
Article is recommended to print as color digital version in recycled paper.

\textbf{ABSTRACT}
\textit{Background:} Oral exfoliated cytology is one of the effective screening and diagnostic tools for early detection of oral mucosal diseases. \textit{Methodology:} This was a cross-sectional case-control study conducted in Ha’il city, Northern Saudi Arabia. Smoked tobacco users were ascertained as cases and non-tobacco users were ascertained as controls. Oral cytological materials were obtained by
Results: Acute inflammatory cells infiltrate were identified in 9 cytological smears, 5 were from the cases, and 4 from controls. Chronic inflammatory cells infiltrate were identified in 10 oral cytological smears 8 were from the cases and 2 from controls. The risk of chronic inflammatory cells infiltrates associated with tobacco smoking, the odds ratio (OR), and 95% confidence interval (95%CI) was, OR (95%CI) = 2.9565 (0.6084 to 14.3667), P = 0.1790, z statistics = 1.344. Fungal infection was identified in 13 cytological smears, 10 belongs to cases and 3 belongs to controls. The risk of oral fungal infection associated with tobacco smoking, OR (95%CI) was 1.344(0.6573 to 9.3678), P = 0.1799, z statistics = 1.341. Conclusion: Exposure to tobacco products can induce inflammatory events in the buccal mucosa, which can be identified by oral exfoliated cytology. Fungal and viral oral infections are common among tobacco users. Further research is needed to explore the hidden causes of inflammation and predict their expected complications.

Keywords: Acute inflammatory cells infiltrate, cytological smears, chronic inflammatory cells infiltrates, oral cytology, HPV, Candida Albicans.

1. INTRODUCTION

Oral cancer represents one of the leading malignancies worldwide (Lingström et al., 2020). The incidence of oral cancer is high in numerous developing countries with declined 5-years survival rates (Shresthaetla., 2020; Nocini et al., 2020). Several risk factors have been linked to the etiology of oral cancer including tobacco usage, alcohol consumption, viral infections, and others (Ahmed, 2013). Tobacco smoking was found to induce oral carcinogenesis through variable processes comprising epigenetic modulation of tumor suppressor genes (Sabi et al., 2020).

Although oral cancer can affect any part of the oral cavity, buccal mucosa is mutually influenced since it is widely exposed to the direct effects of tobacco-related carcinogens (Shresthaetla., 2020).

Screening for the early detection of the oral precancerous and cancerous lesions is an essential step towards oral cancer control and better management ending with good outcomes. Cautious examination of the oral cavity of at-risk individuals followed by appropriate cytological specimens can identify most cases at an early stage with a good chance of treatment (Velleuer et al., 2020).

Besides neoplastic changes, oral cytology can detect several abnormalities. Several infectious agents can be indicated in the oral cytology, such as human papillomavirus (Méndez-Martínez et al., 2020), fungal infections, such as candida abdicant (Gupta et al., 2020). Besides these infectious agents, necrotic effects of tobacco exposure can produce inflammatory processes, which can be evidenced in the oral smear through the presence of variable inflammatory cells infiltrates. Therefore, the present study aimed to assess the patterns of oral inflammatory cells infiltrate associated with cigarette smoking.

2. MATERIALS AND METHODS

This was a cross-sectional case-control study conducted in Ha’il city, Northern Saudi Arabia during the period of October 2018 to October 2019. About 170 volunteers have agreed to participate in the study. A simple random method was used for the selection of the participants regardless of age or sex. Smoked tobacco users were ascertained as cases and non-tobacco users were ascertained as controls. Oral cytological materials were obtained by brushing of the buccal mucosa. The obtained cytological materials were smeared on a cleaned glass-slide and immediately immersed in 95% ethyl alcohol for 15 minutes then sent to the laboratory for cytological staining using Papanicolaou (Pap. Stain).

Smears were evaluated microscopically based on the presence of cytological evidence as follows:

- **Acute inflammatory cells:** identified by the presence of polymorph nuclear cells infiltrate and necrotic cells debris.
- **Acute inflammatory cells:** identified by the presence of mononuclear cells including macrophages, monocytes, and lymphocytes.
- **Fungal infection (Candid Albicans):** it was identified by the presence of hyphae, pseudohyphae.
- **Viral infection (Human Papillomavirus (HPV)):** Koilocytes epithelial cells.

Statistical analysis

Data were analyzed using SPSS software (version 16). Odds ratios and chi-square tests were performed considering a 95% confidence level. A P-value of less than 0.05 was considered statistically significant.

3. RESULTS

In the present study, buccal cytology was done for 170 candidates, ages 19-66 years, with a mean age of 30 years, 121/170(71.2%) were males and 49/170(28.8%) were females (image 1 & 2). The majority of patients were at age group 21-30 years followed by 31-
40 years, representing 92/170 (54%) and 40/170 (23.5%), respectively, as indicated in Table 1, Fig 1. Most of the participants were with secondary education followed university education level, representing 86/170 (50.6%) and 58/170 (34%), in that order, as indicated in Table 1, Fig 1.

Table 1. Distribution of the study population by demographic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>7</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>21-30</td>
<td>70</td>
<td>22</td>
<td>92</td>
</tr>
<tr>
<td>31-40</td>
<td>35</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>41+</td>
<td>9</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>49</td>
<td>170</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic</td>
<td>17</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Secondary</td>
<td>68</td>
<td>18</td>
<td>86</td>
</tr>
<tr>
<td>University</td>
<td>30</td>
<td>28</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>49</td>
<td>164</td>
</tr>
</tbody>
</table>

Figure 1. Participants by demographic characteristics

The males’ females’ ratio was relatively similar between cases and controls. The age and education levels distribution were also relatively similarly distributed between cases and controls, as shown in Table 2, Fig 2.

Table 2. Distribution of the cases/controls by demographic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>cases</th>
<th>controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>69</td>
<td>52</td>
<td>121</td>
</tr>
<tr>
<td>Females</td>
<td>31</td>
<td>18</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>70</td>
<td>170</td>
</tr>
<tr>
<td>age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>8</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>21-30</td>
<td>55</td>
<td>37</td>
<td>92</td>
</tr>
</tbody>
</table>
Acute inflammatory cells infiltrate were identified in 9 cytological smears, 5 were from the cases, and 4 from controls. Chronic inflammatory cells infiltrate were identified in 10 oral cytological smears 8 were from the cases and 2 from controls. The risk of chronic inflammatory cells infiltrates associated with tobacco smoking, the odds ratio (OR), and 95% confidence interval (95%CI) was, OR (95%CI) = 2.9565 (0.6084 to 14.3667), P = 0.1790, z statistics = 1.344 (see image 1).

Fungal infection was identified in 13 cytological smears, 10 belongs to cases and 3 belongs to controls. The risk of oral fungal infection associated with tobacco smoking, OR (95%CI) was 1.344(0.6573 to 9.3678), P = 0.1799, z statistics = 1.341 (see image 2).

Cytological evidence of viral infection was identified in 8 cytological smears, 5 belongs to cases and 3 belongs to controls. The risk of oral viral infection associated with tobacco smoking, OR (95%CI) was 1.1754 (0.2716 to 5.0875), P = 0.8288, z statistics = 0.216, as indicated in Table 3.
All 9 cases with acute inflammatory cell infiltrate were seen among males. The risk of acute inflammatory cell infiltrates associated with men, the OR (95%CI) was 14.6393 (0.8377 to 255.8244), P = 0.066, z statistics = 1.839.

Chronic inflammatory cell infiltrates were seen in 15 males and 4 females. The risk of acute inflammatory cell infiltrates associated with men, the OR (95%CI) was 2.9118 (0.9230 to 9.1856), P = 0.0683, z statistics = 1.823.

Fungal infections were seen in 11 males and 2 females. The risk of fungal infection associated with men, the OR (95%CI) was 4.2022 (0.9014 to 19.5896), P = 0.0676, z statistics = 1.828.

Cytological evidence of viral infections was seen in 7 males and 1 female. The risk of viral infection associated with men, the OR (95%CI) was 5.1935 (0.6244 to 43.1966), P = 0.1274, z statistics = 1.524, as shown in Table 4.

Table 4. Distribution of inflammatory cells infiltrates, fungal and viral infections by gender

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute inflammatory cells infiltrate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>No</td>
<td>112</td>
<td>49</td>
<td>161</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>0</td>
<td>170</td>
</tr>
<tr>
<td><strong>Chronic inflammatory cells infiltrate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>No</td>
<td>106</td>
<td>45</td>
<td>151</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>49</td>
<td>170</td>
</tr>
<tr>
<td><strong>Fungal infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>No</td>
<td>110</td>
<td>47</td>
<td>157</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>49</td>
<td>170</td>
</tr>
<tr>
<td><strong>Viral infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>No</td>
<td>114</td>
<td>48</td>
<td>162</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td>48</td>
<td>170</td>
</tr>
</tbody>
</table>

Table 5, Fig 3, summarized the distribution of inflammatory cells infiltrate, fungal and viral infections by age. The majority of acute inflammatory cells infiltrates were seen in the age group 21-30 years 5/9(56%). The majority of chronic inflammatory cells infiltrates were seen in the age group 21-30 years followed by 31-40 years constituting 9/19(47%) and 5/19(26%) correspondingly. Most fungal infections were evidenced in the age group 21-30 years 8/13(61.5%). Viral infections were indicated in the age range 21-40 years representing 6/8(75%). When calculating the percentage within the entire age group, variable proportions appear, as shown in Fig 3.

Table 5. Distribution of inflammatory cells infiltrates, fungal and viral infections by age
Figure 3. Inflammatory cells infiltrate, fungal and viral infections by age.

Image 1. Buccal smear showing both chronic and acute inflammatory cells infiltrate.
4. DISCUSSION

Besides its deterioration effects on most parts of the body, tobacco consumption tends to have local effects on the oral cavity, particularly buccal mucosa and the dorsal of the tongue. These effects may eventually progress by time into diverse oral abnormalities starting from simple inflammatory changes to precancerous and cancerous disorders. Consequently, tobacco users always at risk of developing oral abnormalities, and this necessitates the continuous inspection of the oral cavity for the detection of any abnormality and treats it as early as possible before progression into a massive disease. Oral exfoliative cytology is one of the effective screening and diagnostic tools for the early detection of oral mucosal diseases (Sahu et al., 2019). For that reason, the present study was assuming that tobacco use can induce necrosis in the mucosa, and these dead cells can attract microorganisms, as well as, various inflammatory reactions, which interns can result in harmful effects.

The present study found increased proportions with elevated relative risks of both acute and inflammatory cells infiltrates (with greater in chronic compared to acute) among tobacco users compared to non-tobacco users (controls). Besides major periodontal abnormalities induced by tobacco smoking, it was reported to influence the oral tissues, attracting bacteria, and stimulating multiple immune-components mostly associated with the mechanism of healing and defense mechanisms responses (Zeller et al., 2019; Javed et al., 2019). Tobacco products were found to produce systemic and local (oral) effects comprising vasoconstriction, and inflammation signifying alterations in cell viability and inspire the production of numerous inflammatory mediators (cytokines) (Aghaloo et al., 2019). These continuous events can result in several consequences including chronic inflammatory conditions and periodontal disease.

In the present study fungal infection (Candida Albicans), was more frequent among cases compared to controls. Some recent studies reported that Candida Albicans species are more common among tobacco users as tobacco products enhance the PH of the oral cavity rendering it more appropriate for the growth of fungi (Negi et al., 2019). It was suggested that the chronic hosting of tissues by Candida Albicans elevates the risk of oral precancerous and oral cancer initiation and progression (Engku et al., 2020).

Cytological evidence of viral infection (mainly human Papillomavirus (HPV)) was identified in a high number of cases compared to females. A recent study has reported HPV in 24.3% of oral specimens (Cossellu et al., 2018). Although infection with high-risk human papillomaviruses subtypes increases the risk of oral precancerous and cancerous lesions, abnormal oral cytology more frequently associated with tobacco usage habits and alcohol consumption (enevelo et al., 2020).

Most abnormalities encountered in the present study were associated with men and a relatively prolonged exposure period. However, this might be attributed to the fact that most tobacco users with high usage intensity were males. Moreover, tobacco use is considered a social stigma in the majority of Arabian populations.
5. CONCLUSION
Exposure to tobacco products can induce inflammatory events in the buccal mucosa, which can be identified by oral exfoliated cytology. Fungal and viral oral infections are common among tobacco users. Further research is needed to explore the hidden causes of inflammation and predict their expected complications. Oral cytology is a simple and non-invasive procedure with outstanding advantages rendering it the most suitable for routine examinations of oral health.

Acknowledgment
The authors would like to thank all participants for their agreement and participation.

Author Contributions:
HGA: Conception, analysis, drafting, approval of the final version.
MAA, GAA, LLA, RKA, GMA, AKA, AFA, AEA, NKA: Conception, design, data acquisition, approval of the final version.

Funding
This research has been funded by Scientific Research Deanship at University of Ha’il – Saudi Arabia through project number RG-191191.

Conflict of interest
Authors declare no conflict of interest

Informed consent:
Written & Oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

Ethical approval for human: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (ethical approval number HREC 00121a/CM-UOH.04/20).

Data and materials availability.
All data associated with this study are present in the paper.

REFERENCES AND NOTES


