DOI:10.31557/APJCC.2024.9.3.431

RESEARCH ARTICLE

Analyzing the Salivary Antioxidant Capacity in Children before and after Dental Caries Restoration: A Comparative Scientific Investigation

Hajira Kunju Mohammed[1], Gopalakrishnan Anjana[1], Edakkadath Raghavan Sindhu[2], Ponnamparambil Purushothaman Binitha[2]

[1]Department of Pediatrics and Preventive Dentistry, Royal Dental College, Chalissery, Palakkad Dist, Kerala, India. [2]Department of Biochemistry, Royal Dental College, Chalissery, Palakkad Dist, Kerala, India.

Abstract

Aim: The purpose of this study is to examine the total antioxidant capacity (TAC) as a biomarker to investigate oxidative stress in different pathological conditions. The primary goal is to compare the TAC levels in saliva before and after the restoration of carious teeth. Additionally, the study aims to explore potential differences in TAC between genders with the intention of establishing a possible link between salivary TAC and dental caries. **Materials and Methods:** In this research, we employed a random selection process to assemble two cohorts: Group I, comprising 27 male individuals, and Group II, encompassing 27 females, all falling within the age bracket of 7 to 10 years. Saliva samples were procured from each participant both thirty days prior to and subsequent to the restorative dental intervention. To gauge the levels of total antioxidant capacity (TAC), we employed Cayman's antioxidant assay kit and quantified the outcomes employing a nanodrop instrument. **Results:** The obtained data was subjected to statistical analysis using SPSS version 18.0. The paired t-test was used to compare the TAC levels before and after the restorative procedure within each group (Group I and Group II). The results revealed that after restoration, both Group I and Group II showed a significant increase in TAC levels compared to before restoration (P = 0.000). Additionally, the Student t-test was employed to compare the TAC levels between Group I (males) and Group II (females). Interestingly, the study observed that the TAC of saliva was significantly higher in males compared to females, both before and after the restoration of carious teeth. **Conclusion**: Our research demonstrated reduced TAC levels in areas affected by dental caries, and these levels exhibited a significant rise following the restoration of all carious teeth. Consequently, it can be deduced that the evaluation of caries activity and the effectiveness of its treatment can be facilitated through the assessment of salivary factors, which holds promise for applications in preventive dentistry. **Clinical Significance:** TAC can be employed as a biomarker and a therapeutic target because a reduced TAC level before restoration is a sign of infection.

Keywords: Dental caries- total antioxidant capacity- oxidative stress- Children, Restoration- saliva

Asian Pac J Cancer Care, **9 (3)**, 431-436

Submission Date: 11/05/2023 Acceptance Date: 05/15/2024

Introduction

Saliva is a complex biological fluid secreted mainly by three pairs of major salivary glands in the oral cavity. It plays an important role in protecting against various agents, such as microorganisms, toxins, and various oxidants. The changes that occur in the salivary composition may have a significant role in controlling and affecting the oxidative damage in the oral cavity [1].

Dental caries is one of the most common chronic, infectious, and inflammatory oral diseases in mankind, affecting all people regardless of their sex, socioeconomic strata, race, and age. This disease is profoundly affected by other factors, like oral hygiene and saliva [2].

Corresponding Author:
Dr. Purushothaman Ponnamparambil Binitha
Department of Biochemistry, Royal Dental College, Chalissery, Palakkad Dist, Kerala, India.
Email: biniprasi@gmail.com

The composition of saliva, either naturally or under certain conditions, varies in different individuals [3]. The carious process is controlled to a large extent by a natural protective mechanism that is inherent within the saliva [4]. The evaluation of total antioxidant capacity (TAC) in biological fluids like saliva is one of the first steps in the search for diseases in biochemistry, medicine, food, and nutritional sciences [5].

Saliva constitutes a first line of defense against free radical-mediated oxidative stress [6]. It also serves as a mirror of the body's health as it contains proteins, hormones, antibodies, and other molecules that are frequently measured in standard blood tests to monitor health and disease. Unlike whole blood, saliva is easy to collect, painless to the patient, and less infectious for the healthcare provider [7]. Evaluation of saliva for those factors that may increase the risk of individuals developing dental caries can pave the way to making recommendations that will cater specifically to the needs of an individual.

TAC (total antioxidant capacity) is a biomarker often used in order to investigate oxidative stress in various pathological conditions [8]. It is the total material in bodily fluids that possesses antioxidant properties [9]. The relationship between infection and TAC depends on the type and site of the infection. The TAC of saliva could also be a marker for dental caries activity among children and adolescents.This study was carried out to compare the TAC of saliva before and after the restoration of carious teeth and between genders in order to determine the potential existence of a relationship between the TAC of saliva and dental caries.

Materials and Methods

The G*Power sample power calculator (version 3.1.9.6 for Mac OS X 13, Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany) was utilized to calculate sample power in the research. The sample size, determined based on an analysis of a previously published study, resulted in 23 participants assigned to each group. To address the lost follow-up data, the sample size was increased by 20% of the minimum estimated value, resulting in a final sample size of 27 participants in each group (28).

54 healthy children (27 males and 27 females) between the ages of 7 and 10 were randomly selected and included in the study. Institutional Ethics Committee approval was obtained prior to the start of the study (RDC/ADMN/2016/550), and written informed consent forms were obtained from the parents of all children prior to examination, and assent from the child was obtained verbally in the presence of the parent before proceeding with examination and saliva collection obtained from all subjects. Exclusion criteria were physically and medically compromised patients, those who are on medications, and children undergoing orthodontic treatment.

Saliva sampling

The selected subjects were requested not to use any oral stimulation, like eating or drinking, for 90 minutes prior to the collection of saliva samples. Each subject was seated in the coachman's position, head slightly down, and was asked not to swallow or move his tongue or lips during the period of saliva collection. Around 2 ml of unstimulated whole saliva specimen was collected using a needleless aspirating syringe directly from the floor of the mouth, transferred into polypropylene tubes, and immediately put into an ice container. The samples were stored at -800C for the analysis of TAC.

Clinical procedure

All clinical examinations were carried out by a single examiner. Caries detection was based only on clinical caries that were observed with plain mouth mirrors and explorers. Subjects who had a DMFT/deft score of 5 or below and an OHI-S score of 3 or below were selected. The saliva samples were collected before the restoration of carious teeth from subjects and estimated for TAC. All the carious teeth were then restored using type IX glass ionomer cement. Saliva samples were collected to estimate the TAC one month after the restorative procedure.

Salivary analysis

TAC measurements of saliva samples were carried out using Cayman's antioxidant assay kit (USA). The reaction was based on the ability of aqueous and lipid antioxidants to inhibit the oxidation of 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS) to ABTS+. The amount of ABTS+ produced can be monitored by reading the absorbance at 750 nm or 405 nm. The antioxidants in the sample cause suppression of absorbance at 750 nm or 405 nm to a degree that is proportional to their concentration. The capacity of the antioxidants to prevent ABTS oxidation is then compared with that of standard Trolox, a water-soluble tocopherol analogue, and quantified as millimolar (mM) Trolox equivalents. Absorbance is measured using NanoDrop Lite (ThermoNonoDrop Lite, USA).

Statistical analysis

The data obtained were statistically analysed using SPSS version 18.0. A paired t test was used for the analysis of TAC before and after the restorative procedure, whereas a student t test was used for the analysis of TAC between Group I (males) and Group II (females). $P \leq 0.05$ was considered statistically significant.

Results

Comparison of TAC before and after restoration of carious teeth

Table 1 showed that the mean salivary TAC of total samples before and after restoration of caries. The mean TAC of saliva before the restoration of carious teeth was 0.73 + 0.06 mM which increased to 0.79+ 0.05 mM one month after restorative procedure. This result was found to be very highly statistically significant with p value of 0.000.

Table 1. Comparison of TAC before and after Restoration of Carious Teeth

	N	TAC (mM)	P Value
Pre-restoration	54	0.73±0.06	0.000[HS]
Post-restoration	54	0.79±0.05	

TAC, Total antioxidant capacity; HS: Highly significant. Values are represented in terms of mean±standard deviation. Comparison between pre-restoration and post-restoration by paired t- test, HS (highly significant).

Table 2. Intra Group Comparison of TAC of Saliva before and after Restoration of Caries in Group I - Males

	N	TAC (mM)	P Value
Pre-restoration	27	0.76±0.05	0.001[HS]
Post-restoration	27	0.82±0.04	

TAC, Total antioxidant capacity; HS, Highly significant. Values are represented in terms of mean±standard deviation. TAC level in saliva is represented in milli molar (mM). Paired t test was used for the analysis of TAC between Group I (males), HS (highly significant).

Table 3. Intra-group Comparison of Mean TAC of Saliva before and after Restoration of Caries in Group II - females

	N	TAC (mM)	P Value
Pre-restoration	27	0.76±0.05	0.001[HS]
Post-restoration	27	0.82±0.04	

TAC, Total antioxidant capacity; HS, Highly significant. Values are represented in terms of mean±standard deviation. TAC level in saliva is represented in milli molar (mM). Paired t test was used for the analysis of TAC between Groups II (females), HS (highly significant).

TAC of saliva before and after restoration of caries in Group I males

The results showed that the mean salivary TAC in Group I before the restoration of carious teeth was 0.76+ 0.04 mM which increased to 0.82+ 0.04 mM one month after restoration(p-value < 0.001) (Table 2).

Comparison of TAC before and after restoration of caries in Group II - Females

The mean salivary TAC in Group II before the restoration was 0.69+ 0.05 mM which increased to 0.76+ 0.04 mM one month after restoration of all carious teeth (Table 3). These results were found to be highly significant (p 0.001).

Inter-group comparison of mean TAC of saliva before restoration of caries

Table 4 showed that the mean TAC of saliva before restoration in Group I was 0.76+0.04 mM where as mean TAC before restoration in Group II was 0.69+0.05mM. There was a statistically significant (p = 0.000) higher salivary TAC in Group I when compared to Group II.

Inter-group comparison of mean TAC of saliva after restoration of caries

Table 5 showed that the mean salivary TAC after restoration of caries in Group I was 0.82+0.04 mM where as mean TAC after restoration in Group II was 0.76+0.04mM. The TAC was significantly higher in Group I with p is 0.000.

Discussion

In our study, the TAC of saliva was found to have an inverse relation with dental caries. TAC significantly increased after restoration of all carious teeth indicating that in a caries free environment, the TAC of saliva seemed to be higher. This result is compatible with the findings of Sikorska–Jaroszyńska et al. where the highest TAC value was observed in children free of dental caries [10].

In the early 1990's, Miller et al. proposed a new test to measure the total antioxidant status, which was designated as TAC. The ability of this test to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound was its major advantage [11]. Since then, TAC has been a biomarker which is often used in order to investigate oxidative stress in many pathological conditions. We determined the TAC because investigating any individual antioxidant activity may be misleading and less representative of whole antioxidant status. Moreover the number of different antioxidant makes it more expensive and difficult to estimate the activity of each of them separately [12]. Also, no statistically significant results were reported in all the previous literatures where individual antioxidants have been evaluated in caries free and caries active individuals, and they suggested that TAC be evaluated rather than individual antioxidants [13].

In this study, we used unstimulated saliva for the determination of TAC because it is reported that TAC is higher in unstimulated saliva [14]. The values of TAC of saliva are dependent on the general health status of patients [15]. In our study, all the subjects were healthy with no active inflammatory processes of gingival or pathological lesions of oral mucosa. Hence, it appeared that caries was the main factor influencing the total antioxidant status of unstimulated saliva.

Our results showed the mean TAC value before restoration of caries was significantly lower when compared to the mean TAC value after restoration, i.e. the TAC of saliva decreased in caries active state. This finding was similar to the results of Krawczyk et al. who reported that in patients with multiple carious lesions, stimulated and unstimulated salivary antioxidant level

Table 4. Inter-group Comparison of Mean TAC of Saliva before Restoration of Caries

Pre-restoration	N	TAC (mM)	P Value
Group I (Male)	27	0.76±0.05	0.000 HS
Group II (Female)	27	0.69±0.05	

TAC, Total antioxidant capacity; HS, Highly significant. Values are represented in terms of mean±standard deviation. TAC level in saliva is represented in milli molar (mM). Student t test was used for the analysis of TAC between Groups I (Males) and Group II (females), HS (highly significant).

Table 5. Inter-group Comparison of Mean TAC of Saliva after Restoration of Caries

Post-restoration	N	TAC (mM)	P Value
Group I	27	0.82±0.04	0.000 HS
Group II	27	0.76±0.04	

TAC, Total antioxidant capacity; HS, Highly significant. Values are represented in terms of mean±standard deviation. TAC level in saliva is represented in milli molar (mM). Student t test was used for the analysis of TAC between Groups I (Males) and Group II (females), HS (highly significant).

significantly decreased [10]. In another study, Krawczyk et al. reported decreased TAC of saliva in subjects with dental caries. The reduction in TAC can probably be explained by the fact that the components of dental plaque bacterial cell wall, i.e. liposaccharides stimulates the immune system to produce inflammatory reaction mediators. An increased metabolism and a significant increase in oxygen consumption occur in the neutrophils, granulocytes and monocytes. This process is referred to as 'respiratory burst'. It is characterized by quick transformation of molecular oxygen into an ion radical superoxide, from which hydrogen peroxide is derived. The latter compound is toxic, not only for bacteria, but also for the surrounding tissues and neutrophils. This, in turn, leads to a decrease of the total antioxidant status value. In other medical conditions like HIV, where the oxidative stress is found to be high, the TAC of saliva was decreased as reported by Padmanabhan et al [16]. The salivary TAC was also reported to have an inverse relation to dental caries in children with Down's syndrome and Cerebral Palsy by Subramaniam et al [17, 18].

There are many more studies that have reported that the decreased TAC of saliva has an important role in the onset and progression of many diseases of oral cavity including oral lichen planus [19] and periodontal disease [20-22], due to the presence of oxidative stress. In the same way, reduction of salivary TAC may have a significant role in dental caries because of the destructive factors like free radicals/ROS [23]. Another observation found in our study was that, the TAC of saliva was found to be significantly higher in males when compared to females, both before and after restoration of carious teeth. This finding was similar to the results of Ahmadi-Motamayel et al., who reported a statistically significant lower TAC in female group compared to male group. It must be noted that the efficacy of total antioxi¬dant system may be related to a number of factor such as amount of free radical produc¬tion, individual's genetic basis, dietary intake, physical activity, hormones and stress. Therefore in females, factors such as hormonal changes and stress can influence TAC of saliva [24].

One of the earliest studies done to correlate between TAC and dental caries was done by Tulunogluet al., they found that the intensification of caries activity coincided with the increase in antioxidant activity of saliva [25]. In another study, Uberos et al., examined 126 patients with mixed dentition and observed that TAC of saliva in patients with dental caries in deciduous teeth, was significantly higher than those without dental caries. But in permanent teeth, no significant relationship was detected between dental caries and TAC of saliva [26]. Studies conducted by Hedge et al., Preethi and Dodward et al., showed, that in children, the TAC level increased with the increase in caries activity [12, 27]. Mahjoub proposed that higher TAC in children with severe early childhood carries (ECC) is a compensatory mechanism against oxidative stress [28]. Moore et al. attributed higher TAC of saliva in patients with dental caries to their diet [14]. These experts stated that salivary TAC is a combination of endogenous and food-derived antioxidants. Uric acid, as the major antioxidant of the saliva composes more than 85% of salivary TAC, is derived from foods and mainly from sugars. Therefore, consumption of sugars not only enhances the risk of dental caries, but also contributes to higher TAC of saliva [23].

On the contrary, TAC was lower in caries active children in the present study. This may be because the radical-scavenging antioxidants are being consumed by the increased free radical activity associated with dental caries and the total antioxidant status may have been used to indirectly assess free radical activity. Hence, superiority of oxidative stress and/or the inadequacy of antioxidant power may attribute to the decreased TAC of saliva in a carious environment [17].

The current study highlights the influence of oxidative stress and antioxidants on oral health. The generation of pro-oxidants in the form of ROS and RNS are effectively kept in check by the various levels of antioxidant defense. However, when it gets exposed to adverse physicochemical, environmental or pathological agents such as atmospheric pollutants, ultraviolet rays, radiation, toxic chemicals and over nutrition; this delicately maintained balance is shifted in favour of pro-oxidants resulting in 'oxidative stress [29]. The conflicts seen in the results by multiple researchers in different studies may be because of the different methods of measuring

TAC, age discrepancy, the severity of dental caries and type of dentition. There is no doubt that antioxidants are necessary components for our health and that they should be in balance with the production of free radicals.

Clinical Significance

TAC can be employed as a biomarker and a therapeutic target because the reduced TAC level before restoration is a sign of infection.

In conclusion, there is evidence that production of excessive free radicals and ROS may be substantially elevated in certain inflammatory diseases including dental caries and it is the antioxidant defense system that maintains the balance between them. Further clinical and laboratory studies must be performed with a wider sample size and longer follow ups to find the exact relationship between TAC and dental caries. Our study showed lower levels of TAC in carious environment which significantly increased after the restoration of all carious teeth. Therefore, it can be inferred that the caries activity and its treatment outcome can be assessed by salivary factors which may be helpful in Preventive dentistry.

Acknowledgments

Statement of Transparency and Principals:

• Author declares no conflict of interest

• Study was approved by Research Ethic Committee of author affiliated Institute .

• Study's data is available upon a reasonable request.

• All authors have contributed to implementation of this research.

References

1. Kumar SV, Kumar RH, Bagewadi N, Krishnan NA. A study to correlate dental caries experience with total antioxidant levels of saliva among adolescents in Mangalore. Journal of Indian Association of Public Health Dentistry. 2015 04 01;13(2):122. https://doi.org/10.4103/2319-5932.159045
2. Shafer WG, Hine MK, Levy BM. ED. A Text Book of Oral Pathology. 5th ed. Philadelphia: WB Saunders company. 1993; 567-658.
3. Reyes E, Martin J, Moncada G, Neira M, Palma P, Gordan V, Oyarzo Jf, Yevenes I. Caries-free subjects have high levels of urease and arginine deiminase activity. Journal of Applied Oral Science. 2014;22(3):235-240. https://doi.org/10.1590/1678-775720130591
4. Mcdonald RE, Avery DR, Stookey GK. Dental caries in child and adolesecent. Dentistry for the Child and Adolescent. 8th edn. St Louis: Mosby; 2004:203–235.
5. Shetty V, Kumari S, Geethashri A, Sneha N. Influence of the total antioxidant content of saliva on dental caries. NUJHS. 2011 09 01;1:19-22. https://doi.org/10.1055/s-0040-1703514
6. Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. Journal of Clinical Periodontology. 2002 03;29(3):189-194. https://doi.org/10.1034/j.1600-051x.2002.290301x.x
7. Dodwad R, Betigeri AV, Preeti BP. Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children. Contemporary Clinical Dentistry. 2011 01;2(1):17-20. https://doi.org/10.4103/0976-237X.79296
8. Peluso I, Raguzzini A. Salivary and Urinary Total Antioxidant Capacity as Biomarkers of Oxidative Stress in Humans. Pathology Research International. 2016;2016:5480267. https://doi.org/10.1155/2016/5480267
9. Shafer WG, Hine MK, Levy BM. A Text Book of Oral Pathology. 6th ed. Philadelphia. WBSaunders; 2009:569–589.
10. Krawczyk D, Błaszczak J, Borowicz J, Mielnik-Błaszczak M. Life style and risk of development of dental caries in a population of adolescents. Annals of agricultural and environmental medicine: AAEM. 2014;21(3):576-580. https://doi.org/10.5604/12321966.1120605
11. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clinical Science (London, England: 1979). 1993 04;84(4):407-412. https://doi.org/10.1042/cs0840407
12. Hegde AM, Rai K, Padmanabhan V. Total antioxidant capacity of saliva and its relation with early childhood caries and rampant caries. The Journal of Clinical Pediatric Dentistry. 2009;33(3):231-234. https://doi.org/10.17796/jcpd.33.3.c730518021m56077
13. Terao J, Nagao A. Antioxidative Effect of Human Saliva on Lipid Peroxidation. Agricultural and Biological Chemistry. 1991 03;55(3):869-872. https://doi.org/10.1080/00021369.1991.10870671
14. Moore S, Calder KA, Miller NJ, Rice-Evans CA. Antioxidant activity of saliva and periodontal disease. Free Radical Research. 1994;21(6):417-425. https://doi.org/10.3109/10715769409056594
15. Krawczyk D, Sikorska-Jaroszyńska MHJ, Mielnik-Błaszczak M, Pasternak K, Kapeć E, Sztanke M. Dental caries and total antioxidant status of unstimulated mixed whole saliva in patients aged 16-23 years. Advances in Medical Sciences. 2012 06 01;57(1):163-168. https://doi.org/10.2478/v10039-012-0015-9
16. Padmanabhan V, Rai K, Hegde AM, Shetty S. Total antioxidant capacity of saliva in children with HIV. The Journal of Clinical Pediatric Dentistry. 2010;34(4):347-350. https://doi.org/10.17796/jcpd.34.4.475137x4n8kj6765
17. Subramaniam P, Girish Babu K, Mohan Das L. Assessment of salivary total antioxidant levels and oral health status in children with Down syndrome. Special Care in Dentistry: Official Publication of the American Association of Hospital Dentists, the Academy of Dentistry for the Handicapped, and the American Society for Geriatric Dentistry. 2014;34(4):193-200. https://doi.org/10.1111/scd.12054
18. Subramaniam P, Mohan Das L, Babu KLG. Assessment of salivary total antioxidant levels and oral health status in children with cerebral palsy. The Journal of Clinical Pediatric Dentistry. 2014;38(3):235-239. https://doi.org/10.17796/jcpd.38.3.tv26g158q7343287
19. Shirzad A, Pouramir M, Seyedmajidi M, Jenabian N, Bijani A, Motallebnejad M. Salivary total antioxidant capacity and lipid peroxidation in patients with erosive oral lichen planus. Journal of Dental Research, Dental Clinics, Dental Prospects. 2014;8(1):35-39. https://doi.org/10.5681/joddd.2014.006
20. Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. Clinical Science (London, England: 1979). 2003 08;105(2):167-172. https://doi.org/10.1042/CS20030031
21. Tóthová L, Celecová V, Celec P. Salivary markers of oxidative stress and their relation to periodontal and dental status in children. Disease Markers. 2013;34(1):9-15. https://

doi.org/10.3233/DMA-2012-00943
22. Azizi A, Sarlati F, Parchakani A, Alirezaei S. Evaluation of Whole Saliva Antioxidant Capacity in Patients with Periodontal Diseases. Open Journal of Stomatology. 2014 01 01;04:228-231. https://doi.org/10.4236/ojst.2014.44031
23. Rahmani M, Ghoorchi V, Rezaei F, Raygani AV. Evaluation of Total Antioxidant Capacity of Saliva in High School Students. Global Journal of Health Science. 2016 04;8(4):89-94. https://doi.org/10.5539/gjhs.v8n4p89
24. Ahmadi-Motamayel F, Goodarzi M, Hendi S, Kasraei S, Moghimbeigi A. Total antioxidant capacity of saliva and dental caries. Medicina Oral, Patologia Oral Y Cirugia Bucal. 2013 07 01;18(4):e553-556. https://doi.org/10.4317/medoral.18762
25. Tulunoglu O, Demirtas S, Tulunoglu I. Total antioxidant levels of saliva in children related to caries, age, and gender. International Journal of Paediatric Dentistry. 2006 05;16(3):186-191. https://doi.org/10.1111/j.1365-263X.2006.00733.x
26. Uberos J, Alarcón JA, Peñalver MA, Molina-Carballo A, Ruiz M, González E, Castejon J, Muñoz-Hoyos A. Influence of the antioxidant content of saliva on dental caries in an at-risk community. British Dental Journal. 2008 07 26;205(2):E5. https://doi.org/10.1038/sj.bdj.2008.520
27. Preethi BP, Reshma D, Anand P. Evaluation of Flow Rate, pH, Buffering Capacity, Calcium, Total Proteins and Total Antioxidant Capacity Levels of Saliva in Caries Free and Caries Active Children: An In Vivo Study. Indian journal of clinical biochemistry: IJCB. 2010 Oct;25(4):425-428. https://doi.org/10.1007/s12291-010-0062-6
28. Mahjoub S, Ghasempour M, Gharage A, Bijani A, Masrourroudsari J. Comparison of total antioxidant capacity in saliva of children with severe early childhood caries and caries-free children. Caries Research. 2014;48(4):271-275. https://doi.org/10.1159/000355581
29. Sies H. Biochemistry of Oxidative Stress. Angewandte Chemie International Edition in English. 1986 01 01;. https://doi.org/555555