Comparative evaluation of salivary levels of malondialdehyde and lactate dehydrogenase in active and passive smokers

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Abstract

BACKGROUND AND AIM: This study is conducted aiming to assess and compare the salivary levels of malondialdehyde (MDA) and lactate dehydrogenase (LDH) in active and passive smokers to determine the correlation between environmental tobacco smoke and health condition.

METHODS: This study evaluated 75 healthy individuals including 25 active smokers, 25 passive smokers, and 25 non-smokers with an equal percentage of males and females. Unstimulated saliva samples were collected from all participants. The salivary level of cotinine was first measured for correct allocation of participants to the aforementioned three groups using enzyme-linked immunosorbent assay (ELISA) technique. The salivary levels of MDA and LDH were then measured.

RESULTS: The salivary level of cotinine was 19.1, 8.12, and 3.36 nmol/ml in active smokers, passive smokers, and non-smokers, respectively. The salivary level of MDA was 4.78, 2.67, and 2.63 nmol/ml while the salivary level of LDH was 508.33, 364.98, and 271.63 nmol/ml in active smokers, passive smokers, and non-smokers, in the order given.

CONCLUSION: Acceding to the results, the salivary levels of cotinine, MDA, and LDH had significant correlations with cigarette smoking. The salivary levels of MDA and LDH were significantly higher in active smokers than passive smokers, and also the values in passive smokers were higher than the corresponding values in non-smokers (P < 0.05).

KEYWORDS: Malondialdehyde; Lactate Dehydrogenase; Smokers; Passive Smoking; Saliva


Tobacco use is a serious public health dilemma that can lead to many systemic conditions and premature death. Despite the warnings regarding the strong association of cigarette smoking and increased morbidity and mortality due to related disease conditions, around 35% to 40% of the World’s population still smoke. Thus, an increasingly higher number of individuals is exposed to environmental tobacco smoke. Cigarette smoking is a major risk factor for many chronic conditions such as pulmonary diseases, cardiovascular diseases (CVDs), cancer, and many other conditions such as tuberculosis (TB).3-5

A passive smoker or involuntary smoker refers to a non-smoker individual who is continuously exposed to cigarette smoke in a closed environment. According to another definition, passive smoker refers to an individual who is exposed to the smoke of at least one cigarette daily or is exposed to air polluted with cigarette smoke for 2 hours daily. Evidence shows that passive smokers are susceptible to the adverse effects of smoking.
cigarette smoke as much as active smokers.\textsuperscript{4,6,7} The cigarette smoke includes over 7000 different chemical agents; out of which, 70 constituents are carcinogenic.\textsuperscript{3,8} Cotinine is a biomarker used for the detection of passive smokers. It is derived from the break-down of nicotine absorbed by the human body in the previous 2-3 days. The half-life of cotinine is longer than other nicotine metabolites and is detectable in body fluids such as saliva, serum, and urine, and even hair.\textsuperscript{9-11}

Several extrinsic factors such as cigarette smoke and radiation can result in the generation of free radicals.\textsuperscript{12} Free radicals are highly reactive molecules with an unbound electron that are frequently generated in cells as a metabolic byproduct or due to leakage during mitochondrial cell respiration.\textsuperscript{13}

Reactive oxygen species (ROS) such as H$_2$O$_2$ and superoxide radicals are generated by the effect of cigarette smoke and play an important role in the pathogenesis of many conditions such as severe tissue damage, cancer, CVDs, diabetes mellitus (DM), pulmonary diseases, renal diseases, rheumatoid arthritis, cataract, and many nerve tissue conditions.\textsuperscript{14,15} Increased level of ROS leads to oxidative stress, which indicates an impaired balance between the generation of free radicals and antioxidant defense mechanisms following tissue injury.\textsuperscript{8,16} The process of lipid peroxidation is initiated upon the exposure of ROS to unsaturated fatty acids or lipoproteins in the cell membrane. As the result of lipid peroxidation, fatty acids are converted to primary products such as lipid peroxide and secondary products such as hydroxyl aldehyde, light-weight hydrocarbons, ketone, alkenes, and alkanes such as malondialdehyde (MDA).\textsuperscript{16,17} Two important factors namely MDA and lactate dehydrogenase (LDH) are present in the saliva that are used as markers for assessment of the level of oxidative stress.\textsuperscript{3,18} MDA is one of the indicators of lipid peroxidation, and evidence shows that its salivary and serum levels are correlated with several diseases and inflammatory and pathological conditions.

Binding of MDA to purine bases in the deoxyribonucleic acid (DNA) structure causes mutagenesis and consequent carcinogenesis.\textsuperscript{4,19} LDH, as an intracellular enzyme, is detectable in the cytoplasm of all human cells and is released from the cells immediately after cell death. Thus, its extracellular presence indicates cell death and tissue destruction, and its elevated levels indicate initiation of disease. LDH plays a fundamental role in the clinical diagnosis of many conditions and has been studied as a general marker for cellular health.\textsuperscript{20}

Similar to blood, saliva contains many hormones, antibodies, antimicrobial agents, and growth factors. Evidence shows that saliva is comparable to serum in revealing the physiological status of the human body (hormonal, nutritional, and metabolic). On the other hand, collection, storage, transfer, and sampling of saliva are easier than the urine and serum. Additionally, its application for diagnostic purposes is non-invasive and cost-effective.\textsuperscript{21}

This study is performed aiming to measure the salivary levels of MDA and LDH in active smokers, passive smokers, and non-smokers. Considering the scarcity of studies on passive smokers, this study mainly focused on passive smokers.

**Methods**

This cohort study was approved by the ethics committee of Zanjan University of Medical Sciences, Zanjan, Iran (IR.ZUMS.REC.1398.002). A total of 75 individuals between 20 to 45 years were enrolled after signing informed consent forms. The participants included 25 non-smoker controls, 25 active smokers that smoked a minimum of 20 cigarettes daily and had started smoking at least 1 year earlier,\textsuperscript{3} and 25 passive smokers who were exposed to cigarette smoke of a minimum of 1 cigarette daily or exposed to air polluted with cigarette smoke for a minimum of 2 hours daily. An equal number of males and females were enrolled to eliminate the confounding effect.
of gender on the results.

The exclusion criteria were patients with DM, patients with seizure or sialoliths, those taking anti-hypertensive medications, alcoholics, periodontal pocket depth > 3 mm, active gingivitis/periodontitis, history of radiotherapy or chemotherapy, Sjögren’s syndrome, active caries, soft or hard tissue disorders impairing the salivary gland function (quality and quantity of saliva), and poor patient cooperation.

For saliva collection, the participants were requested to refrain from eating and drinking for one hour before saliva collection. Moreover, they were requested to rinse their mouth with water before saliva collection. For standardization, samples were collected between 10 a.m. to 12 p.m. and the unstimulated saliva samples were collected in a resting seated position by the spitting method. The patients were requested to spit into a 20-cc test tube for 1 minute. Next, each sample was centrifuged at 3000 rpm for 10 minutes to isolate the debris. Pure samples were subjected to the enzyme-linked immunosorbent assay (ELISA) technique.

The salivary cotinine level of all participants was first measured to more accurately assign the subjects to the three groups. Next, the salivary levels of MDH and LDH were measured and analyzed using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA) at P < 0.05 level of significance.

### Results

Active and passive smoking had significant correlations with the salivary levels of MDA and LDH compared with the control group. Table 1 compares the mean salivary level of cotinine (for more accurate allocation of participants to the three groups) and salivary levels of MDA and LDH in the three groups. The salivary levels of MDA and LDH in non-smokers were lower than the corresponding values in other groups.

### Discussion

Cigarette smoking increases the risk of many diseases including some fatal conditions such as CVDs, cancers, and obstructive pulmonary diseases. The cigarette smoke has adverse effects on the oral cavity, ranging from discoloration of teeth and restorations to serious, life-threatening conditions such as oral cancer. Passive smoking or secondhand smoking is a public health dilemma and an environmental risk factor compromising general health. Evidence suggests that cigarette smoke generally increases the risk of cancers. Passive smoking, in particular, can significantly increase the risk of lung cancer and breast cancer in females. There are two types of secondhand smoke: mainstream smoke exhaled by an active smoker and sidestream smoke released from a burning cigarette; 80% of individuals are exposed to sidestream smoke.

Cotinine is the main metabolite of nicotine, which is commonly used as a diagnostic biomarker for passive smoking. Its use as a diagnostic biomarker is because of the conversion of 72% of nicotine to cotinine. Furthermore, half-life of cotinine in human body is 17 hours, while that of nicotine is 2-3 hours. On the other hand, cotinine can be identified and measured in the saliva, plasma, urine, and hair. The use of saliva in this study was because saliva is the first body fluid exposed to cigarette smoke.

Long-term cigarette smoking and inhaling cigarette smoke increase the level of free radicals and antioxidants; as mentioned earlier, the role of free radicals in the development of many systemic conditions has been widely accepted.

### Table 1. Salivary levels of cotinine, malondialdehyde (MDA), and lactate dehydrogenase (LDH) in active smokers, passive smokers, and non-smokers

<table>
<thead>
<tr>
<th>Group</th>
<th>Active smokers</th>
<th>Passive smokers</th>
<th>Non-smokers</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary level of cotinine</td>
<td>19.10 ± 7.90</td>
<td>8.12 ± 1.58</td>
<td>3.36 ± 1.35</td>
<td>0.0001</td>
</tr>
<tr>
<td>Salivary level of MDA</td>
<td>4.78 ± 4.70</td>
<td>2.67 ± 1.00</td>
<td>2.63 ± 2.22</td>
<td>0.0060</td>
</tr>
<tr>
<td>Salivary level of LDH</td>
<td>508.33 ± 322.92</td>
<td>364.98 ± 89.51</td>
<td>271.63 ± 117.57</td>
<td>0.0010</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde; LDH: Lactate dehydrogenase
Free radicals can cause oxidative stress and subsequent lipid peroxidation, with LDH and MDA being the two biomarkers produced in this process. The role of these biomarkers is to show the changes caused by toxic factors. Increased level of LDH was reported in patients with Oral squamous cell carcinoma (OSCC), and this increase was correlated with histopathological grading of the tumor. Additionally, the level of LDH in patients with precancerous lesions was higher than that in healthy individuals. Increased level of MDH in smokers is expected because the carcinogens present in cigarette smoke cause oxidative stress and the generation of free radicals and consequent lipid peroxidation. Due to this cycle, the levels of MDA and LDH, as lipid peroxidation markers, rise in smokers. This study assessed the effect of cigarette smoke on oxidative stress, generation of free radicals, lipid peroxidation, and the resultant increase in salivary levels of MDA and LDH. In this study, aside from asking some questions to differentiate between active and passive smokers, the salivary level of cotinine was also measured for a more accurate grouping of participants. Next, the salivary levels of MDA and LDH were compared among active smokers, passive smokers, and non-smokers. The results indicated that the salivary levels of LDH and MDA in active smokers were higher than the corresponding values in passive smokers, and the values in passive smokers were higher than those in non-smokers. The difference in this respect was significant among the three groups. According to these findings, passive smoking, similar to active smoking, can play a role in oxidative stress and lipid peroxidation.

The literature review by the authors revealed that previous studies paid less attention to passive smoking and its comparison with active smoking. Thus, information regarding the adverse consequences of passive smoking is limited. Previous studies on this topic mainly focused on only one biomarker (MDA or LDH) while this study measured the salivary level of cotinine for more accurate grouping of participants and measured the salivary levels of both MDA and LDH, in addition to comparing them among the three groups.

According to Singh and Kaur, cigarette smoking can increase the plasma level of MDA. In their study, only two groups of smokers and non-smokers were compared, however in the current study, three groups of active and passive smokers and non-smokers were compared. Lymperaki et al. demonstrated that acute exposure to cigarette smoke negatively affected the hematological indices and oxidative stress biomarkers. Besides, short-term exposure to cigarette smoke could impair the function of endothelial cells. According to their study, it seems that the outcome would be worse for active smokers considering the oxidative stress factors and anti-oxidative protective markers compared to passive smokers.

This study also assessed the correlation between the salivary level of LDH and cigarette smoking. The results showed a significant correlation between cigarette smoking and salivary level of LDH. These findings were in agreement with those of the study by Rao et al. In their study, the salivary level of LDH was significantly higher in smokers compared to non-smokers. Plus, the significance of this difference was greater than that of serum LDH. However, they did not find a significant correlation between the rate and years of smoking and an increase in the serum level of LDH. They concluded that saliva was suitable for the detection of tissue injury in smokers. Greabu et al. found that the salivary antioxidant system was significantly influenced by the gas phase and particles of cigarette smoke. They reported a higher level of salivary LDH in smokers in comparison to non-smokers. Similarly, Kumar et al. measured the salivary LDH of active and passive smokers as an oral cancer biomarker and found a significant association between the salivary level of LDH and active and passive smoking. The results of the study by
Demirtash et al. were also in line with our findings. They reported a higher salivary level of MDA in smokers compared to non-smokers and passive smokers. Nonetheless, they did not measure the level of LDH.\(^4\) As mentioned earlier, only one biomarker (MDA or LDH) was investigated in the abovementioned study and many others.\(^{26,27,29}\)

In this study, the salivary level of cotinine was measured to ensure the correct grouping of individuals. This was performed to prevent recall bias and social desirability bias. This study revealed a significant correlation between the salivary levels of MDA and LDH and cigarette smoking in participants. In addition, the salivary levels of MDA and LDH in smokers were significantly higher than the values in other groups, with these values being the lowest in non-smokers.

Considering the higher salivary levels of MDA and LDH in active and passive smokers compared to the control group, it may be claimed that passive smokers are not safe from the adverse effects of cigarette smoke and eventually suffer from its adverse consequences.

Considering the current results and those of previous studies, cigarette smoking in closed environments and public areas should be banned to protect others. A small sample size (due to the high cost of the tests) was one limitation of this study.

**Conclusion**

Considering the current findings, the salivary levels of MDA and LDH increase in smokers as the result of lipid peroxidation, which indicates oxidative stress. The high level of these biomarkers in the saliva of passive smokers reveals that cigarette smoke has adverse effects on non-smokers as well. In general, the salivary levels of MDA and LDH have a significant correlation with the level of oxidative stress due to cigarette smoke in 20 to 45-year-olds.

**Conflict of Interests**

Authors have no conflict of interest.

**Acknowledgments**

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**References**


