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Comparative Evaluation Of Salivary Cortisol Levels And Dental Anxiety In Children With Autism

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I. INTRODUCTION

Autism is a severe neurodevelopmental disorder characterized by qualitative impairment before the age of three in verbal and nonverbal communication, reciprocal social interaction, and a markedly restricted repertoire of activities and interests[1].

Observable changes in the behaviour of an individual experiencing stress and the marked increase in the stress related hormones like Adrenocorticotropic hormone (ACTH), corticotropin releasing hormone (CRH) and cortisol are the main indicators for the response to stress[2].

Any type of physical or mental stress can result in an elevation of cortisol; however, the type of situation that elicits a stress response can vary from person to person. Cortisol hormone is dispersed in all body fluids like urine, serum, or saliva and has a diurnal variation and rhythm which is mainly unaffected by age, gender, or pubertal status[3]. It is observed that autistic individuals react distinctively to environmental changes or unfamiliar situations unlike healthy individuals[4]. So it is important to evaluate those stress related hormonal changes in both healthy as well as in children with special health care needs.

In the present study saliva was used for estimation of cortisol levels, as collection of the saliva is easy and non-invasive in children.

Anticipation of dental treatment is a common source of anxiety and fear amongst children[5]. It has been suggested that this may result in increased salivary cortisol concentration in response to increased level of dental anxiety. Dental anxiety can also be assessed using Modified dental anxiety scale (MDAS) which is designed to identify fearful and non fearful patients so as to examine their relative reactions to dental procedures, both real and imagined[6].

Hitherto there are no published comparative research work related to dental anxiety and the salivary cortisol levels in autistic and healthy children. Thus, the present study was conducted to compare the salivary cortisol levels and dental anxiety in autistic and healthy children during dental examination.

II. MATERIALS AND METHODS

The sample consists of twenty children of both genders, aged between 11-15 years. children were categorized in to two groups. Group 1 consisted of ten autistic children, diagnosed according to DSM-IV Criteria, from Deepika school - school for special children, in Bangalore. Group 2 consisted of ten healthy children who visited the Department of Pediatric and Preventive Dentistry , V S Dental college, Bangalore for a routine dental check up. Signed written informed consent was obtained from the school authorities and parents of the children prior to the commencement of the study.

In both the groups, pre and post dental examination Salivary cortisol (SC) levels were estimated using Electrochemiluminescence immunoassay (ECLIA) method for which about 1-2ml of

unstimulated salivary samples were collected. All the children were instructed not to eat or drink anything half an hour prior to the sample collection as it may impact the test results. Salivary samples were collected by asking the children to expectorate into disposable plastic containers, one prior and one immediately after dental examination, which took place between 9am to 11am to avoid diurnal variations in the cortisol levels. In Group 2, sample collection was done in the recall visit. The pre and post dental examination salivary samples were then centrifuged for 15 minutes at 3000 rpm and the supernatant separated after centrifugation was transferred in screw capped bottles and tested for salivary cortisol levels.

MDAS was used to measure the dental anxiety. Questionnaires were distributed immediately after the pre examination salivary sample collection. Children were asked to fill the questionnaire and were helped by the trained school teachers and the examiner. Questionnaire comprised of five multiple choice questions dealing with the individual's reaction and expectations of going to and being treated by a dentist. Each question consisted of five choices, ranging from 1(not anxious) to 5 (extremely anxious), with a total score ranging from 5 to 25. A MDAS score of ≥19 indicates high dental anxiety.

Descriptive and statistical analysis were done. The Shapiro-Wilk test (sample size <50) was used for testing normality of data. The independent sample Student's t-test was used to check differences between the groups. SPSS (Statistical Package for Social Sciences) Version 20.1 (Chicago, USA Inc.) was used.

III. RESULTS

The study was conducted to compare the salivary cortisol levels and dental anxiety in children with autism and healthy children. The age of the study population ranged between 11-15years with a mean age of 13.30 (Table 1). Based on gender distribution, the study population consisted of 70% males and 30% females.

Pre examination and Post examination Salivary cortisol levels were estimated using ECLIA method. Pre SC levels in autistic children were noted to be 0.23nMol/L(mean) which were higher when compared to the post SC levels 0.16nMol/L(mean), results were not statistically significant(p-0.056). Pre SC levels in healthy children were observed to be 0.018nMol/L (mean) which were almost equal to the post SC levels 0.017nMol/L(mean) , the results were not statistically significant(p - 0.334). (Table-2 , fig-2)

Pre SC levels were found to be increased in autistic children (0.23) when compared to that of healthy children (0.18) and post SC levels were found to be almost equal in both the groups. (Fig-1)

MDAS was used to measure the dental anxiety. When the participants were asked the MDAS question no. 1, four answered not anxious, 9 answered slightly anxious, and 7 answered fairly anxious; it was observed that results were not statistically significant (p-0.067). Statistically significant value (p-0.018) was observed in the MDAS question 2, where the study population was asked about how they would feel if they were sitting in the waiting area before the treatment. 4 participants answered slightly anxious, 12 answered fairly anxious, and 4 answered very anxious. For MDAS question no. 3, two answered fairly anxious, 10 answered very anxious and 8 answered extremely anxious; results were not statistically significant (p - 1.000) . Similarly, when they were asked MDAS question no. 4, one child answered not anxious,10 answered slightly anxious,5 answered fairly anxious and 4 answered very anxious; it was noted that the results were not statistically significant for this question.(p-0.079). For MDAS question no. 5, four children answered fairy anxious, 2 very anxious and 14 extremely anxious; results were not statistically significant (p - 0.076) (Table 3).

According to MDAS criteria, a MDAS score of ≥19 indicates high dental anxiety. In the present study four autistic children and 1 healthy child, were found to be highly anxious.

Table 1: Age description of the study population

Age	N	Range	Mean	S.D.
Autistic	10	11-15	13.70	1.41
Normal	10	12-14	12.90	0.56
Total	20	11-15	13.30	1.12

Table 2: Comparison of pre and post dental examination salivary cortisol (nMol/L) levels in autistic and normal children

Variables		N	Mean	S.D.	S.E.	M.D.	95% C.I.	t-Value	P-Value*
Autistic	Pre SC	10	**0.23**	0.11	0.03	0.07	-0.002-0.144	2.189	0.056
	Post SC	10	**0.16**	0.08	0.02				
Normal	Pre SC	10	0.18	0.08	0.02	0.01	-0.015-0.041	1.020	0.334
	Post SC	10	0.17	0.08	0.02				

SC, Salivary cortisol in nMol/L; *P-value derived from paired sample t-test

Table 3: Responses to Modified Dental Anxiety Scale (MDAS) questions by the study population

Questions	Groups	Not Anxious		Slightly Anxious		Fairly Anxious		Very Anxious		Extreme Anxious		X^2-Value	P-Value
		N	%	N	%	N	%	n	%	n	%		
How would you feel if you went to your dentist for treatment tomorrow?	Autistic	0	00.0	5	50.0	5	50.0	0	00.0	0	00.0	5.397	0.067
	Normal	4	40.0	4	40.0	2	20.0	0	00.0	0	00.0		
How would you feel if you were sitting in the waiting room for treatment?	Autistic	0	00.0	0	00.0	6	60.0	4	40.0	0	00.0	8.000	**0.018**
	Normal	0	00.0	4	40.0	6	60.0	0	00.0	0	00.0		
How would you feel if you were about to have a tooth drilled?	Autistic	0	00.0	0	00.0	1	10.0	5	50.0	4	40.0	0.000	1.000
	Normal	0	00.0	0	00.0	1	10.0	5	50.0	4	40.0		
How would you feel if you were about to have your teeth scaled and polished?	Autistic	0	00.0	3	30.0	3	30.0	4	40.0	0	00.0	6.800	0.079
	Normal	1	10	7	70.0	2	20.0	0	00.0	0	00.0		
How would you feel if you were about to have a local anaesthetic injection in your gums?	Autistic	0	00.0	0	00.0	0	00.0	1	10.0	9	90.0	5.143	.076
	Normal	0	00.0	0	00.0	4	40.0	1	10.0	5	50.0		

Figure 1: Comparison of pre and post dental examination salivary cortisol (nMol/L) levels among the study population

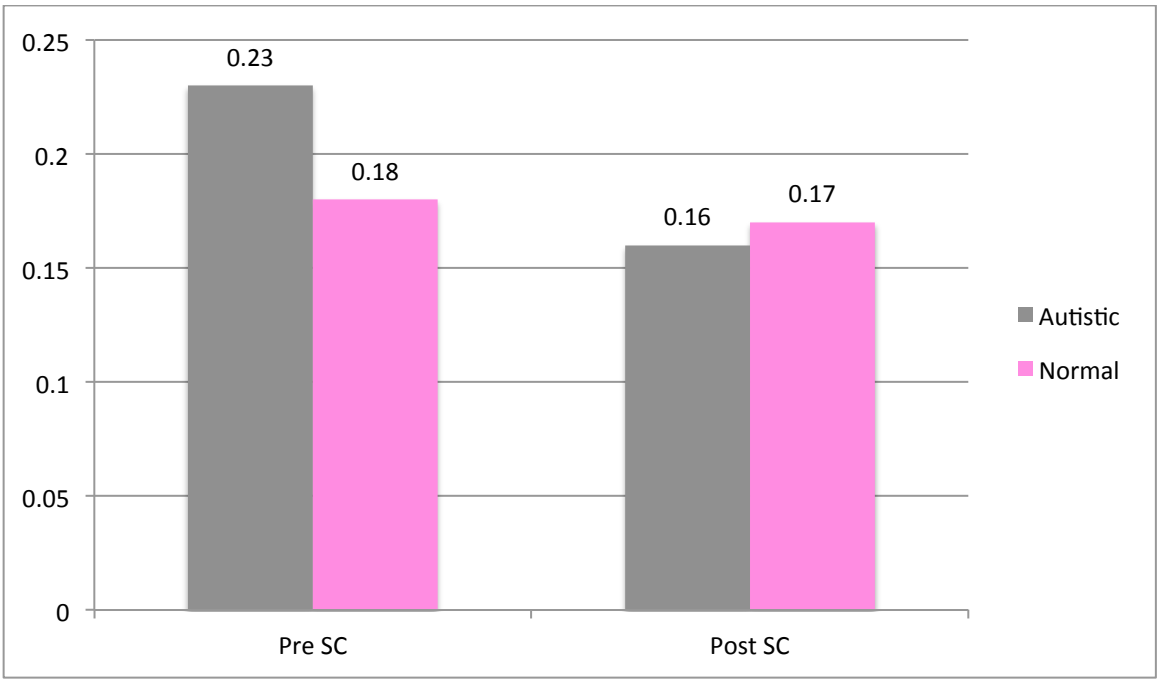
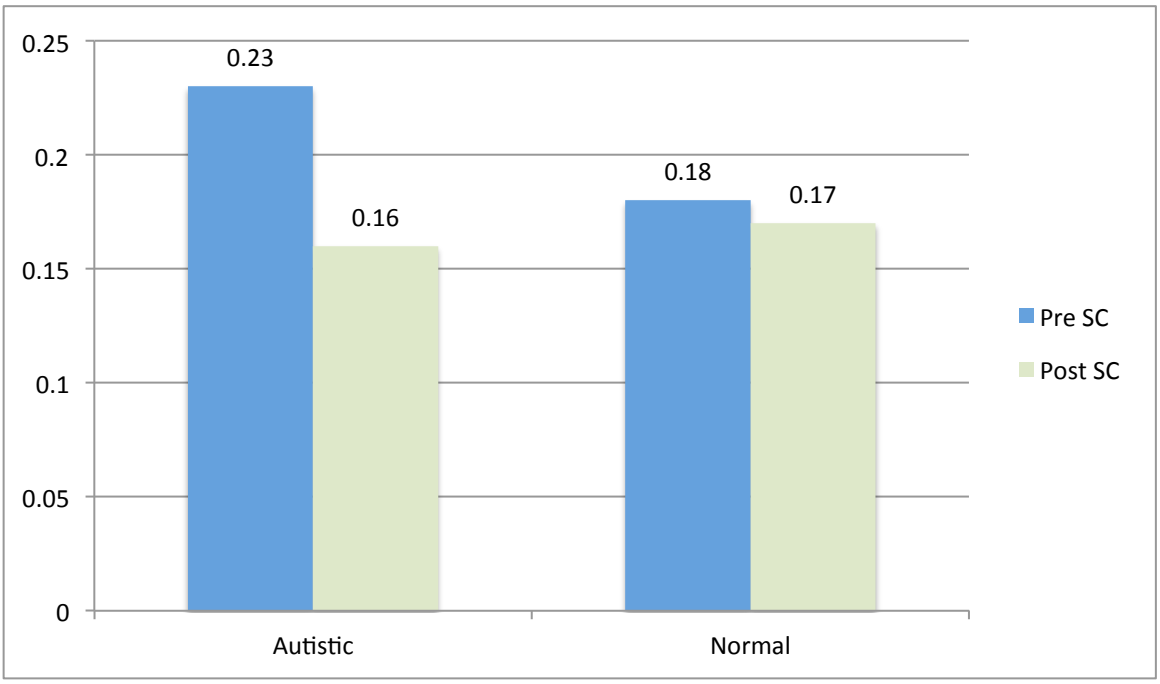


Figure 2: Comparison of pre and post dental examination salivary cortisol (nMol/L) levels in autistic and normal children



IV.DISCUSSION

Individuals with autism are clinically described as being more vulnerable to stress. This increased vulnerability is known to arise from deficits in social skills, in communication, and in adapting to unfamiliar situations. Autistic children can show hyperreactions like tantrums, stereotyped behaviour, and self-injurious behaviour on one hand and hyporeactions like unresponsive to their environment on the other hand[7].

Children are often subjected to numerous situations that test their ability for adaptation and interplay. A visit to the dental office is one such situation which, for some children, is associated with fear and anxiety. The prevalence of dental anxiety in children has been reported to be between 5.7 and 6.7%, with anxiety decreasing with increasing age[8].

Thought of receiving dental treatment acts as a stimulus for the adrenal cortex to release cortisol and measurement of its salivary concentration indicates rapid changes in the adrenocortical activities. Therefore, salivary cortisol concentration may be related to the level of dental anxiety. Cortisol is the major glucocorticoid produced and secreted by the adrenal cortex which affects the metabolism of fat, carbohydrates and proteins; the maintenance of muscle and myocardial integrity; and the suppression of inflammatory and allergic activities[5].

It has been reported that, cortisol levels in saliva closely resemble serum free cortisol levels and are independent of flow rate[9]. Collection of saliva is a non-invasive, stress free procedure, sample storage is very simple. In contrast collection of serum and blood samples is stressful; thereby, directly elevating free cortisol concentration due to psychological stress of sample collection and distorting the results of the tests. The importance of circadian rhythm of cortisol has been emphasised in numerous studies considering which, all the samples for the present study were collected from children between 9:00am and 11:00am.

In the present study pre and post dental examination salivary cortisol levels were measured and compared in a group of autistic and healthy children. It was observed that the pre dental examination SC levels were higher than the post dental examination SC levels in children with autism. However, the difference was not statistically significant.(p – 0.056)

Biological range of salivary cortisol levels in healthy individual is 0.37-0.99 nmol/l

The pre dental examination SC levels of autistic children were higher than both pre and post SC levels in healthy children. In healthy children both pre and post SC levels were found to be equal however, the differences were not statistically significant(p – 0.334).

These findings may be attributed to increased anxiety due to anticipation of dental examination as well as exposure to an unknown individual in the form of the dentist which may have led to higher levels of pre examination SC levels. However, performing the dental examination in a known environment and subsequent acclimatisation to the examiner might have reduced the stress and anxiety in the study population, thus showing low post examination SC levels.

In contrast Corbett et al, reported that majority of children with autism demonstrated increased SC levels after exposure to mock MRI, a non social stressor. Whereas non autistic children showed no response or reduction in SC levels [10].

This is the first study showing comparative evaluation of salivary cortisol levels and dental anxiety in children with autism.

In the present study 5 out of 20 children (4-autistic and 1-healthy child respectively) showed high dental anxiety according to the MDAS score. However, there was no statistically significant co-relation of MDAS score with that of pre and post SC levels. The reason for this may be difficulty in obtaining the view point of very young children who may not be able to appropriately fill the questionnaire either by themselves or when interviewed by the teacher or the examiner.

This result was consistent with the work of Patil et al who reported that no association existed when Corah's dental anxiety scale was correlated with salivary cortisol levels when response to dental procedures such as dental examination, restoration and extraction were measured[5].

An interesting statistically significant result (p - 0.018) was observed when the children were asked how they would feel if they were sitting in the waiting area for the treatment, thus implying that majority of the children would feel highly anxious when subjected to the given scenario.

V.SUMMARY AND CONCLUSION

Within the limitations of the present study the following conclusions were drawn.

i. Children with autism showed increased pre dental examination SC levels and decreased post dental examination SC levels

ii. Healthy children showed equal levels of salivary cortisol in both pre and post dental examination
iii. Children were highly anxious waiting in the reception area before the procedure
iv. MDAS score shows no correlation with salivary cortisol levels in both the groups. It is important to predict a child's behaviour, especially of special health care needs children, in the dental office. This would help not only to provide optimum treatment but also positively influence their perception towards dental treatment as an adult. No correlation exists in between MDAS and salivary cortisol levels. Measurement of salivary cortisol levels can be used as a successful predictor of dental anxiety and contribute towards behaviour modulation in children with special health care needs, thus enhancing patient care and subsequent success of dental treatment.

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