# BIOCHEMICAL ANALYSIS OF SALIVARY MALONDIALDEHYDE LEVELS IN 14-15-YEAR-OLD ADOLESCENTS WITH ORAL PATHOLOGY

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## ABSTRACT

Elevated oxidative stress can contribute to inflammation, which is believed to play a role in oral manifestation such as GT or AU mainly in ages of 14 and 15 years. During this period, children are undergoing various physical, mental, and emotional changes due to their transition from childhood to adolescence. As GT or AU are more frequent in this ages, **our aim of the study** was to determine if there is any positive correlation between the salivary levels of Malondialdehyde (MDA) considered a biomarker of oxidative stress and the severity and progression of mucosal pathology. **Materials and methods:** Using the High-Performance Liquid Chromatography (HPLC) technique, we assessed the MDA concentrations which were statistically evaluated and compared between a study group of participants diagnosed with GT or AU and a control group of healthy age-matched subjects. **Results:** The study group has a significantly higher mean value (26.285) compared to the control group (21.425), with a p-value of 0.0017, indicating a statistically significant difference between the two groups. However, the gender distribution between the study group (51.4% female, 48.5% male) and the control group (52% female, 48% male) does not significantly differ, as evidenced by a p-value of 0.9016. **Conclusions:** The levels of salivary MDA were higher in the study groups with GT and AU, with statistically significant differences ($p<0.05$) compared to healthy subjects, which questions weather oxidative stress might be linked to geographic tongue pathogenesis or aphthous ulcers.

**Key words:** Geographic tongue, Malondialdehyde, HPLC.

## INTRODUCTION

Around the ages of 14 and 15, children undergo various physical, mental, and emotional changes as a consequence of their transition from childhood to adolescence. The most notable medical milestones during this period are puberty, menstruation in girls, bone growth, brain development, mental health, and not least, vision, dental, and oral changes [1,2]. Due to ongoing brain development and socio-emotional factors, some teenagers may engage in risk-taking behaviours like drug/alcohol use. This period is also a time of vulnerability for the onset of pathological conditions, such as depression, anxiety, eating disorders, and more [3,4].

By the age of 14 to 15, most adolescents will have their complete set of permanent teeth, except for the third molars. This includes incisors, canines, premolars, and the first and second molars. The third molars are typically in the development stage in the jawbone during this period [5, 6]. This age is common for orthodontic treatment; many adolescents either have braces or are being evaluated for



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braces or other orthodontic appliances to correct misaligned teeth and bite issues. Also, with increased independence in food choices and potentially busy schedules, some may neglect proper oral hygiene or consume more sugary snacks and drinks, increasing their risk for cavities. Habits like nail-biting, which can lead to chipping of teeth or place additional stress on the teeth, are common at this age.

The combination of these changes can place stress on the body, which may manifest in various ways [7-10].

In terms of oral mucosa and soft tissues, stress and other factors associated with adolescence can indeed lead to several pathological or temporary changes and increased oxidative stress.

This results from an imbalance between the production of free radicals (ROS) and the body's ability to counteract their harmful effects through neutralization by antioxidants [11, 12].

Malondialdehyde (MDA) is commonly used as a biomarker to measure the level of oxidative stress in an organism. MDA is a byproduct of lipid peroxidation, a process in which polyunsaturated fatty acids in cell membranes are attacked by reactive oxygen species, particularly during stress conditions [13, 14].

Saliva, like other biological fluids, can contain MDA due to the presence and breakdown of lipid structures within cells lining the oral cavity and from systemic sources [15].

The oral epithelial cells lining the oral cavity are continually exposed to various internal and external stressors [16, 17]. These stressors can cause oxidative damage to the lipids within these cells, leading to lipid peroxidation and the subsequent production of MDA. As these cells turnover or undergo damage, they can release MDA into the saliva. Saliva also contains an array of antioxidant systems to combat oxidative stress; however, when the balance between oxidants and antioxidants is skewed, it can lead to increased oxidative damage and MDA production [18-19].

An additional source of this biomarker is from systemic circulation making its way into the saliva via the gingival crevicular fluid or through passive diffusion from blood vessels supplying the salivary glands. Hence, oxidative stress in other parts of the body can be reflected, at least in part, in salivary MDA levels [20-23].

Elevated oxidative stress can contribute to inflammation, believed to play a role in the oral manifestation like geographic tongue (GT) or aphthous ulcers (AU). Also known as benign migratory glossitis, GT is a benign condition characterized by smooth, red, irregularly shaped patches on the dorsal surface of the tongue, surrounded by a slightly raised, white or yellowish border [24].

The pattern and location of these patches can change over time, hence the term "migratory." While the exact cause of GT is unknown, several factors and theories are associated with its occurrence, such as oxidative stress [25]. Aphthous ulcers commonly known as canker sores, are a frequent oral condition in adolescents.

The exact etiology is not fully understood, being considered a multifactorial pathology, involving a complex interplay of genetic, immunological, nutritional, hormonal, psychological, traumatic, infectious, and allergic factors. Managing these ulcers often requires a comprehensive approach that addresses potential triggers and underlying conditions [26, 27].

Adolescence, with its changes and challenges, can be a period of increased stress, which can be associated with systemic oxidative stress and inflammation and might indirectly influence the manifestation or exacerbation of GT, AU or other soft tissue pathology. While these conditions are asymptomatic, some may experience mild



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discomfort, burning sensations, or increased sensitivity, especially to spicy or acidic foods. Currently, there is no cure for geographic tongue, but symptoms can be managed by avoiding irritants like spicy or acidic foods. For those who experience discomfort, topical treatments like over-the-counter pain relievers or anti-inflammatory agents can be recommended [28, 29].

Regular dental check-ups can help monitor and manage the oral conditions, especially if it's causing any discomfort. Starting with the hypothesis that adolescents with geographic tongue and aphthous ulcers will demonstrate higher levels of oxidative stress markers compared to those without, mucosal pathology, our objective was to determine these levels and the positive correlation with the severity and progression of the condition.

**MATHERIALS AND METHODS**

We conducted a clinical and biochemical study to investigate the potential link between GT, AU pathology and intraoral oxidative stress, focusing on biomarkers like Malondialdehyde.

The study was approved by the Ethics Committee of Private Dental Clinic StoicaMed in Tîrgu-Mureș (approval no. 15A/13 December 2021) and lasted from January 2022 to July 2023.

The study included 4 essential steps, namely: (1) the creation of the study group by diagnosing the participants with GT or AU, and the control group, both to meet the inclusion criteria; (2) the collection of saliva from all study participants; (3) testing and finding out the concentration of Malondialdehyde in saliva by HPLC technique; (4) the statistical analysis of the results and drawing the conclusions of this study.

**(1). Creating study population:** the inclusion criteria for the participant to the study group were: adolescents aged 14 and 15 years; willingness to participate in the study; consent from parents or guardians; the positive diagnosis of GT or AU.

The exclusion criteria were: systemic illnesses or conditions that may affect oral health; medications or treatments known to influence the study variables; those who do not provide written consent. The selection of the control group followed the same inclusion and exclusion criteria, consisting of age-matched children without geographic tongue.

To create and gather the young members of the two groups, we have approach 3 high schools administrations from the Romanian town Tîrgu-Mureș with a formal proposal detailing the study's objectives, procedures and benefits, and conducted an information session to introduce the study to teachers, parents, and students. We also distributed the informed consent forms for all parents or guardians to sign, ensuring that they understand the study's nature and their child's involvement.

We have created using specific medical guidance and followed, a protocol that describes the clinical approach to diagnosing GT and AU, by taking in consider each patient's unique medical presentation and included the following steps:

A. Patient medical history: we obtained a comprehensive medical history to rule out associated systemic conditions or any medication intake that may cause tongue alterations.

B. Clinical examination: we began with a broad inspection of the entire oral cavity using a dental light or flashlight, dental mirror in conditions of full medical equipment, all to also ensure the patient's comfort.

C. Differential diagnosis: it was important to differentiate GT from other conditions that can affect the tongue's appearance like: fissured tongue that appears with deep grooves or fissures but can also coexist with GT; median rhomboid glossitis which is a red, smooth, and



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oval or rhomboid lesion in the central part of the dorsal tongue; oral candidiasis which might look similar but is often associated with a burning sensation and can be wiped off, leaving a red, bleeding surface and Lichen Planus that can also cause tongue alterations, but the pattern is usually lacelike (reticular). The differential diagnosis for AU includes recurrent aphthous stomatitis, Behçet's disease, systemic lupus erythematosus, inflammatory bowel disease, celiac disease, , medication side effects, trauma, viral infections, erythema multiforme, malignancies, and other autoimmune conditions like pemphigus vulgaris and lichen planus.

**(2). Saliva collection:** the saliva was collected for all participants included in both study and control groups, at 8:00 a.m. after confirming that they followed the instructions given beforehand like: avoiding high-sugar, acidic, or high-caffeine foods prior to sample collection, as these can compromise the test by lowering saliva pH and increasing bacterial growth.

Saliva is a valuable source of biomarkers for oxidative stress and has the advantage of being collected in a non-invasive and painless way. We have collected the passive saliva, which is a method of collecting whole saliva (also called "mixed"), and it is considered by many researchers to be the gold standard for samples for biological testing.

We used the Salimetrics containers (Fig.1) that also include the patented Saliva Bio Collection Aid system, allowing participants to easily collect up to 1.8 ml of whole saliva on their own without supervision.

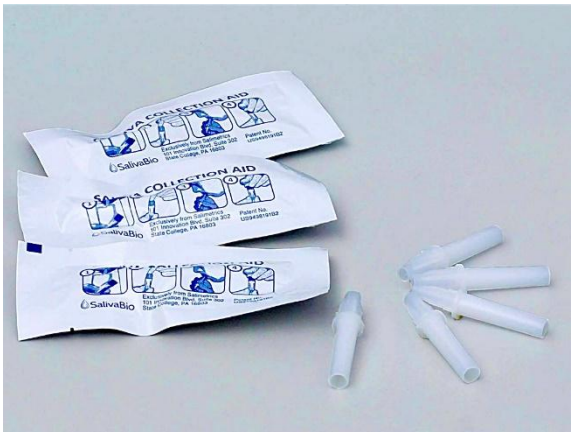


**Figure 1. Salimetrics containers**

We asked the participants to rinse the mouth with water for removing food debris and we waited at least 10 minutes after rinsing to avoid sample dilution before collecting saliva. We prepared the saliva collection container by securely attaching the ribbed end of the collection system to the prelabelled Saliva Bio Collection Aid container.

The participants had to let the saliva flow into the mouth and then, with the head tilted forward, gently guide the saliva into the containers without forcing the saliva as it can create foam or bubbles. After the bottles were filled to the required volume, we removed and discard the Saliva Bio Collection Aid and seal the collection container with a lid. Until the testing of the saliva sample using the HPLC method, the containers were stored according to the recommendations at -80$^{o}$ C.

**(3). MDA biochemical analysis:** the technique used to determine the presence of Malondialdehyde in the collected saliva was the HPLC (High Performance Liquid Chromatography) technique which is an analytical method used to separate, identify and quantify chemical components in a mixture of substances which follows a specific work protocol composed of the following stages: (1) centrifuging the samples for 5 minutes at 10,000 rpm (Fig.2); (2) sampling 500 µl of sediment-free saliva and pipetting this amount into PCR Clean Eppendorf Safe



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Lock tubes with a capacity of 2 ml; (3) adding a quantity of 250 µl of acetonitrile; (4) shaking the test tube and centrifuging them for 5 minutes at 12,000 revolutions/min; (5) adding a quantity of 200 µl of the supernatant solution, (6) adding a quantity of 300µl of thio-barbituric acid solution; (7) adding a quantity of 500 µl of sulfuric acid; (8) inserting the test tubes in the thermo-shaker and keeping them at a temperature of 90$^{o}$ for 30 minutes; (9) keeping the samples cold for 2 hours at a temperature of -10$^{o}$. Afterwards, the prepared solution is pipetted and transferred into the glass tubes specific to the chromatography technique, and then inserted into the HPLC device (Fig.3) for 12 hours. At the end of this time interval, the system provides the data and MDA concentrations (ng/ml) of each sample taken from the patients included in the study [30-35].

**Figure 2. Inserting saliva samples for centrifuging**


**Figure 3. HPLC unit.**

**(4). Data collection and statistical analysis:** IBM SPSS Statistics for Windows software, Version 26.0, was used for the statistical processing of the study data. The independent samples t-test was used to compare means according to the dichotomous variables in the study. A statistical significance coefficient value of $p<0.05$ was considered significant.

## RESULTS AND DISCUSSIONS

The study was approved by the Ethics Committee of Private Dental Clinic StoicaMed in Tîrgu-Mureș (approval no. 15A/13 December 2022) and lasted from January 2023 to July 2023. All participants were pupils in Târgu-Mureș schools, and were included in the study only after obtaining the written consent of their parents or legal guardians.

To achieve the research objectives, data from a number of 120 participants were used, divided into two groups: the study group, made up of 68 participants, included subjects of both genders, the female representatives being 51.4%, while the male representatives are 48.5%.

In the control group (subjects without oral pathology) were included 52 participants, of which 52% are girls and 48% are boys. From 68 participants, 38 presented GT (52.6% boys and 47.3% girls) and 30 AU (56.6% girls and 43.3% boys).

Table I presents the Malondialdehyde minimum and maximum concentration (ng/ml) in the collected saliva.



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**Table I. Malondialdehyde concentration (ng/ml) in saliva**

| | Study group | Control group |
|---|---|---|
| **Minimum value** | 15.37 | 13.22 |
| **Maximum value** | 37.20 | 29.63 |

**Table II. Descriptive statistics of Malondialdehyde concentration (ng/ml) in saliva**

| | Mean Value | Standard deviation |
|---|---|---|
| **Study group** | 26.285 | ≈10.91 |
| **Control group** | 21.425 | ≈8.21 |

Descriptive statistics of Malondialdehyde concentration (ng/ml) in saliva is included in table II. T-test for mean comparison ≈3.22 had the p value calculated based on t-statistic and degrees of freedom (approx. 0.0017). Since the p-value is less than 0.05, there is a significant difference between the means of the study group and the control group. The mean values of the study group (26.285) and the control group (21.425) are significantly different, with a p-value of 0.0017 indicating a statistically significant difference. There is no significant difference in the gender distribution between the study group and the control group, with a p-value of 0.9016.

The study group shows a significantly higher mean value compared to the control group, suggesting a difference in the variable being measured. However, the gender distribution between the two groups does not significantly differ, indicating that the gender balance is relatively similar in both groups. These findings highlight the importance of considering both the quantitative measures and demographic characteristics when comparing different study groups.

By understanding how oxidative stress contributes to oral diseases, researchers can gain insights into their underlying mechanisms. Oral diseases such as periodontitis, oral cancer, AU, lichen planus and GT are common and can have significant health implications. This could lead to the development of new treatments or preventive measures. While through our study on GT, AU and oxidative stress we can draws parallels with research on other oral diseases, each condition has unique characteristics in its association with oxidative stress.

Scientific studies, by Wang, Yang et al. [36], have found decreased levels of anti-oxidants such as glutathione, superoxide dismutase, and catalase in soft oral tissues pathology, or in the saliva of patients with oral mucosal pathology. This suggests that the antioxidant defence mechanisms might be compromised in these individuals, contributing to oxidative damage. Evidence indicates that there is increased DNA damage in mucosal lesions, which could be attributed to oxidative stress.

Oxidative stress can enhance inflammation by activating various cellular pathways. Therefore, the interplay between oxidative stress and immune responses could influence the severity and progression inflammatory chronic oral pathologies.

Recent studies by Shang, Liu et al. [37] have confirmed that understanding the role of oxidative stress in periodontitis has led to the exploration of antioxidant therapies as a potential adjunct to conventional periodontal treatment. Antioxidants might help to mitigate the damage caused by oxidative stress, reduce inflammation, and promote tissue healing.

The link between oxidative stress and periodontitis highlights the importance of managing oxidative stress as part of a comprehensive approach to treating and preventing periodontal disease. This includes promoting good oral hygiene practices, encouraging a healthy lifestyle, and potentially



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using antioxidant therapies when appropriate.

Oxidative stress can induce the formation of new blood vessels (angiogenesis), providing nutrients to the growing tumour, enhancing may enhance the invasiveness of cancer cells, contributing to metastasis [38]. It can also modulate the immune response, helping cancer cells evade detection and destruction by the immune system and being associated with chronic inflammation, which can promote cancer development.

Geographic tongue (Fig.5 a,b,c) is a benign inflammatory condition with an unknown exact etiology, but various contributing factors including genetics, hormonal fluctuations, and even environmental factors. Since only few scientific studies have suggested just a potential link between oxidative stress and the pathology of GT, our study brings mor information and statistical data to support this hypothesis [39, 40].

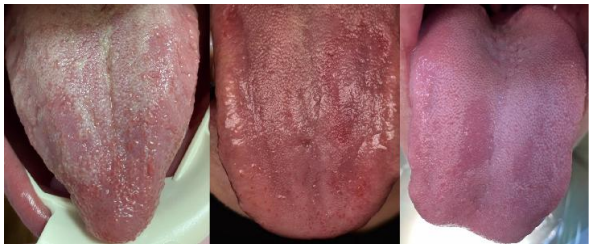

**Figure 5a. GT in 15 years old girl; 5b. GT in 15 years old boy; 5c GT in 14 years old boy.**

All our results have an essential role in emphasizing the importance of assessing oxidative stress in the context of oral health. This link between MDA levels and GT or AU opens new opportunities for the development of personalized general prevention and possible treatment strategies. If we can understand and control the factors that contribute to oxidative stress, we could have a significant impact on the incidence of GT and AU affecting young population and we could sustain a healthy medical development.

The revelation of oxidative stress's role in geographic tongue introduces the potential for antioxidant-based therapies. Strategies aimed at bolstering the body's anti-oxidative defences, or directly quenching ROS, could emerge as promising therapeutic options [41-43]. However, it is imperative to approach this with caution, ensuring thorough validation and optimization of any potential interventions.

Understanding the relationship between oxidative stress and oral diseases is also crucial for developing preventive strategies, improving diagnostic accuracy, and creating more effective treatments.

Research continues to delve into these connections to better understand how oxidative stress contributes to oral diseases and how its modulation could lead to improved patient outcomes. This knowledge not only broadens our understanding but also paves the way for innovations in oral healthcare. Ultimately, these advancements hold the promise of significantly enhancing patient well-being and overall health.

## CONCLUSIONS

1. Oxidative stress plays a pivotal role in the pathogenesis of various oral diseases like geographic tongue or aphthous ulcers, and it exacerbates chronic inflammatory conditions by causing cellular and DNA damage by disrupting immune regulation.
2. Effective management of oxidative stress through lifestyle choices, antioxidant therapies, and proper oral hygiene is essential for enhancing both oral and systemic health.
3. Elevated reactive oxygen species and reduced antioxidant defences in individuals with oral pathologies underscore the harmful impact of oxidative imbalance.



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