Malondialdehyde and Nitrous oxide as Salivary Biomarkers for Malignant and Premalignant Oral lesions

Maha Abdelkawy1, Sahar El Refai2, Olfat Gamil Shaker3, Weam Elbattawy4

1Department of Oral Medicine and Periodontology, Faculty of Dentistry, Beni-Suef University, Beni-Suef, Egypt.
2 Department of Oral Pathology, College of Dentistry, princess Nourah Bint Abdulrahman University.
3 Department of Biochemistry, Faculty of Medicine, Cairo University.
4 Department of Oral Medicine and Periodontology, Faculty of Dentistry, Cairo University, Cairo, Egypt.

Email: weamelbattawy@dentistry.cu.edu.eg

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Abstract

Objectives: the current study investigated the levels of malondialdehyde & nitric oxide in saliva in oral premalignant and malignant lesions in order to determine their diagnostic value for the malignant and potentially malignant lesions.

Subjects and Methods: A total of 100 subjects diagnosed with keratosis (n=20), leukoplakia (n=20), oral lichen planus (n=20), oral squamous cell carcinoma (n=20) & control group of healthy age- and gender-matched individuals (n=20) were included in this study. Clinical and histopathological examinations were performed to assure diagnosis then salivary samples were collected. Malondialdehyde and nitric oxide were measured by enzyme linked immunosorbent assay technique.

Results: Salivary malondialdehyde and nitric oxide were significantly high in the oral squamous cell carcinoma and oral lichen planus groups, where both showed 100% diagnostic accuracy. Pair-wise comparison between areas under the receiver operating characteristic (ROC) curve revealed 90% for malondialdehyde and 85% for nitric oxide in the leukoplakia group, showing that malondialdehyde had statistically significantly higher mean areas under the ROC curve than nitric oxide. On comparing both markers in the keratosis group, malondialdehyde and nitric oxide showed 85% and 80% respectively with no statistically significant difference.

Conclusion: malondialdehyde & nitric oxide are noteworthy diagnostic markers that could be utilized in diagnosis of oral squamous cell carcinoma.

Keywords: malondialdehyde, nitric oxide, oral potentially malignant disorders, malignant oral lesions, salivary biomarkers

Introduction

Lesions that are prone to turn malignant; oral potentially malignant disorders (OPMDs) or premalignant lesions, are recently being greatly considered as they set a big challenge for researchers. This owes to their increased liability and significant risk to easily transform into cancer (Senghore et al., 2018).

The potentiality of dysplastic premalignant lesions as keratosis, oral lichen planus (OLP) and leukoplakia for malignant transformation has been strongly debatable.
Leukoplakia is one of the most common OPMDs, nevertheless, oral lichen planus (OLP) has been a relatively common, immunologically mediated, inflammatory disease that has also been placed on the premalignant list with many different views. However, tendency of keratosis for malignant change is relatively rare in most of its types. An exception to this, are those occurring with oral snuff and tobacco chewing, which could progress to carcinoma but still demand a long-term use (Farah et al., 2014) (Scully, 2013). Oral squamous cell carcinoma (OSCC) accounts for nearly 90% of malignancies of the oral cavity. Studies have revealed that more than 70% of the patients have a premalignant lesion prior to cancer (Awasthi, 2017).

Being a detrimental disease, cancer development is a very complicated process that requires many sequential steps and depends on a various number of genetic alterations and factors (Senghore et al., 2018). Among these factors are the free radicals which are derived either from endogenous or exogenous sources (Deepankar et al., 2016). Free radicals result in an oxidative stress of the affected cells generating a body imbalance between oxidants and antioxidants (Khoubnasabjafari, Ansarin and Jouyban, 2016).

In this regard, saliva has been currently used to assess such possible oxidative imbalance through various salivary biomarkers including nitric oxide (NO) and malondialdehyde (MDA). Saliva could be used as a reliable rich medium, known for its role in diagnosis, prognosis and treatment that has become more prominent in oral diseases (Khoubnasabjafari, Ansarin and Jouyban, 2016).

Nitric oxide (NO) is an intercellular messenger molecule having many important biological functions. The levels of nitric oxide in salivary secretions could be used to monitor the severity of many underlying disease processes. Moreover, malondialdehyde (MDA) has been the most frequently used biomarker of oxidative stress in many pathological conditions such as cancer. It results from reactive oxygen species lipid degradation and induced toxic stress in affected cells, making both biomarkers a point of focus in monitoring clinical progress of suspected lesions (Sundar et al., 2013) (Khoubnasabjafari, Ansarin and Jouyban, 2015) (Farmer and Duvoine, 2007).

Subjects and Methods:
This study started on February 2019 till November 2019 and it included 100 patients. They were divided into five groups equally; OSCC, atrophic-bullous erosive OLP, leukoplakia, keratosis (frictional) and control group of healthy age- and gender-matched individuals.

The study protocol was registered in ClinicalTrials.gov (ID:NCT04267419) and approved by the Research Ethics Committee of Faculty of Dentistry Beni-Suef University (ID #FDBSUREC/17022019/AM) and was conducted in full accordance with the World Medical Association Declaration of Helsinki 1975, revised in 2003. Each patient received the appropriate treatment after finishing their role in the study.

Inclusion Criteria:
All included subjects were assessed medically in conformity with the Systemically free patients, American Society of Anesthesiologists I; (ASA I,2019). The patients were recruited from the diagnostic center, Faculty of Dentistry, Cairo University. Based on patient history and clinical examination, all included individuals were not under any current medication and were not suffering from any other systemic condition or any other oral mucosal disorder. The diagnosis of patients was based on clinical findings and was confirmed by histopathological examination based on criteria published by the World Health Organization (WHO, 2005).

Exclusion Criteria
Subjects were excluded from this study based on the following criteria which included: subjects having systemic disorders or taking medications; pregnant or lactating females; subjects who suffered from any other mucosal lesions; and severe periodontal inflammation.

Salivary sample collection:
Collection of whole unstimulated saliva, using standard techniques was done as described by (Navazesh, 1993). Concisely, individuals refrained from eating, drinking, chewing gum etc., for at least half an hour prior to the evaluation. Samples were collected by asking subjects to swallow first, tilt their head forward and expectorate all saliva in a tube for 5 minutes without swallowing. After collection, all
samples were immediately stored at -20°C until assayed.

Determination of NO

The nitric oxide level in saliva was measured using ELISA kit provided by My BioSource, USA.

Principle of the Assay:

This experiment used the double-sandwich ELISA technique and the ELISA Kit provided was typical. The pre-coated antibody was human NO monoclonal antibody and the detecting antibody was polyclonal antibody with biotin labeled. Samples and biotin labeling antibody were added into ELISA plate wells and washed out with PBS or TBS. Then Avidin-peroxidase conjugates were added to ELISA wells in order; Used TMB substrate for coloring after reactant thoroughly washed out by PBS or TBS. TMB turned into blue in peroxidase catalytic and finally turned into yellow under the action of acid. The color depth and the testing factors in samples were positively correlated.

Determination of MDA

MDA was measured in saliva using ELISA kit provided by SunLong Biotech Co.,LTD (China). This Quantitative Sandwich ELISA kit is only for in vitro research use only, not for drug, household, therapeutic or diagnostic applications. It is intended to determine MDA concentrations in Human serum, plasma and other body fluids. Using Purified Human MDA antibody to coat Microelisa Stripplate wells to make solid-phase antibody, then add MDA and MDA antibody which has been labeled with HRP to wells, then the reactants become antibody-antigen-antibody-enzyme complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color under HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of MDA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Sample Size Calculations

This power analysis used MD level as the primary outcome. The effect size (f = 0.34) for comparison between the five groups was calculated based upon the results of (Kaur, Politis and Jacobs, 2016). Using alpha (α) level of (5%) and Beta (β) level of (20%) i.e. power = 80%; the minimum estimated sample size was 20 subjects per group giving a total of 100 subjects. Sample size calculation was performed using G*Power Version 3.1.9.2.

Statistical Analysis

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Age data showed normal (parametric) distribution while markers levels showed non-normal (non-parametric) distribution. Parametric data were presented as mean, standard deviation (SD) and 95% confidence interval values (95% CI). Non-parametric data were presented as median and Inter-Quartile Range (IQR).

For parametric data; one-way ANOVA was used to compare between the five groups. For non-parametric data; Kruskal-Wallis test was used to compare between the five groups. Dunn’s test was used for pair-wise comparisons. Qualitative data were presented as frequencies and percentages. Chi-square test was used for the comparisons.

Receiver operating characteristic (ROC) curve was constructed to determine the cut-off values of the two markers for differentiation between the different groups. Areas under the ROC curve (AUCs) were compared using z-statistic. The significance level was set at P ≤ 0.05. Statistical analysis was performed with IBM (Corporation, NY, USA) SPSS (Inc., an IBM Company) Statistics Version 20 for Windows. ROC curve analysis was performed with MedCalc Version 11.3 for Windows (MedCalc Software bvba).

Results

Baseline characteristics

There was no statistically significant difference between mean age values in the five groups. There was also no statistically significant difference between gender distributions in the five groups (Table 1).
Table (1): Descriptive statistics and results of one-way ANOVA and Chi-square tests for comparisons between baseline characteristics in the five groups

<table>
<thead>
<tr>
<th></th>
<th>Keratosis (n = 20)</th>
<th>Leukoplakia (n = 20)</th>
<th>OLP (n = 20)</th>
<th>OSCC (n = 20)</th>
<th>Control (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>41.7 (6.4)</td>
<td>47.6 (12.8)</td>
<td>40.1 (12)</td>
<td>47.2 (11.5)</td>
<td>41 (7.7)</td>
<td>0.056</td>
</tr>
<tr>
<td>95% CI</td>
<td>38.7 – 44.6</td>
<td>41.6 – 53.5</td>
<td>34.5 – 45.7</td>
<td>41.8 – 52.6</td>
<td>37.4 – 44.6</td>
<td></td>
</tr>
<tr>
<td>Gender [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12/20 (60%)</td>
<td>11/20 (55%)</td>
<td>6/20 (30%)</td>
<td>7/20 (35%)</td>
<td>4/20 (20%)</td>
<td>0.055</td>
</tr>
<tr>
<td>Female</td>
<td>8/20 (40%)</td>
<td>9/20 (45%)</td>
<td>14/20 (70%)</td>
<td>13/20 (65%)</td>
<td>16/20 (80%)</td>
<td></td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05

Markers

The Level of Malondialdehyde (MDA) and Nitric Oxide (NO) in Each Group:

Considering the level of MDA in the salivary samples, the OSCC group showed the statistically significantly highest mean level, however, OLP group showed statistically significantly lower mean level. There was no statistically significant difference between keratosis and leukoplakia groups; both showed statistically significantly lower mean levels. The control group showed the statistically significant lowest mean MDA level in saliva (Table 2).

On investigating the level of NO in saliva, still the OSCC group showed the statistically significantly highest mean level. OLP group showed statistically significantly lower mean level. However, there was no statistically significant difference between keratosis, leukoplakia and control groups, where all showed statistically significant lower mean levels than the other two groups (Table 2).

Table (2): Descriptive statistics and results of Kruskal-Wallis test for comparisons between markers’ levels in the five groups

<table>
<thead>
<tr>
<th></th>
<th>Keratosis (n = 20)</th>
<th>Leukoplakia (n = 20)</th>
<th>OLP (n = 20)</th>
<th>OSCC (n = 20)</th>
<th>Control (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>1.89 (1.38–1.91)</td>
<td>1.54 (1.47–2.04)</td>
<td>2.62 (2.56–3.11)</td>
<td>3.33 (3.22–4.32)</td>
<td>0.97 (0.87–1.24)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>NO (µmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>11.7 (10.3–13.2)</td>
<td>12.3 (10.9–14.3)</td>
<td>39.5 (32.2–48.4)</td>
<td>68.9 (50.2–116.7)</td>
<td>9.1 (8.3–10.1)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different.
Diagnostic accuracy of the two markers (MDA and NO) by ROC curve analysis:

A. Differentiation between keratosis and control

ROC curve analysis of the two markers for differentiation between keratosis and control subjects showed cut-off values of 1.02 and 10.1 µmol/l for MDA and NO, respectively. MDA showed higher diagnostic accuracy (85%) compared with NO (80%). However, pair-wise comparison between areas under the ROC curve of the two markers did not show statistically significant difference (P-value = 0.157) (Table 3, Figure 1).

B. Differentiation between leukoplakia and control

ROC curve analysis of the two markers for differentiation between leukoplakia cases and control subjects as presented in table (3) and figure (1) showed cut-off values of 1.24 and 10.1 µmol/l for MDA and NO, respectively. MDA showed higher diagnostic accuracy (90%) compared with NO (85%). Pair-wise comparison between areas under the ROC curve (AUC) of the two markers revealed that MDA showed statistically significantly higher mean AUC than NO for differentiation between leukoplakia cases and normal subjects (P-value <0.001).

Table (3): Cut-off values for different markers and the corresponding sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for differentiation between keratosis, Leukoplakia, OLP and OSCC with the control subjects

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>+PV (%)</th>
<th>-PV (%)</th>
<th>Diagnostic accuracy (%)</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Keratosis &amp; control groups</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>1.02</td>
<td>100</td>
<td>70</td>
<td>76.9</td>
<td>100</td>
<td>85%</td>
<td>0.925</td>
<td>0.796-0.984</td>
</tr>
<tr>
<td>NO</td>
<td>10.1</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80%</td>
<td>0.830</td>
<td>0.678-0.930</td>
</tr>
<tr>
<td><strong>Leukoplakia &amp; control groups</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>1.24</td>
<td>100</td>
<td>80</td>
<td>83.3</td>
<td>100</td>
<td>90%</td>
<td>0.927</td>
<td>0.800-0.985</td>
</tr>
<tr>
<td>NO</td>
<td>10.1</td>
<td>90</td>
<td>80</td>
<td>81.8</td>
<td>88.9</td>
<td>85%</td>
<td>0.890</td>
<td>0.751-0.967</td>
</tr>
<tr>
<td><strong>OLP &amp; control groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>1.68</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100%</td>
<td>1.00</td>
<td>0.912-1.000</td>
</tr>
<tr>
<td>NO</td>
<td>12.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100%</td>
<td>1.00</td>
<td>0.912-1.000</td>
</tr>
<tr>
<td><strong>OSCC &amp; control groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>1.68</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100%</td>
<td>1.00</td>
<td>0.912-1.000</td>
</tr>
<tr>
<td>NO</td>
<td>12.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100%</td>
<td>1.00</td>
<td>0.912-1.000</td>
</tr>
</tbody>
</table>

+PV: Positive Predictive Value, -PV: Negative Predictive Value
Figure (1): ROC curves of the two markers (MD and NO) for differentiation between control subjects and keratosis cases and leukoplakia cases.

C. Differentiation between OLP and control
   ROC curve analysis of the two markers for differentiation between OLP and control subjects showed cut-off values of 1.68 and 12.6 µmol/l for MDA and NO, respectively. Both markers showed 100% accuracy for differentiation between OLP and normal subjects (Table 3).

D. Differentiation between OSCC and control
   ROC curve analysis of the two markers for differentiation between OSCC and control subjects showed cut-off values of 1.68 and 12.6 µmol/l for MDA and NO, respectively. Both markers showed 100% accuracy for differentiation between OSCC and normal subjects (Table 3).

E. Differentiation between keratosis and leukoplakia
   ROC curve analysis of the two markers for differentiation between keratosis and Leukoplakia subjects showed cut-off values of 1.68 and 10.3 µmol/l for MDA and NO, respectively. Although MDA showed higher diagnostic accuracy (70%) compared with NO (65%) as shown in table (4), however, pairwise comparison between areas under the ROC curve of the two markers showed non-statistically significant difference (P-value = 0.396) as represented in figure (2).
Table (4): Cut-off values for different markers and the corresponding sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for differentiation between keratosis and Leukoplakia.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cut-off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>+PV</th>
<th>-PV</th>
<th>Diagnostic accuracy</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratosis &amp; leukoplakia</td>
<td>MDA</td>
<td>1.68</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70%</td>
<td>0.527</td>
<td>0.364-0.687</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>10.3</td>
<td>90</td>
<td>40</td>
<td>60</td>
<td>80%</td>
<td>0.608</td>
<td>0.441-0.758</td>
</tr>
</tbody>
</table>

+PV: Positive Predictive Value, -PV: Negative Predictive Value

Figure (2): ROC curves of the two markers for differentiation between keratosis and leukoplakia

F. Differentiation between keratosis and OSCC

ROC curve analysis of the two markers for differentiation between keratosis and OSCC subjects showed cut-off values of 2.17 and 15.8 µmol/l for MDA and NO, respectively. Both markers showed 100% diagnostic accuracy for differentiation between keratosis and OSCC (Table 5).

G. Differentiation between leukoplakia and OSCC

ROC curve analysis of the two markers for differentiation between leukoplakia and OSCC subjects showed cut-off values of 2.34 and 15.5 µmol/l for MDA and NO, respectively. Both markers showed the same diagnostic accuracy (84.5%), however, pair-wise comparison between areas under the ROC curve of the two markers showed non-statistically significant difference (P-value = 0.840) as represented in table (5) and figure (3).

H. Differentiation between OLP and OSCC
ROC curve analysis of the two markers for differentiation between OLP and OSCC subjects showed cut-off values of 3.11 and 48.4 µmol/l for MDA and NO, respectively. MDA showed lower diagnostic accuracy (80%) compared with NO (82.2%), which is shown in table (5). However, pair-wise comparison between areas under the ROC curve of the two markers showed non-statistically significant difference (P-value = 0.361) as shown in figure (3).

**Table (5):** Cut-off values for different markers and the corresponding sensitivity, specificity, predictive values, diagnostic accuracy, Area Under the ROC curve (AUC) and 95% confidence interval (95% CI) of the (AUC) for differentiation between each of keratosis, leukoplakia and OLP with OSCC.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cut-off</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>+PV %</th>
<th>-PV %</th>
<th>Diagnostic accuracy %</th>
<th>AUC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>keratosis and OSCC</td>
<td>MDA</td>
<td>2.17</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100%</td>
<td>1.000</td>
<td>0.912-1.000</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>15.8</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100%</td>
<td>1.000</td>
<td>0.912-1.000</td>
</tr>
<tr>
<td>Leukoplakia and OSCC</td>
<td>MDA</td>
<td>2.34</td>
<td>100</td>
<td>55</td>
<td>69</td>
<td>84.5%</td>
<td>0.795</td>
<td>0.638-0.906</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>15.5</td>
<td>100</td>
<td>55</td>
<td>69</td>
<td>84.5%</td>
<td>0.785</td>
<td>0.627-0.899</td>
</tr>
<tr>
<td>OLP and OSCC</td>
<td>MDA</td>
<td>3.11</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80%</td>
<td>0.837</td>
<td>0.687-0.935</td>
</tr>
<tr>
<td></td>
<td>NO</td>
<td>48.4</td>
<td>85</td>
<td>80</td>
<td>81</td>
<td>84.2%</td>
<td>0.900</td>
<td>0.763-0.972</td>
</tr>
</tbody>
</table>

+PV: Positive Predictive Value, -PV: Negative Predictive Value

**Figure (3):** ROC curves of the two markers (MD and NO) for differentiation between OSCC with leukoplakia and OLP.

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Discussion:

Cancer development in humans is a complex, multistep process, therefore detecting oral cancer at an early stage has been mandatory to improve the prognosis of the disease. This has been challenging for oral clinicians and oral medicine specialists as different clinical forms of the same disease as well as similar clinical forms of different diseases have always been an obstacle (Lodi et al., 2019).

Several actions including molecular and cellular alterations could contribute to cancer development. The overall process is mediated by different types of endogenous and exogenous stimuli such as generation of reactive oxygen species (ROS). Oxidative stresses have been proven to play a role in premalignant and malignant oral lesion development, with the suspected role in the conversion process. This showed how essential the indicators could be for early cancer detection (Lodi et al., 2019).

Although histopathological examination could give the definitive diagnosis, several noninvasive techniques has been targeted as salivary biomarkers where saliva was being used as diagnostic medium. MDA and NO levels have been found to be increased in OSCC indicating the deficiency of the antioxidant mechanism in the malignant conditions (Khournasabjafari, Ansarin and Jouyban, 2015).

The current study results showed MDA and NO levels to be the highest in OSCC group followed by OLP group. Keratosis and leukoplakia showed lower results while the control group recorded the lowest levels of MDA and NO. These results were in agreement with (Gokul et al., 2010) who revealed that MDA and NO levels were found to be high in blood and tissues of OSCC patients. Moreover, (Ratajczak-Wrona et al., 2013) stated that NO and its end products, one of which is MDA, were found to be higher in serum of OSCC patients than control subjects indicating that they might have a role in the disease progression.

Concerning OLP, the results of this study were also in agreement with (Lopez-Jornet, Martinez-Canovas and Pons-Fuster, 2014), whose study showed high salivary MDA in OLP group than control group. They stated that their findings showed that the mechanism of antioxidant defense was disturbed leading to increased ROS production, which resulted in high lipid peroxidation along with MDA as its end product.

The definition of leukoplakia agreed upon by the WHO is; “White plaque of questionable risk, having excluded other known diseases or disorders that carry no risk”. Frictional lesions have been considered as a different category as there is a cause for their presence (Farah et al., 2014) (Warnakulasuriya, Johnson and van der Waal, 2007). Accordingly, this study included two different groups;
leukoplakia and keratosis, where the levels of salivary MDA and NO in these two groups were found to be lower than OSCC and OLP groups but still higher than the control group with no statistically significant difference. This was found to be in consensus with (Güven et al., 2005), where salivary MDA in patients with oral leukoplakia were studied and found to be higher than the control group with no statistically significant difference.

On the other hand, (Khoubnasabjafari, Ansarin and Jouyban, 2016) stated that despite there is no doubt that oxidative stress had a role in the etiology of systemic and oral diseases, MDA was not to be considered a biomarker representing oxidative stresses in saliva, serum or plasma due to wide MDA concentration ranges in different studies. Also, (Khoubnasabjafari, Ansarin and Jouyban, 2016) together with (Cheng, Rees and Wright, 2014) attributed the unreliability of MDA salivary biomarker to the deficient standardization of saliva sample collection and lack of validation criteria for it. Therefore, in the current study, it was mandatory to assess the diagnostic accuracy of MDA and NO salivary biomarkers by using the ROC curve and plotting the sensitivity and specificity of each test to every possible cut off point (Akobeng, 2007).

In this study, NO and MDA biomarkers showed 100% accuracy in OSCC and OLP groups on differentiation with control group with cut off values 1.68 and 12.6 Umol for MDA and NO respectively. This implies the possibility of using them as a diagnostic test. However, on testing the diagnostic accuracy for leukoplakia with the control group, it was 90% and 85% for MDA and NO respectively with MDA levels showing statistical significance. Also, the diagnostic accuracy for the keratosis group showed 85% for MDA and 80% for NO with no statistical significance for neither of them. This indicates that using MDA and NO biomarkers for diagnosis in cases of leukoplakia and keratosis is still questionable and would need further investigations.

On comparing the diagnostic accuracy of both markers for keratosis and OSCC groups, it revealed a 100% accuracy. However, both NO and MDA showed the same diagnostic accuracy (84.5%) on differentiating the OSCC and leukoplakia groups with no statistical significance. Additionally, the same was found in case of comparing the diagnostic accuracy of OLP and OSCC where MDA and NO gave almost the same diagnostic accuracy (80% MDA and 84.2 % NO). The results of these comparisons would favor using MDA and NO salivary biomarkers in OSCC suspected lesions but still make the differentiation between an OLP and OSCC patient questionable.

In agreement with our results (Kaur, Politis and Jacobs, 2016) reported that MDA levels were significantly higher in OSCC with sensitivity and specificity of 61% and 58% versus control. On comparing MDA of potentially malignant lesion versus control, both sensitivity and specificity were 55% while on comparing OSCC and OPML it was only 43% and 45% for sensitivity and specificity respectively. They concluded that lipid damage and oxidative DNA are present in pre-cancerous and SCC patients and hence detection of MDA can act as suitable diagnostic biomarker.

Conclusion: MDA & NO are noteworthy diagnostic markers that can be utilized in the early diagnosis of oral squamous cell carcinoma.

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