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Comparative Evaluation of Alpha Amylase Levels In Autistic and Normal Children: A Pilot Study.

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ABSTRACT

Human saliva is an attractive body fluid for diagnosis of disease and prognosis because testing of saliva is simple, safe, low-cost and noninvasive which maintains the homeostasis of the oral cavity. It consists mainly of water, essential electrolytes, glycoproteins, antimicrobial enzymes and numerous other important constituents like glucose, amylase and thiocyanate. The most abundant protein in saliva is amylase, accounting for approximately 20% of salivary proteins. Salivary ALPHA-AMYLASE (SAA) has been investigated during past thirty years as a biomarker for dental caries (locally) and stress (systemically) stress To evaluate and compare salivary ALPHA-AMYLASE levels in autistic and normally developing children. Unstimulated whole saliva samples were collected FROM 24 children, 12 autistic and 12 normally developing healthy children. Biochemical analysis was done by using Liquipath-Alpha Amylase kit with UV spectrophotometer. The study showed that there was significant difference seen in the levels of salivary ALPHA-AMYLASE among autistic children and normal children. Though the present study is a preliminary study, further research could possibly prove efficacy of salivary ALPHA-AMYLASE levels as a chair side indicator for various local and stress related diseases.

Keywords: SALIVARY ALPHA-AMYLASE, AUTISTIC CHILDREN.

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INTRODUCTION

Autism disorder (AD) was first described in 1943 by the American child psychologist, Leo Kanner. It is defined as “A complex developmental disability that typically appears during the first 3 years of life and is the result of a neurological disorder that affects the normal functioning of the brain, impacting development in the areas of social interaction and communication skills.” given by Autism Society of America[1].

Autistic children generally have limited attention span, low frustration levels which may lead to temper tantrums [2]. These children exhibit hypersensitivity to touch and noise. They are very hyperactive and have a tendency to repeat particular body moments [1]. Some authors have reported about a strong urge for soft, sticky, and sweet foods in autistic children [3, 4]. Oral conditions of these children vary from person to person. The majority of autistic children have poor oral hygiene, and almost all of them have gingivitis. This is due to improper brushing habits and also due to the medication taken such as antidepressants, stimulants, and antipsychotics [1].

THERE IS NO SPECIFIC MEDICAL TEST FOR THE DIAGNOSIS OF THE AUTISM SUCH AS BLOOD OR BIOPSY TEST. IT CAN ONLY BE DIAGNOSED CLINICALLY BY THE DEVELOPMENTAL OR BEHAVIORAL PATTERN OF THE CHILD. THIS MAY TAKE SEVERAL YEARS CAUSING A DELAY IN THE DIAGNOSIS. Hence, it is important to have a specific biomarker for the diagnosis of the autistic disease. Early detection will help in early information about education and support; earlier access to target social, communication, behavioral interventions and also identification of associated medical, developmental and psychiatric conditions [1].

Salivary biomarkers have been used as a tool for the diagnosis of oral and systemic conditions. Salivary alpha amylase is one of the biomarkers which has been studied in past few years but still have doubtful utility [5]. Salivary ALPHA-AMYLASE activity is an increasingly investigated biomarker for the activation of the autonomic nervous system. There are few reported studies which show measurement of ALPHA-AMYLASE activity in the saliva of children has been used as an indicator of the aggressive response involving sympathetic nervous system [3].

CORRELATION OF SALIVARY ALPHA-AMYLASE WITH DENTAL CARIES HAVE ALSO YIELDED ON SPECIFIC RESULTS THOUGH some studies have found that salivary ALPHA-AMYLASE concentration was higher for caries active group as compared to caries-free[6]. Thus, the present study was designed with the aim to revisit and evaluate ALPHA-AMYLASE levels in saliva of autistic children AS COMPARED TO NORMALLY DEVELOPING CHILDREN AS A CONTRIBUTING FACTOR FOR DENTAL HEALTH.

Null hypothesis - Levels of salivary ALPHA-AMYLASE in autistic and normally developing children are COMPARABLE AND DOES NOT CONTRIBUTE TO ORAL DISEASES.

MATERIALS AND METHODS

Patient selection

Saliva samples were collected from 24 children ranging from 4-15 years of age from Ankur School, Near Fergusson College, Pune, and patients visiting the department of Pedodontia in Dr. D. Y. Patil dental college. Informed consent forms were obtained from the parent or guardian of the subject after explaining the procedure to the parent or guardian. A separate permission letter was collected from school. Samples were collected in two groups:

Group A – includes 12 children those are clinically diagnosed with autism at least one year back;

Group B – 12 samples of normally developing children with no relevant medical history. Children with other systematic conditions were excluded. Age and sex matching were done.

Method of saliva collection:

A brief case history of all children was recorded. To minimize the effect of circadian rhythms, children were instructed not to eat or drink for at least 1 hour prior to collection of the samples. Collection of saliva samples was carried out between 9 to 10.30 am. Subjects were advised to rinse mouth with tap water and after 10 minutes saliva samples were collected to avoid sample dilution. The subject was seated in a well-

ventilated and well-lit room. The head was kept at 45 degrees flexion with one hand holding onto a 4ml cryoprecipitation vial with a funnel inserted into it, in a calm atmosphere to simulate unstimulated conditions. The saliva was allowed to drip into the funnel held to the lower lip (Colin Dawe’s method) [10]. For each trial, the collection continued for 2 minutes but if the saliva sample was insufficient within 2 minutes, the collection was continued until 2 ml of saliva per subject was obtained. The salivary samples were labeled according to the group to which the subject belonged.

Methods of laboratory analysis:

Samples were stored at -80^oc until biochemical assays were performed. Samples were thawed before the biochemical analysis started. The analysis was done for ALPHA-AMYLASE levels in saliva using Liquipath-Alpha Amylase kit (PATHOZYME DIAGNOSTICS) with UV-1800 spectrophotometer (SHIMADZU). Thawed saliva samples were centrifuged, 20 µl of it was added to ALPHA-AMYLASE reagent and enzyme activity was analyzed by measuring the absorbance at 405 nm using a UV spectrophotometer. Readings were recorded at 1min, 2min, and 3min interval. The mean of all three reading was taken as the final reading.

Amylase Activity was calculated using the following formula
 Amylase Activity in U/L= (mean reading×3806) ×dilution factor

RESULTS

The children studied in our group were mostly boys (83%) and were in the age range of 7-13 years with the mean age being 11 years. The salivary ALPHA-AMYLASE levels were studied and we found that in the autistic individuals the average value of ALPHA-AMYLASE is 38009 whereas the average value of ALPHA-AMYLASE is 25,883. A comparative analysis (Table 3) using non-parametric Mann- Whitney U test, the p value was significant (p= 0.002). Thus, it is seen that salivary ALPHA-AMYLASE levels were significantly higher in autistic individuals than normally developing children.

Table 1: Salivary ALPHA-AMYLASE levels in autistic children

Sample No	Age/Sex	Salivary ALPHA-AMYLASE
1	11/M	33,994
2	11/M	40,767
3	11/M	16,645
4	12/M	49,123
5	13/M	39,725
6	5/M	42,866
7	7/F	30,023
8	10/M	47,119
9	5/M	31,389
10	12/M	42,133
11	10/F	35,980
12	7/M	46,344

Table 2: Salivary ALPHA-AMYLASE levels in Normally Developing Children

Sample No.	Age/Sex	Salivary ALPHA-AMYLASE levels
1	11/M	41,105
2	11/M	28,220
3	11/M	25,122
4	12/M	27,192
5	13/M	22,855
6	5/M	18,644
7	7/F	28,671
8	10/M	24,559
9	5/M	21,376
10	12/M	20,179

11	10/F	28,446
12	7/M	24,235

Table 3: Comparative analysis between the salivary alpha- amylase in Autistic and normally developing children.

Group	N	Mean Rank	Mann-Whitney U	p-value
1	12	17.00	18.000	0.002
2	12	8.00		
Total	24			

DISCUSSION

The National Institute of Child Health and Human Development defined Autism Disorder (AD) as:

“A complex biological disorder that generally lasts throughout a person’s life, as it starts before age three, in the developmental period, and causes delay or problems in many different ways in which a person develops or grows [1].”

POSSERUD ET AL AND WONG ET AL IN A WORLDWIDE SURVEY STUDIED APPROXIMATE PREVALENCE RATES OF AUTISM DIAGNOSED IN CHILDREN. IN INDIA THE PREVALENCE RATE IS APPROXIMATELY 1 out of 250[1, 7, 8]. Diagnosis of autistic conditions involves a two-step process; the first step is a developmental screening, which is followed by a comprehensive diagnostic evaluation. Many children present to clinics with extensive caries and periodontal pathologies as training of oral hygiene practices is difficult in autistic patients.

Thus, a salivary screening tool could aid in

- a. Predicting future dental needs of these patients
- b. Better diagnosis and management of autistic children.

Literature evidence says that changes in salivary ALPHA-AMYLASE levels have implications for dental health. Also saliva collection being a non-invasive method, samples collection was easier in the present study to deal with the autistic children.

Many of the studies carried out by Bosch et al., 1996, 2003; Nater et al., 2006, 2005; Rohleder et al., 2006; Skosnik et al., 2000 have stated that the salivary ALPHA-AMYLASE has been found to correspond to psychological stress[7]. Granger et al. (2006) found that this finding is also true in children. He also suggested a link between salivary ALPHA-AMYLASE and disease. This study also found that there is an increase in salivary ALPHA-AMYLASE in response to laboratory challenges as observed in children with more parent-reported illnesses. The another study carried out by Granger et al. (2007) revealed that higher levels of salivary alpha amylase after acute laboratory stressors were associated with increased health problems in children such as respiratory problems⁷.

Kidd et al.2013 carried out one study to evaluate the salivary ALPHA-AMYLASE levels in autistic (26) and normally developing children (26). They divided autistic children into high, low, and extremely low functioning groups and collected the saliva samples at 3 different times of the day. The results suggested that there was an increase in cortisol levels, and particularly salivary ALPHA-AMYLASE levels when compared with diagnostic groups stratified by IQ[8].

It is also mentioned that there is a correlation between salivary ALPHA-AMYLASE and dental caries incidence. Mojarad F et al. in 2013 carried out a study, in which they have included 84 early childhood caries active cases were compared with 84 early childhood caries-free controls. In this study they found out that the mean level of salivary alpha- amylase in the control groups was higher than that of case groups; 42.08 kU/L versus 28.37kU/L respectively. They also observed the salivary ALPHA-AMYLASE values after the emergency caries treatment, in which they noticed increased level of salivary ALPHA-AMYLASE after the treatment but it was not up to the levels of the control group [9].

It has been seen that increased incidence of salivary ALPHA-AMYLASE levels is associated with increased stress levels. There is also a correlation between salivary alpha- amylase and dental caries.

Based on these findings we designed a pilot study including 12 autistic children and 12 normally developing children. Oral screening for all the children was done DMFT and the periodontal index was recorded.

In this study we have found an increased level of salivary ALPHA-AMYLASE in autistic children as compared to the normally developing healthy children although there was no difference in the DMFT status in this both the group.

This study was undertaken to estimate the use of salivary ALPHA-AMYLASE as a marker for the autistic condition and oral manifestation of this internal stressed condition, as poor periodontal status was one of the major and common finding in autistic children group. This study was a preliminary research on the association between salivary ALPHA-AMYLASE and oral conditions. Study on the larger sample size and a longitudinal study design will be required to confirm the findings. This could prove important in the management of autistic children.

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