Original Article

Comparative Assessment of Total Antioxidant Capacity of Unstimulated Saliva Which Can Reflect the Oxidative Stress between Autism Children and Normal Children

Manikandan Sundaram[1], Baskaran Kumarasamy[2], Kavitha P. Subbian[3], Mohan Kumar Perumal[2], Kiruthika Balu[4], Saranya Manikandan[5]

[1]District Early Intervention Centre/ Department of Dental Surgery, Government Mohan Kumaramangalam Medical College Hospital, Salem, Tamil Nadu, India, [2]Department of Dental Surgery, Government Mohan Kumaramangalam Medical College Hospital, Salem, Tamil Nadu, India, [3]Department of Dental Surgery, Government Dindigul Medical College and Hospital, Dindigul, Tamil Nadu, India, [4]Consultant Orthodontist, Erode, Tamil Nadu, India, [5]Department of Oral Pathology, Vivekanandha Dental College for Women, Elayampalayam, Thiruchengode, Tamil Nadu, India

Submitted: 16-Nov-2023
Revised: 22-Dec-2023
Accepted: 17-Jan-2024
Published: 16-Apr-2024

Abstract

Aim: To assess the level of total antioxidant capacity (TAC) in the unstimulated saliva of autism children and normal children. **Methods:** A total of 60 children participated in the study (autism children—30; normal children—30) in the age-group of 7–14 years, at a Special Needs Children's Home in Salem district. Both groups were further subdivided into subgroups of 15 children each (caries-free and caries-active). All children were given instructions regarding saliva sample collection, and unstimulated saliva samples were collected in sterile cryovials and transported to the laboratory at 4°C, where the TAC of saliva was evaluated using the spectrophotometric assay. **Results:** TAC of autism children was comparable to that of normal children due to personal care and improved diet. TAC was increased in caries-active children when compared to caries-free children in both groups. When comparing the mean values of TAC in caries-active and caries-free children of both groups, it was not statistically significant. So, from the above results, it can be assumed that the diet, age, and caries activity have a definitive influence on the TAC level of saliva. **Conclusion:** Personal hygiene, diet, age, and caries activity have a definitive influence on the level of TAC in saliva.

Keywords: *Autism, oxidative stress, reactive oxygen species, TAC*

Introduction

Several recent reports have indicated high levels of reactive oxygen species, causing "oxidative stress," in the pathogenesis of dental caries. "Children with Special Needs" is a broad spectrum under which an array of diagnoses are wedged.[1] Children with special needs are those who are affected from a chronic physical, developmental, behavioral, or emotional disability, due to which they demand more care and concern than normal children. They usually fall victim to several systemic and oral impairments.

Frequent conditions, such as tooth decay, gum diseases, delayed eruption, and malocclusion, are commonly noted among all children, whereas children with special needs are prone to several other oral impairments. Excessive drooling, gagging, and swallowing problems are commonly encountered by disabled children due to

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jpbs

DOI: 10.4103/jpbs.jpbs_1182_23

Address for correspondence: Dr. Saranya Manikandan, Department of Oral Pathology, Vivekanandha Dental College for Women, Elayampalayam, Thiruchengode, Tamil Nadu, India.
E-mail: drsaranyamani@gmail.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Sundaram M, Kumarasamy B, Subbian KP, Perumal MK, Balu K, Manikandan S. Comparative assessment of total antioxidant capacity of unstimulated saliva which can reflect the oxidative stress between autism children and normal children. J Pharm Bioall Sci 2024;16:S1811-4.

Downloaded from http://journals.lww.com/jpbs by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 04/25/2024

Downloaded from http://journals.lww.com/jpbs by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 04/25/2024

impaired cognition and uncontrolled body movements with behavioral and neuromuscular problems.[2] Their treatment involves regular usage of numerous drugs which directly or indirectly has an impact on their immune status. As most of the drugs are centrally acting, they result in reduced salivary flow, which in turn results in xerostomia thereby altering the oral microbiome. Sweetening agents used in the medication and restricted diet also tend to alter the gut and the oral microbiome which impairs their immunity rendering them more susceptible to several infections. Under immunocompromised status and frequent exposure to drugs, it destroys the gut microbiota resulting in the overgrowth of microbes, which when multiplies produces toxins and impairs the central nervous system and immune system.[3]

Recently, it has been claimed that oxidative stress plays an important role in the onset and the development of several inflammatory oral pathologies including dental caries.[4] There are epidemiological evidences correlating higher intake of components, or foods with antioxidant abilities have lowered the incidence of various human morbidities and mortalities. This research has revealed the potential applications of antioxidant/free radical manipulations in prevention and/or control of the disease.[3]

Total antioxidant capacity (TAC), defined as the moles of oxidants neutralized by one liter of solution, is a biomarker measuring the antioxidant potential of the body's fluids. This study was conducted to evaluate TAC in the unstimulated saliva of autism children and normal children and their relationship with dental caries.

Materials and Method

A total of 60 children participated in the study (autism children—30; normal children—30) in the age-group of 7–14 years, at a Special Needs Children's Home in Salem district. Informed consent was obtained before enrolment from the Special Needs Children's Home authority who was the guardian of all the children participating in the study. Both groups were further subdivided into subgroups of 15 children each, those being caries-free, that is, their dmft/DMFT score "0," and the other 15 children having active carious lesions with dmft/DMFT scores of ≥5. The caries status was assessed according to World Health Organization (WHO) criteria (2004).[5]

All the children were given a uniform controlled diet from their central kitchen 1 day before the collection of saliva for investigation so as to rule out any bias in the results. All the children were brought in for saliva collection 2 hours after the morning breakfast. The unstimulated whole salivary samples were collected 2 hours after any oral or visual exposure to foodstuffs. All the salivary samples were collected in sterile cryovials between 10 and 11 am to prevent any bias in the concentration of the saliva due to circadian rhythm.

After the collection of saliva, it was stored at a temperature of 4ºC using dry ice pack. Immediately, it was transported to the laboratory where the TAC was evaluated based on the spectrophotometric assay. TAC was evaluated using the TAC Assay Kit (BioAssay Systems, USA) and Spectro Max Nano spectrophotometer [Figures 1 and 2].

This assay is based on the principle of the cupric ion-reducing antioxidant capacity (CUPRAC) method, in which Cu^{2+} is reduced by antioxidant (in sample) to Cu^{+}. The resulting Cu^{+} specifically forms a colored complex with a dye reagent. The color intensity at 570nm is proportional to the TAC of sample. The collected data were subjected to statistical analysis using the Chi-squared test with the Statistical Package for the Social Science (SPSS) package, and the test of significance *P* value of less than 0.05 was taken to be statistically significant.

Results

The study and control groups were compared for the TAC of saliva. It was found that the antioxidant capacity of saliva was lower in the study group when compared to the controls. The mean TAC of saliva was compared, and the results were statistically significant.

Table 1 and Graph 1 shows the descriptive statistics of mean TAC in caries-free and caries-active children. In caries-active children, the mean level of TAC was increased when compared to caries-free children in both groups, but it was not statistically significant.

Table 2 and Graph 2 depicts the age-wise comparison with mean TAC of saliva in the study group and control groups, and as the age increases, there is an increase in TAC noted. When comparing TAC values between the two groups, the TAC value of the control group was



Figure 1: TAC Assay Kit (BioAssay Systems, USA)

Downloaded from http://journals.lww.com/jpbs by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 04/25/2024

Table 1: Mean TAC of caries-free and caries-active children

Group	TAC			
	Caries-free		Caries-active	
	Mean	SD	Mean	SD
Study group	266.13	182.49	310.87	271.45
Control	296.00	178.21	361.07	367.25
Overall	281.07	177.87	335.97	318.33

Table 2: Comparison of mean TAC of saliva

Children	*n*	Mean	SD	SE	*t*	*P*
TAC (mM)						
Normal	30	1.7398	0.2911	0.0531	6.586	0.001**
Autism	30	1.1015	0.4440	0.0811		

**Highly significant

higher in all age-groups which is highly significant with a *P* value of <0.001.

Discussion

Saliva is a complex and dynamic biologic fluid, which over the years has been recognized for its numerous functions in the oral cavity. Recently, there has been a significant interest in saliva analyses because the collection of saliva is simple and noninvasive.[6] Sampling of saliva is safe for both the operator and the patient, and its storage is comparatively cost-effective.

Unstimulated saliva samples were used in this study as it is preferred over stimulated saliva in the determination of antioxidant defense parameters and it is claimed that TAC is higher in unstimulated saliva.[7] The TAC of saliva was evaluated as it is suggested that free radicals/reactive oxygen species (FR/ROS) and antioxidant system appear to act in concert rather than alone. Investigations of individual antioxidant activity may be misleading, and the measurement of any individual antioxidant may be less representative of the whole antioxidant status. Moreover, the number of different antioxidants makes it difficult and also expensive to measure each of them separately.[8]

In this study, TAC assay is based on the principle of the CUPRAC method, as it offers distinct advantage over other electron transfer-based assays (ABTS/TEAC, DPPH, Folin-Ciocalteu, and FRAP), namely, the selection of working pH at physiological pH, applicability to both hydrophilic and lipophilic antioxidants, and completion of the redox reactions for most common flavonoids.[9]

The physical constraints associated with disease with a continuous overload of systemic medications, dietary

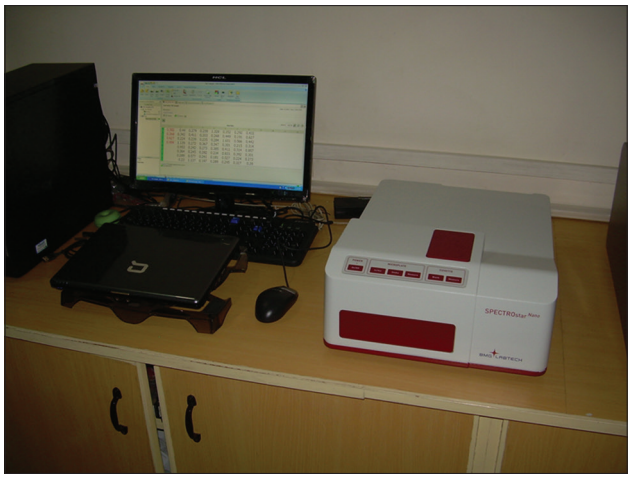
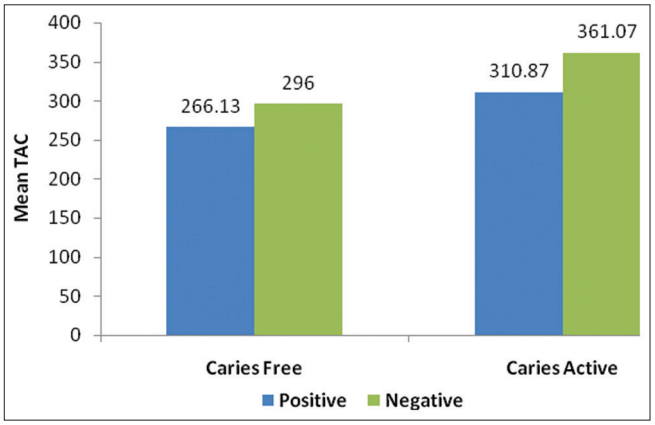
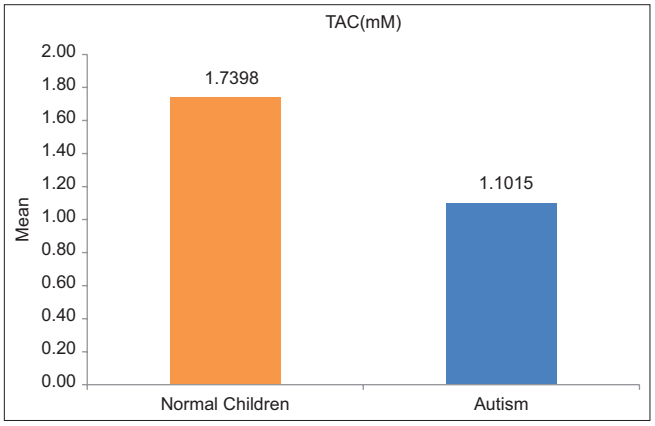


Figure 2: Spectro Max Nano spectrophotometer



Graph 1: Mean TAC of caries-free and caries-active children



Graph 2: Comparison of mean TAC of saliva

limitations, and inability to maintain hygiene predispose conditions favorable for yeast multiplication and invasion. Conditions, such as dental caries, periodontal diseases, malocclusion, and delayed tooth formation, are commonly noted among special needs children; however, the possibility of yeast overgrowth was to be investigated.

Downloaded from http://journals.lww.com/jpbs by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 04/25/2024

Dykens and Volkmar (1997) noted that there was also evidence of yeast overload among children affected with autism which may be a result of a weakened immune system. Few scientists had concerns regarding prenatal and postnatal infectious diseases and its subsequent impact on child's immune system. Following which, studies were also conducted by Kennedy *et al.* (2004) to investigate any correlation between pre-/postnatal infections and suppressed immunity to be held as reason for worsening behavioral pattern among children with autism. Increasing research has also focused on the connections between the immune system and the nervous system, indicating its possible role in the development of autism.

The survey held by the Autism Research Institute of over 25,000 parents reports that parents find antifungal to be one of the most effective medications for improving behavior. It is possible that children with autism are more sensitive.

The response of children with autism treated with antioxidant therapy showed improvements, such as decreased hyperactivity, more eye contact, increased vocalization (more words and more usage), better sleep patterns, better concentration, and increased imaginative play, reduced stereotypical behaviors (such as spinning objects), and better academic performance.

In this study, TAC of saliva when observed in relation to age, it was noted that the antioxidant levels gradually increased with age both in the study and control groups and the correlation was highly significant (0.698) [Table 2 and Figure 2], and this was similar to the findings of previous studies by Amitha M. Hegde *et al*.[7] (2009) and Vivek Padmanabhan *et al.* [7] (2010). The increase in levels of TAC could be due to the levels of nutrition that can vary between the ages. Children of the younger age-group could be having adequate nutrition for their age but the volume of food consumed is lesser than older children. Another plausible reason for the increasing levels of antioxidants with age may be attributed to the fact that the immune status of a child improves with age, and therefore, children who are older can have higher antioxidant levels when compared to the children of the younger age-group.[9]

The presence of an infectious challenge in the form of caries or poor oral hygiene as observed in caries-active children could be one of the reasons for the comparatively increased levels of TAC in saliva. In this study, overall mean level of TAC in caries-free children was 281.07 (standard deviation (SD) 177.87), and in caries-active children, it was 335.97 (SD 318.33), indicating a linear relation between TAC of saliva and caries as reported in earlier studies by Amitha M. Hegde *et al*.[7] (2009) and Reshma Dodwad *et al*. (2011).[10]

Conclusions

The results revealed that the mean TAC was significantly reduced in autism children when compared to the normal children. When comparing the mean values of TAC in caries-active and caries-free children of both groups, it was not statistically significant. So, from this study, it can be assumed that the diet, age, and caries activity have a definitive influence on the TAC level of saliva. This study also indicates a need for improvement in home care measures, parents, and caretakers on importance of oral hygiene maintenance among autism children. Further studies with larger samples and long-term follow-up are essential for confirmation of these findings.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Yamazaki I, Piette LH. ESR spin trapping studies on the reaction of Fe2+ ions with H2O2 reactive species in O2 toxicity in biology. J Biol Chem 1990;265:189-94.
2. Mc Donald RE, Avery DR, Dean JA. Dentistry for the Child and Adolescent. 10th ed. St. Louis: Mosby Publications; 2015.
3. Devasagayam TP, Tilak JC, Boloor Kennedy K, Sane KS. Free radicals and antioxidants in human health: Current status and future prospects. J Assoc Physicians India 2004;52:794-804.
4. Lenora R.Bigler, Dubinsky WP. Salivary biomarkers for the detection of malignant tumors that are remote from the oral cavity. Clin Lab Med 2009;29:71-85.
5. Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. Clin Chim Acta 2007;383:30-40.
6. John T, McDevitt. Saliva as the next best diagnostic tool. J Biochem 2006;45:23-5.
7. Hegde AM, Rai K, Padmanabhan V. Total antioxidant capacity of saliva and its relationship with ECC and rampant caries. J Clin Pediatr Dent 2009;33:231-4.
8. Prior R.L, Cao G. *In vivo* total antioxidant capacity: Comparison of different analytical methods. Free Radic Biol Med 1999;27: 1173-81.
9. Apak RK, Demirata B, Ozyurek M, Celik SE, Bektaşoglu B, Berker KI, *et al*. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. J Biology 2007;12:1496-547.
10. Dodwad R, Betigeri AV, Preeti BR. Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children. Contemp Clin Dent 2011;2:17-20.