



Comparison of Oral Health Status and Saliva CRP Levels in Autistic and Non-autistic Individuals

Dr. Joseph Devasia¹, Dr. Sham S. Bhat¹, Dr. Nivedita L. Rao^{2*}, Dr. Vidya Bhat S³, Dr. Lyn Mary⁴

¹Department of Paedodontics, Yenepoya Dental College, Mangalore, Karnataka, India.

^{2*}Department of Biochemistry, Yenepoya Medical College, Mangalore, Karnataka, India.

³Department of Prosthodontics and Maxillofacial prosthetics, Yenepoya Dental College, Mangalore, Karnataka, India.

⁴Department of Orthodontics, K V G Dental College, Sullia, Karnataka, India.

*Corresponding author's E-mail: nldr@yahoo.com

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ABSTRACT

Autism is a neurodevelopment disorder characterized by impaired social interaction and behaviour which can have adverse influence on dental care and oral health in the affected individuals. C - Reactive Protein (CRP) is a biomarker of inflammation with clinical utility in inflammation assessment and disease management. The present study was undertaken to assess and compare the oral health status and saliva CRP levels in autistic individuals and non-autistic individuals. 20 autistic and 20 healthy non-autistic individuals were examined for oral health status, oral hygiene index and their saliva CRP concentrations were estimated using ELISA method. Oral health status of autistic group was found to be similar to that of non-autistic group. However, saliva CRP levels were found to be significantly elevated in autistic individuals compared to non-autistic individuals ($p=0.014$). Elevated saliva CRP levels in autistic individuals demonstrated in the present study indicates the presence of underlying systemic inflammatory process and has important implication for elaborating the role of immune system dysfunction in autism. It may be concluded that increased saliva CRP level has the potential to serve as a marker for the diagnosis of autism.

Keywords: Autism, Oral health status, Oral hygiene, Saliva CRP.

INTRODUCTION

Autism is a spectrum of disorders, which includes the pervasive developmental disorders and Asperger syndrome.¹ Autism, also referred to as autistic disorder, usually presents in young children below age of 3 years and is characterized by deficits in reciprocal social interaction, deficits in communication, restricted, repetitive behaviours, interests and activities.² This complex developmental disability impairs social, behavioural and intellectual functioning. The prevalence is estimated to be 1-2 per 1,000 for autism, with about four times as many males as females.³ Some autistic individuals may express abnormal emotional and linguistic development with visual and hearing impairment; others also have coexisting disabilities such as mental retardation or epilepsy, which may complicate dental care for the affected children.⁴

The risk of dental caries and gingivitis can be expected to be higher in autistic individuals due to improper brushing and flossing because of the difficulties the trainers and parents encounter when they brush their teeth. It could also be due to a lack of necessary manual dexterity in autistic individuals.

C - Reactive protein (CRP) is an acute-phase protein and a saliva-based biomarker of inflammation whose concentration is increased during inflammatory conditions such as gingivitis or periodontitis.⁵ Autistic children are known to have acute-phase systemic inflammation, demonstrated by elevated levels of serum

CRP and S100 proteins which, is believed to precede inflammation of the brain.⁶

There are only few studies describing oral health and dental needs of children with autism. Information on the oral health patterns in the population with this disease is important because it acts as foundation for the planning of public oral health policies.⁷

The present study was conducted to assess the oral health status, salivary CRP levels in autistic individuals and compare them with those of healthy non-autistic individuals.

MATERIALS AND METHODS

Selection of subjects

The study group consisted of 20 autistic individuals (males and females) attending the Autism Centre at Saanidhya, Special Children Care Centre, Mangalore, aged between 9 – 24 years. All the individuals were previously examined and diagnosed medically as autistic patients according to the Centre's medical records.

The study was conducted according to the ethical standards approved by the Institutional Ethics Committee of Yenepoya University.

Consent for examining the autistic individuals was obtained from their caregivers and school authorities. Those individuals who underwent dental prophylaxis in the last 6 months or had other systemic diseases were excluded from the study.



The control group consisted of 20 healthy non-autistic individuals from Ullal Government School, Mangalore and volunteers from Yenepoya Dental College, Mangalore who were age and sex matched with the autistic individuals. All controls were medically fit, not undergoing any antibiotic or anti-inflammatory therapy or not undergone such therapy in the previous 6 months.

Assessment of oral health status and oral hygiene

After taking the complete medical history, one examiner conducted the assessment of all subjects for oral health status and oral hygiene index. Examination of the soft and hard tissues was done under flash light and regular room light. Each individual accompanied by his/her caregivers was brought to the examination room and was seated on an adjustable chair. "Tell-Show-Feel and Do" technique were used with the individuals. This methodology was used systematically for all the examined autistic and healthy non-autistic individuals and the findings were recorded.

The gingival status was evaluated according to the gingival index of Loe and Silness.⁸ Gingival status was recorded as generalized or localized gingival inflammation depending on the amount of gingival redness and bleeding during the examination.

Oral hygiene recording was done as good, fair or poor according to the Simplified Oral Hygiene Index (SOHI).⁹

Saliva specimen collection

The subjects were asked to rinse their mouths 2-3 times with water. 10 minutes later whole saliva was collected by allowing the saliva to pool on the floor of the mouth by tilting the head forward, then passing the saliva through a short straw into a polypropylene vial.

Sample collection was avoided within 60 minutes after eating a major meal. The time and date of specimen collection were recorded. After collection, samples were kept in the cold at -20°C , in order to avoid bacterial growth and loss of CRP in the specimen.

Estimation of saliva C- Reactive Protein (CRP)

On day of assay, the collected saliva samples were thawed completely, vortexed and centrifuged at 3000 rpm for 15 minutes. Clear supernatants of samples were used for CRP estimations. The concentration of CRP in saliva was estimated by Enzyme Linked Immunosorbent Assay (ELISA) method using Salimetrics C-Reactive Protein ELISA kit (USA), which had very high sensitivity and lower detection limit of 10 pg/mL.

Statistical analysis of data

Unpaired Students t test was used to compare saliva CRP levels between the groups. Chi-square test was employed for comparison of gender differences, oral hygiene scores, gingival index scores between the two groups and also for the correlation between oral health status and saliva CRP levels in autistic and non-autistic individuals. P values

lower than 0.05 were considered to be statistically significant. SPSS Version 16 (SPSS Inc., Chicago, IL) software was used for the analysis.

RESULTS AND DISCUSSION

Age of the individuals in the study groups ranged from 9 - 24 years. The gender distributions are given in Table 1.

Table 1: Gender distribution of autistic and non-autistic groups

Group		Frequency (Percent)	Chi-square	P-value
Autistic	Male	14 (70.0)	0.00	1.00*
	Female	6 (30.0)		
Non-autistic	Male	14 (70.0)		
	Female	6 (30.0)		

*denotes statistically not significant

Childhood Autism Rating Scale (CARS), a diagnostic assessment method that rates children on a scale from one to four for various criteria, ranging from normal to severe, were obtained from the records of autistic individuals. The composite score ranges from non-autistic to mildly autistic, moderately autistic or severely autistic. The CARS score characteristics of the autistic individuals are given in Table 2.

Table 2: Childhood autism rating scale (CARS) score among autistic group

CARS score	Autistic individuals Numbers	Autistic individuals %
1	8	40
2	11	55
3	1	5

CARS Score indications: 1 = Normal for individual's age; 2 = Mildly abnormal; 3 = Moderately abnormal

Table 3: Simplified oral hygiene scores of autistic and non-autistic individuals

Group		Frequency (Percent)	Chi-square	P-value
Autistic	Good	2 (10.0)	0.407	0.204*
	Fair	18 (90.0)		
Non-autistic	Good	5 (25.0)		
	Fair	15 (75.0)		

*denotes statistically not significant

Majority of the individuals presented with a fair oral hygiene in both the groups. The oral hygiene status of autistic and non-autistic groups did not show significant differences (Table 3).

80 - 85% of the individuals in both the groups presented with moderate gingivitis but there was no statistically significant difference in gingival index scores between the groups (Table 4).

Table 4: Gingival index scores of autistic and non-autistic individuals

Group		Frequency (Percent)	Chi-square	P-value
Autistic	Mild	4 (20.0)	0.173	1.00*
	Moderate	16 (80.0)		
Non-autistic	Mild	3 (15.0)		
	Moderate	17 (85.0)		

*denotes statistically not significant

Table 5: Comparison of saliva CRP levels between autistic and non-autistic group using student's t-test

Group	Saliva CRP levels (pg/mL)		t-value	P-value
	Mean	Std. Deviation		
Autistic (n=20)	1202.56	974.52	2.576	0.014*
Non-autistic (n=20)	586.96	438.49		

* denotes statistically significant

There was a statistically significant increase in the saliva CRP levels of autistic individuals (Table 5).

There was no statistically significant association between the oral health status and saliva CRP levels in the autistic and non- autistic individuals.

Autism is a severe childhood neuropsychiatric disorder. Studies comparing individuals with disabilities to similarly aged individuals with normal development have shown poorer oral hygiene and increased periodontal disease within the handicapped group.¹⁰ Generalized gingivitis might occur as a side effect of medications used to control the manifestations of autism such as psychoactive drugs or anticonvulsants, with the most common drug classes being antidepressants, stimulants, and antipsychotics.⁴ It is well known that good oral hygiene, particularly tooth brushing with fluoride toothpaste is important for keeping the teeth free from caries. Maintenance of good oral hygiene in an autistic individual can prove to be a formidable task for both parents, care givers of the individual and dental staff.

Studies on oral health conditions in autism individuals in India are sparse. The present study was conducted to

systematically identify the oral health problems of autistic individuals in Mangalore, Karnataka State, India in comparison with non-autistic individuals. In the present study the autism group had more males than females, with a male to female ratio of 2.3:1 (Table 1), which might reflect the higher prevalence of autism in males as reported in other studies.^{3,11}

Scores obtained from Childhood Autism Rating Scale (CARS), a behaviour rating scale used to help diagnose autism, showed that majority of autistic individuals in the present study had score 2 which is mildly abnormal and many had score 1 which is normal for individuals age with only one individual who had score 3 which is moderately abnormal (Table 2).

Majority of autistic individuals in this study had fair oral hygiene with moderate gingivitis. Similar findings were observed in the non-autistic individuals (Tables 3, 4). This could be related to special attention and regular dental care given to autistic individuals residing in the special care school in Mangalore. It can be observed that these results on oral health status of autistic individuals of Mangalore, India are different from some reports on autistic individuals in UAE, Saudi Arabia and India where in majority of autistic individuals had poor oral hygiene with moderate gingivitis.^{4,11,12} The UAE study reported significant differences in oral hygiene status and gingival status between autistic individuals and non-autistic controls.⁴ Results of the present study are similar to those reported in other studies which concluded that individuals with autism had no statistically significant differences in oral health status and degree of oral hygiene in comparison with non-autistic individuals.^{13,14}

Serum CRP levels have been used to aid clinical assessment of inflammation and disease management.¹⁵ But, there are no reports on saliva CRP levels in autistic individuals. The present study demonstrates significantly elevated saliva CRP levels in autistic individuals compared to non-autistic individuals (Table 5). This, according to the authors' current knowledge, is the first report on saliva CRP levels in autistic individuals and it clearly indicates an underlying inflammatory process in these individuals. There was no significant correlation between oral health status and saliva CRP levels in both autistic and non-autistic individuals (Table 6).

Table 6: Correlation between oral health status and saliva CRP levels in autistic and non- autistic individuals

Dependent Variable			n (%)	df	Chi square	P value
Autistic	Saliva CRP Levels	Oral Hygiene Index	Good	2(10)	15	13.691
			Fair	18(90)		
	Gingival Index		Mild	4 (20)	15	19.00
			Moderate	16 (80)		
Non- Autistic	Saliva CRP Levels	Oral Hygiene Index	Good	5 (25)	14	13.843
			Fair	15 (75)		
	Gingival Index		Mild	3 (15)	14	16.078
			Moderate	17 (85)		

denotes statistically not significant



Elevated serum CRP levels have been reported in autistic individuals.⁶ Increase in saliva CRP levels in autistic individuals of the present study in the absence of significant oral inflammation could be related to their possibly increased serum CRP levels due to systemic inflammation.

Autism, considered to be a multi-factorial disorder, is influenced by genetic, environmental, and immunological factors. Oxidative stress is believed to be a mechanism linking these factors. Several reports have shown that autistic individuals have oxidative stress. Circulating cytokines and xanthine oxidase (XO) are greater in autism, and both generate free radicals.¹⁶ Cytokines and XO can be both cause and effect of oxidative stress.

A recent report has investigated the association between maternal CRP and childhood autism from a large national birth cohort and concluded that elevated maternal CRP during pregnancy was related to a significant increase in risk of childhood autism in offspring.¹⁷ Plasma Nitric oxide free radical (NO^{*}) was observed to be elevated in autism in that report and excess NO^{*} is known to increase inflammation. Elevated proinflammatory cytokines in plasma and a variety of other immunologic anomalies have also been observed in children with autism, shedding light on the complex, multifaceted relationship between immune dysfunction and behaviour.¹⁸ Elevated saliva CRP levels in autistic individuals obtained in the present study confirms the presence of inflammatory process in these individuals and has important implication for elaborating the role of immune system dysfunction in autism.

Saliva-based CRP estimation in autistic individuals is advantageous as collection of saliva involves procedures that are considered to be non-invasive, painless and therefore, convenient to perform several times to monitor the inflammatory status of these individuals under circumstances where it may be difficult to collect blood specimens.

CONCLUSION

There was no significant difference between the oral health status and oral hygiene index scores of autistic and non-autistic individuals. Saliva CRP levels were significantly increased in autistic individuals in comparison with non-autistic individuals. No significant association was found between oral health status and saliva CRP levels in both autistic and non-autistic individuals. The main finding of this study is that saliva levels of the inflammatory marker CRP are significantly increased in autistic individuals possibly due to systemic inflammation and elevated serum CRP. Increased saliva CRP level may, therefore, serve as a non-invasive, potential marker for the diagnosis of autism.

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Corresponding Author's Biography: Dr. Nivedita L. Rao



Dr. Nivedita Rao obtained her Masters and PhD degrees in Medical Biochemistry from Manipal University, in Manipal. She is currently working as Professor of Biochemistry at Yenepoya Medical College, Yenepoya University in Mangalore, India. Her research areas include, saliva based diagnostics, measurement of relative changes in free calcium at cellular stores and has several publications to her credit. Her first report on saliva CRP levels in Hashimoto's and Subacute Thyroiditis has several citations and the saliva CRP test has received distinction as an emerging diagnostic test in the Best Practice website of the British Medical Journal.