Assessment of Salivary Total Antioxidant Levels and Oral Health Status in Children with Cerebral Palsy

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Oxidative stress plays a pivotal role in the pathogenesis of neurological disorders. Saliva may constitute a first line of defence, against free radical-modified oxidative stress. The ***objective*** *of the present study was to evaluate Total Antioxidant Capacity(TAC), levels of Nitric Oxide(NO), and Sialic Acid (SA) in saliva of cerebral palsied children.* ***Study design:*** *Thirty four non-institutionalized children in the age group of 7-12 years having cerebral palsy formed the study group. The control group consisted of thirty three normal, healthy children. The W.H.O. criteria was used for diagnosis and recording of dental caries. Oral hygiene status was assessed using the Simplified Oral Hygiene Index OHI-S. Estimation of Total Antioxidant Capacity, levels of Nitric Oxide and Sialic Acid in saliva was done. Data obtained was subjected to statistical analysis.* ***Results:*** *Children with CP had higher deft scores than that of normal children. Oral hygiene of children with CP was significantly poorer than that of normal children. Total Antioxidant Capacity of saliva was significantly higher in normal children than cerebral palsied children. Levels of Sialic Acid in saliva were significantly higher in cerebral palsy children.* ***Conclusions:*** *In children with CP, TAC of saliva showed an inverse relation with dental caries.*

Keywords: *cerebral palsy, TAC, SA, NO, dental caries*

INTRODUCTION

Cerebral Palsy (CP) is defined as a non- progressive disorder that manifests as an abnormality of motion and posture and results from a central nervous system injury sustained in the early period of brain development. Cerebral Palsied children face a number of dental problems including dental caries, periodontitis, malocclusion and bruxism. The difficulty in swallowing in these patients leads to drooling. Inability to perform oral hygiene procedures contributes to the increased incidence of dental diseases in these patients.[1]

There is contradictory information in literature regarding the incidence of oral diseases in patients with Cerebral Palsy.[2-5] These are mainly due to failure in selection criteria of study group, absence of control groups, use of non-standardized criteria for diagnosis, inexperienced investigators, and imporper analysis of the results. [6] The management of cerebral palsied children not only includes prevention and treatment of disease, but also identification and assessment of associated risk factors.

Oxidative stress plays a pivotal role in the pathogenesis of neurological disorders. Oxidative stress occurs as a consequence of imbalance between the formation of free oxygen radicals and inactivation of the extracellular constituents.[7] The imbalance in the levels of free radicals and reactive oxygen species with antioxidants may play an important role in the onset and development of several chronic inflammatory degenerative diseases.[8] Antioxidants are present in all body fluids and tissues, protecting against endogenously formed free radicals.[9] It has been suggested that the levels of antioxidants could be altered in response to an infection or disease.[10]

Saliva 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.[11] Saliva may constitute a first line of defence, against free radical-modified oxidative stress. It contains many biochemical systems known to be involved in soft-tissue repair, and many antibacterial components including lysozyme, lactoferrin and salivary peroxidase. There are only few studies[8,12,13] on the antioxidant defence system of saliva and their relation with oral disease. Hence, the objective of the present study was to evaluate Total Antioxidant Capacity (TAC) of saliva, levels of Nitric Oxide(NO), and Sialic Acid (SA) in saliva of children with Cerebral Palsy and its relation to their oral health status.

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MATERIALS AND METHOD

The study was carried out at different institutions and schools for special children in Bangalore city, India. These included Bethany Special School, Cluny convent Special School, Arunachethana, and Nachikethana. Ethical clearance to conduct the study was obtained from the institutional review board. Prior to the study, consent was obtained from the authorities of special schools. Thirty four non-institutionalized children in the age group of 7-12 years having cerebral palsy formed the study group. The selected CP children were not further categorized based on the type of neuromuscular dysfunction and their motor disabilities were limited to staggering gait, monoplegia, and paraplegia. The control group consisted of thirty three normal, healthy children visiting the Department of Pedodontics and Preventive Dentistry. These children were matched for age and gender with children of the study group. Prior to examination the nature of the study was explained and written consent was then taken from the parents/caretakers of all children.

Children with associated medical conditions, mental retardation and those on regular long term medication were not included. Children who did not get parental consent and who were unable to cooperate sufficiently for collection of saliva were excluded from the study. Only those children who obtained written consent from their parents/caretakers were subjected to dental examination and assessment of salivary parameters. A proforma was used to gather demographic data and other relevant medical information of each child.

Intra oral examination of children with cerebral palsy was done with the assistance of a school teacher or parent/ caregiver to gain cooperation from the children. A single investigator conducted the oral examination with the help of an assistant to record the data. The W.H.O. criteria was used for diagnosis and recording of dental caries.[14] The CPI probe was used to confirm visual evidence of caries on the occlusal, buccal and lingual surfaces. Training and calibration for examination of dental caries was carried out in the Department of Pedodontics and Preventive Dentistry. Dental caries was recorded by a dental surgeon sitting besides the examiner, so that the codes given by the examiner could be easily heard. Ten percent of children were examined twice for intra-examiner reliability. The kappa value for intra-examiner agreement of the tooth status was 0.88.

Oral hygiene status was assessed using the Simplified Oral Hygiene Index OHI-S given by Green and Vermilion[15] and its modification for the deciduous dentition as given by Miglani et al.[16]

COLLECTION OF SALIVA

Prior to collection of saliva, the child was instructed to rinse with 15ml of distilled water in order to wash out any food debris and exfoliated cells.[17] Unstimulated saliva was collected during the day, one hour after breakfast by requesting the subject to sit in a quiet environment in the "coachman" position and expectorate into a graduated jar.[18,19] The child was seated on the chair with the head slightly down and was asked not to swallow or move his tongue or lips during the period of collection.[20,21] Saliva was allowed to accumulate in the floor of the mouth and the child was asked to spit into a sterile graduated jar until 3ml of saliva was collected. During their transfer to the laboratory, the salivary samples were taken in sterile eppendorf tubes stored at a temperature of -4^{0}C. At the laboratory, each sample was centrifuged at 3000 rpm for 20 minutes.[21]

Estimation of Total Antioxidant Capacity, levels of Nitric Oxide and Sialic Acid in saliva was done using spectrophotometry analysis (phosphomolybdenum method),[22] Griess method[23] and Diphenylamine method[24] respectively.

Table 1. Comparison of salivary TAC, SA and NO levels between cerebral palsy and normal children

Groups	TAC Mean ±SD (µg/ml)	SA Mean ±SD (mg/dl)	NO Mean±SD (µm/l)
Cerebral palsy	74.40±44.39	34.23 ±28.82	48.50±31.22
Normal	165.54±53.09	17.25± 5.11	47.00±38.60
P value	<0.001*	<0.001*	0.543

*p≤0.001 highly significant

Data obtained was subjected to statistical analysis using Kruskal-Wallis to find the significance of the study parameters on continuous scale between the two groups. Mann-Whitney test was used for pair-wise comparisons. Spearman's Rank Correlation test was used to find the correlation between dental caries, OHI-S and the levels of total antioxidant capacity, nitric oxide and sialic acid. ($p \leq 0.001$ was considered as highly significant)

RESULTS

In comparison to normal children, TAC of saliva in CP children was significantly lower and with significantly higher salivary SA levels (Table 1). In both groups of children, dental caries was higher in primary dentition when compared to their permanent dentition. Mean deft score was higher in CP children; whereas mean DMFT score was higher in normal children. Oral hygiene of CP children was poorer than that of normal children (Table 2). In CP children, there was an inverse relationship between salivary parameters assessed and dental caries in the primary dentition (Table 3). An inverse relationship between oral hygiene and TAC of saliva and salivary SA levels was observed only in normal children (Table 4). In both groups of children, there was an inverse relationship with salivary levels of NO and permanent dentition caries (Tables 3 and 4).

DISCUSSION

Cerebral Palsy (CP) is the most common form of neuromuscular disability affecting children.[18] This handicapping condition has specific motor skills problems, delay in developmental milestones as well as physical limitations that might include abnormal muscles tonus, reflexes and persistent infantile reflexes.

Access to these special children has always been challenging. Dental examination poses an additional challenge due to neuromuscular in-coordination. In our study, radiographs were not taken to aid in the diagnosis of caries due to lack of sufficient patient cooperation. The main factor related to gingival or periodontal problems in disabled individuals is the inadequacy of dental plaque removal which is impaired by learning disabilities, motor incoordination and muscular limitation, particularly in neuromuscular disorders. The

Table 2. Comparison of dental caries and oral hygiene status between cerebral palsy and normal children

Groups	deft Mean±SD	DMFT Mean±SD	OHI-S Mean±SD
Cerebral Palsy	3.19 ±1.65	1.44 ± 0.58	1.58 ±0.72
Normal	2.90 ± 1.60	1.84 ± 1.12	1.35 ±0.75
p value	0.484	0.280	0.177

Table 3. Correlation between dental caries, oral hygiene status and salivary parameters assessed in cerebral palsied children

Salivary levels	deft		DMFT		OHI-S	
	r	p value	r	p value	R	p value
TAC	-0.154	0.401	0.161	0.421	0.078	0.659
SA	-0.143	0.436	0.157	0.433	0.132	0.458
NO	-0.215	0.237	-0.178	0.374	0.138	0.438

Table 4. Correlation between dental caries, oral hygiene status and salivary parameters assessed in normal children

Salivary levels	deft		DMFT		OHI-S	
	r	p value	r	p value	R	p value
TAC	0.163	0.390	0.018	0.941	-0.245	0.169
SA	0.249	0.185	0.055	0.822	-0.180	0.316
NO	0.196	0.298	-0.045	0.854	0.041	0.822

manual dexterity of these children is reduced, there by compromising their ability to maintain proper oral hygiene.[18]

Most studies in literature have reported unsatisfactory oral hygiene in individuals with CP.[2,5,25-28] This was in accordance with the present study, wherein oral hygiene of CP children was poorer than that of normal children. In CP children, the lack of neuromuscular co- ordination, inability to brush well, soft consistency of diet and retention of food may contribute to poor oral hygiene. Due to dietary restrictions, essential nutritional factors often are supplied in inadequate amounts. Inadequacy of vitamin C undoubtedly is one of the predisposing nutritional factors in the incidence of gingival disease in these patients.[29] Position of the child at the time of oral hygiene, presence of pathological oral reflexes, such as biting and vomiting and alterations in intraoral sensitivity are also very important factors that influence mechanical removal of plaque.

The incidence of dental caries in cerebral palsied children is usually anticipated to be greater because of factors such as inadequate nutrition, consistency of diet and poor oral hygiene. However, studies on dental caries in CP children have given varying results.[2-5,25] In our study, dental caries was higher in the primary dentition in normal as well as CP children. Developmental disturbances in tooth enamel and longer exposure of primary teeth to cariogenic environment may be the reason for higher caries in the primary dentition of CP children. Lack of or difficulty in providing oral hygiene at a very young age, inability to rinse effectively, delayed oral clearance are other contributing factors. With increasing age, there is a difference in the nutritional requirements and dietary changes from softer, semisolid foods to more solid foods.

Pope and Curzon[4] observed that CP children had more untreated decay than normal children. This was indicative of the difficulties that disabled individuals often have in accessing dental care. In developing countries, dental neglect is an important factor in the widespread destruction of the teeth noted in many cases of CP. However, Nielsen, in Denmark, found lower decayed, missing and filled tooth surfaces in adolescents who had CP than in a control group.[3] Significantly higher DMFS were observed in children who had CP when compared with a control group of children who did not have disabilities.[30] This was in contrast to our study, where in lower DMFS were observed in the permanent dentition of CP children.

All the CP children in our study were not institutionalized. They were probably prevented from residing in such institutions because of stigmatisation and low expectations among families. According to Selwitz *et al*,[31] higher control of dental caries occurs due to fluoride exposure by means of water fluoridation, mouth rinses, dentifrices, topical applications and food rich in fluoride. Water fluoridation has been considered as a determinant factor for the decline of caries, especially due to its topical action.[31] Apart from the influence of water ingestion, lower incidence of dental caries observed in the present study may have occurred due to easy availability and wide use of fluoridated dentifrices in the community.

Saliva is rich in antioxidants, mainly uric acid with lesser but definite contributions from albumin, ascorbate, and glutathione. All of them are proteins or have protein in their structure. It has been reported that uric acid is the major antioxidant in saliva accounting for more than 85% of TAC of both unstimulated and stimulated saliva.[10] Stimulated saliva contains a lower concentration of antioxidants but when flow rates are taken into consideration, the antioxidant capacity is higher than in unstimulated saliva.[17] Previous studies have used both stimulated and unstimulated saliva in the analysis of antioxidants.[9,32,33] Unstimulated whole saliva was collected in this study because it often yields valuable information in the determination of antioxidant defence parameters and usually correlates to clinical conditions more accurately than stimulated saliva. [34] It has been claimed that Total Antioxidant Capacity is higher in unstimulated saliva.[21]

Total Antioxidant Capacity of saliva must be evaluated as it is suggested that free radical/reactive oxygen species and antioxidant system appear to act in concert rather than alone, and measurement of any individual antioxidant may be less representative of whole antioxidant status. Moreover, due to the number of different antioxidants, it is difficult and also expensive to measure each of them separately.

In our study, the significantly lower TAC of saliva observed in CP children implies that there is an impaired oxidative/antioxidant balance due to marked oxidative stress. Deficiency in vitamin and food intake, environmental factors and epileptic seizures may cause oxidative stress in children with CP.

In normal healthy individuals, total antioxidant capacity of saliva has been shown to have a linear relation with dental caries.[10,35] However, TAC levels in saliva showed an inverse relation to dental caries in children with CP. This is possibly due to high oxidative stress at a very young age and increased caries risk in these children together with reduced total antioxidant capacity of saliva.

Nitrate and nitrite in saliva play a role in the maintenance of certain oral protective functions, in particular, the production of nitric oxide. It is also one of the most powerful antibacterial compounds acting either through inhibition of bacterial growth or through enhancement of macrophage-mediated cytotoxicity. The concentration of nitrite in saliva varies according to dietary nitrate intake, activity of bacterial nitrate reductase, salivary flow rate and endogenous production of nitrate.[36] No production might be a host defense mechanism when dental caries increases or oral hygiene deteriorates.[37]

Sialic Acid iss a family of acidic sugar derivatives of neuraminic acid (a 9-carbon acidic sugar) and is an important component of the glycoproteins. In the present study, salivary levels of SA were significantly higher in children with CP, which may be due to a high degradation of glycoproteins by enzymatic hydrolysis. An earlier study also found total protein and Sialic Acid concentration in saliva to be higher in children with Cerebral Palsy.[38]

This study highlights the influence of oxidative stress and antioxidants on oral health. It further emphasises the importance of saliva as a diagnostic tool. The physicochemical properties of saliva can influence the development of oral disease. Since, saliva can be easily collected, measurement of salivary total antioxidant capacity may prove a cost effective method for screening large populations.[9] Due to the imbalance between oxidative stress and antioxidants in children with Cerebral Palsy, antioxidants in the form of micronutrients should be included as nutritional supplements in their diets. Regular consumption of foods rich in antioxidants can help in combating oxidative stress in these individuals. Further studies could be carried out on evaluation of nutritional status and its impact on oral defence factors.

CONCLUSIONS

1. Primary dentition caries was higher in both cerebral palsied and normal children. Children with CP had higher deft scores than that of normal children.
2. Oral hygiene of children with CP was significantly poorer than that of normal children.
3. Total Antioxidant Capacity of saliva was significantly higher in normal children than cerebral palsied children.
4. In children with CP TAC of saliva showed an inverse relation with dental caries.
5. Levels of Sialic Acid in saliva was significantly higher in cerebral palsy children.

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