Salivary Cortisol Levels and its Implication on Behavior In Children with Autism during Dental Treatment

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*The **aim** of the study was to estimate the diurnal variations of salivary cortisol in children with autism and healthy children and it's implication on behavior during non-invasive dental procedures. **Study design:** 50 children with autism and 50 healthy children in the age group between 6 to 12 years of both genders with the need for dental treatment were included in the study. Whole unstimulated saliva was collected from them during early hours of the day and during evenings for 2 consecutive days . The collected saliva was then subjected to electrochemiluminescence assay . Minimum invasive dental procedures like hand scaling, pit and fissure sealants and glass ionomer cement restorations were performed for the participants each time after the saliva sample collection and their behavior during the procedures was rated using Frankl's Behavior Rating Scale. **Results:**Significant correlation was seen between cortisol levels and behavior in children with autism. As cortisol levels increased in children with autism, behavior worsened and as the cortisol levels decreased they showed positive behaviour. **Conclusion:** Cortisol acts as a stress marker and studying the diurnal variations of salivary cortisol can help us in attaining better knowledge about the behavior pattern and thereby assist us in modifying the behavior modification procedures and treatment planning in this group of special children.*

Key words: Autism, Cortisol, Stress, Diurnal Variation, children.

INTRODUCTION

One of the greatest challenges a paediatric dentist face is to provide effective and efficient treatment to children with special health care needs. Pediatric dentists have shifted from one group of special children to the next, often failing to provide proper dental care to this population. In dentistry, we have moved through the special need patients, but still a large fraction of this population is deprived of the basic treatment needs.

Much have been known and done about special conditions such as Down's syndrome, Cerebral palsy, intellectual and learning disabilities but recently more emphasis is being laid on autism, a condition which once was considered to be rare. But recent reports from across the globe indicating the increased prevalence of autism has gained profession's attention as another potential challenge in dentistry.

Autism is a type of pervasive developmental disorder that is defined by (a) the presence of abnormal or impaired development that is manifested before the age of 3yrs and (b) the characteristic type of abnormal functioning in all the three areas of psychopathology: reciprocal social interaction, communication and restricted, stereotyped, repetitive behavior. In addition to these diagnostic features, a range of other non-specific problems are common such as phobias, sleeping and eating disturbances, temper tantrums and (self directed) aggression.[1]

Autism is categorized under children with special health care needs, it is important for the practitioner to be fully aware of the developmental disability, systemic conditions compromising the physical well-being, and the signs and symptoms expressed by the patient, as it may all hinder in providing effective dental treatment during the dental procedures.[2]

Various studies have shown altered cortisol response in children with autism.[3,4] Cortisol is the principal circulating glucocorticoid and its release by the adrenal gland is controlled by adrenocorticotropin (ACTH) produced by corticotrophs in the anterior pituitary gland. Their main function is to help the body to maintain the chemical status quo against all forms of stresses including illness, injury, infection, mental strain and allergic reactions. Without adrenal cortex and their products the body would succumb to the impact of such stress. Cortisol follows a circadian rhythm, with high concentrations in the morning and a decline throughout the day, with the

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lowest levels in the evening and at night. This diurnal pattern is well established by the 3rd month of infancy. Individual differences in cortisol secretion, especially in the morning, may be an important variable for typically developing children as well as children with neurodevelopmental disorders, such as autism[5].

But whether this individual differences in cortisol secretion had an impact on the behavior of children with autism needed to be explored along with the diurnal variation of cortisol in them.

It has been found that salivary cortisol is a better measure of adrenal cortical function than serum cortisol and is particularly useful in studies with children.[6]Hence in the present study it was decided to evaluate cortisol levels in saliva rather than cortisol levels in serum or urine.

MATERIALS AND METHOD

A total of 100 children (50 children with autism and 50 healthy children) in the age group between 6 to 12 years of both genders with the need for dental treatment were included in the study. The children with autism(study group) were selected from among the students of the special school. The Healthy children (control group) were selected from among students of a normal school. Children who were medically compromised and on medication were excluded from the study group. Children who needed pulp therapy and minor surgical procedures like extraction of teeth were excluded from both the study and control groups. Informed consent were obtained from parents, caretakers, guardians and the concerned authority of the school to conduct the study. Ethical clearance was obtained from the ethical committee of teaching institute.

In the present study the whole unstimulated saliva was collected from the study group and control group during early hours of the day (approx. 8 – 8.30 am) and during evenings (approx. 4 – 4.15 pm) using the Zunt method[7] for 2 consecutive days to assess the diurnal variation of salivary cortisol. This was done in order to validate the diurnal variation of salivary cortisol. The subjects were put on fast one hour before saliva collection. The selected children were seated head slightly down, and was asked not to swallow or move their tongue or lips during the collection period. The saliva was allowed to accumulate in their mouth for 2 minutes, and he or she was then asked to spit the accumulated saliva in to the receiving vessel[8,9]. 2ml of saliva was collected and refrigerated at a temperature of 0^0 celsius. In children who were uncooperative the saliva was collected on a graduated cylinder over period of 5 minutes using plastic droppers.The collected saliva was then subjected to electrochemiluminescence assay to assess the levels of salivary cortisol[8,10] using ELECSYS 1010. Oral examination and minimum invasive dental procedures like hand scaling, pit and fissure sealants and glass ionomer cement restorations were performed for the participants each time after the saliva sample collection,that is during morning and in the evening and their behaviour during the procedures was rated using Frankl's Behaviour Rating Scale[11]. Since the saliva collection was done 4 times in an individual(two mornings and two evenings) dental treatments in one quadrant were performed at a time for convenience. The data collected were statistically evaluated using paired t test, ANOVA test and Kappa analysis with SPSS software version 11.0.

RESULTS

The mean salivary cortisol levels in children with autism were lower in the mornings and higher in the evenings and this difference was statistically significant (Table.1),However there was no significant difference in the mean salivary cortisol levels in morning and evening for the children with autism from day 1 to day 2(Table. 2).

The salivary cortisol levels in Healthy children were within the normal limits and were higher in the mornings and lower in the evenings for both the days and this difference was statistically significant(Table.1),and there was no significant difference in the cortisol levels from day 1 to day 2(Table. 2).

The behavior of the control and study group were assessed during minimum invasive dental procedures like hand scaling, pit and fissure sealants and glass ionomer cement restorations using Frankl's behavior rating scale.[11]

During minimum invasive dental treatment, it was found that out of the 50 children with autism:

- First day morning: 19 showed negative behavior and 31 showed positive behavior.
- First day evening: 33 showed definitely negative behavior and 16 showed negative behavior.(Table.3)
- Second day morning: 20 showed negative behavior and 30 showed positive behavior.
- Second day evening: 31 showed definitely negative behavior and 17 showed negative behavior. (Table.3)

On both the days in the study group, morning to evening, generally the behavior worsened from positive to negative or definitely negative during minimum invasive dental treatment.

During treatment, it was found that out of 50 healthy children:

- First day Morning - 35 showed positive behavior and 13 showed negative behavior.
- First day Evening - 24 showed positive behavior ,22 showed definitely positive behavior and 4 showed negative behavior. (Table.4)
- Second day morning - 34 showed positive behavior and 14 showed negative behavior in the morning.
- Second day evening and 25 showed positive behavior, 21 showed definitely positive behavior and 4 showed negative behavior in the second day evening. (Table.4)

On both the days in the control group, morning to evening, generally there was an improvement in behavior from negative to positive or definitely positive during minimum invasive dental treatment.

Behaviour in the healthy children improved statistically from positive to definitely positive from morning to evening for the dental procedures, whereas children with autism showed positive behavior in the morning and the behavior worsened in the evening and this was statistically significant.

Significant correlation was seen between cortisol levels and behaviour in children with autism for both the days (Table.3).

As cortisol levels increased in children with autism their behavior worsened and as the cortisol levels decreased they showed positive behavior.

Table.1:Cortisol levels in healthy children(control) and children with autism (study) during morning and evening.

	group	N	Mean	Std.d	t	df	Sig. (2-tailed)
cortisol morning 1	HEALTHY CHILDREN	50	96.16	15.694	13.023	97	<0.001
	CHILDREN WITH AUTISM	50	57.12	14.108			
cortisol evening 1	HEALTHY CHILDREN	50	66.39	11.735	-11.646	85.436	<0.001
	CHILDREN WITH AUTISM	50	101.4	17.647			
cortisol morning 2	HEALTHY CHILDREN	50	96.73	16.569	12.631	97	<0.001
	CHILDREN WITH AUTISM	50	57.96	13.883			
cortisol evening 2	HEALTHY CHILDREN	50	66.55	12.099	-11.597	86.401	<0.001
	CHILDREN WITH AUTISM	50	102	17.827			

Table.2: Comparison of day 1 and day 2 cortisol levels in each group separately

Paired Samples Statistics						Paired Differences				
Group			Mean	N	Std. Deviation	Mean	Std. Deviation	t	df	Sig. (2- tailed)
HEALTHY CHILDREN	Pair 1	cortisol morning 1	96.16	50	15.694	-0.571	6.134	-0.652	48	0.517
		cortisol morning 2	96.73	50	16.569					
	Pair 2	cortisol evening 1	66.39	50	11.735	-0.163	5.109	-0.224	48	0.824
		cortisol evening 2	66.55	50	12.099					
CHILDREN WITH AUTISM	Pair 1	cortisol morning 1	57.12	50	14.108	-0.84	4.991	-1.19	49	0.24
		cortisol morning 2	57.96	50	13.883					
	Pair 2	cortisol evening 1	101.4	50	17.647	-0.6	5.949	-0.713	49	0.479
		cortisol evening 2	102	50	17.827					

Table.3:Comparison of Cortisol levels and behaviour in children with autism(study) during minimum invasive dental treatment.

	group	Frankl's Behavior rating scale	N	Mean	Std. Deviation	Mean Square	F	Sig.
Evening day 1	CHILDREN WITH AUTISM	DEFINITELY NEGATIVE	33	106.27	14.399	1482.727	5.668	0.006
		NEGATIVE	16	93.5	19.425			
		POSITIVE	1	67	.			
Evening day 2	CHILDREN WITH AUTISM	DEFINITELY NEGATIVE	31	106.42	14.667	1241.095	4.456	0.017
		NEGATIVE	17	94.88	20.34			
		POSITIVE	2	70				
		Total	50	102	17.827			
Morning day 1	CHILDREN WITH AUTISM	NEGATIVE	19	60.95	14.049	1.522	48	0.135
		POSITIVE	31	54.77	13.846			
Morning day 2	CHILDREN WITH AUTISM	NEGATIVE	20	62.35	14.317	1.872	48	0.067
		POSITIVE	30	55.03	13.008			

There was no significant correlation between cortisol levels and behavior in healthy children for both the days; which indicates that cortisol levels does not affect the behavior in normal children (Table. 4).

Table.4:Comparison of Cortisol levels and behaviour in healthy children(control) during minimum invasive dental treatment.

	group	Frankl's Behavior rating scale	N	Mean	Std. Deviation	Statistic	df1	Sig
1st day morning	HEALTHY CHILDREN	NEGATIVE	13	108.54	21.942	4.752	2	0.089
		POSITIVE	35	91.26	9.854			
		DEFINITELY POSITIVE	2	99	4.243			
		Total	50	96.16	15.694			
1st day evening	HEALTHY CHILDREN	NEGATIVE	4	77.75	14.863	348.822	2.714	0.077
		POSITIVE	24	67	13.125			
		DEFINITELY POSITIVE	22	63.52	8.023			
2nd day morning	HEALTHY CHILDREN	NEGATIVE	14	109.71	22.445	5.346	2	0.072
		POSITIVE	34	91.09	9.986			
		DEFINITELY POSITIVE	2	99	4.243			
		Total	50	96.73	16.569			
2nd day evening	HEALTHY CHILDREN	NEGATIVE	4	79.75	17.347	404.969	2.997	0.06
		POSITIVE	25	66.38	12.772			
		DEFINITELY POSITIVE	21	64.24	8.837			
		Total	50	66.55	12.099			

DISCUSSION

Cortisol is a hormonal marker of stress which has received considerable attention in studies of psychological and physical health[12,13,14]. In the present study salivary cortisol levels were used to mark the stress levels in children with autism during dental procedures. It is observed that during exposure to an acute stressful event, the hypothalamic-pituitary-adrenal (HPA) axis is activated, in other words HPA axis is the main neuroendocrine component of stress. Cells in hypothalamus produce hormone corticotrophin-releasing factor (CRF) in humans in response to most any type of stress physical or psychological. The hypothalamus secretes CRF, which in turn binds to specific receptors on pituitary cells, which produce adrenocorticotropic hormone (ACTH). ACTH is then transported to its target the adrenal gland stimulates the production of adrenal hormones. The adrenal glands that are located on top of the kidneys then increase the secretion of cortisol. The release of cortisol initiates a series of metabolic effects aimed at alleviating the harmful effects of stress through negative feedback to both the hypothalamus and the anterior pituitary, which decreases the concentration of ACTH and cortisol in the blood once the state of stress subsides. HPA axis regulation involves 3 interrelated mechanisms: the maintenance of a diurnal rhythm, activation in response to stress or threat and the restoration of basal activity via negative feedback mechanisms. One form of dysregulation of the HPA axis is manifested by disruptions in circadian rhythms .[3]

This may have detrimental impacts on health and well-being, such as suppression of bone growth[15]as well as poorer cognitive performance[16].

A major methodological breakthrough for psychological research was achieved when it was shown that salivary cortisol level reliably reflected circulating hormone levels[17,18], which enabled assessment of adrenal activity across the day in a non-invasive manner. Even in the present study cortisol levels were measured in autistic children and healthy children via saliva samples collected at 2 times, that is during the day and evening over a 2-day period.

Salivary cortisol levels typically followed a clear daily rhythm, with peak values seen in the morning and a decline occurring throughout the day until evening in the healthy children.

Whereas the salivary cortisol levels in children with autism was lower during morning and increased considerably during evening. This was in accordance with previous studies done by Vahdettin *et al* [5] and Corbett *et al* [3,4]. This was due to the dysregulation of the HPA axis which manifested in the form of disrupted diurnal variation of cortisol levels.

Also in the present study by rating the behavior of participants during dental treatment we were able to extend the research on the impact of cortisol levels on subjective stress.

It was found that behavioral pattern of the children with autism varied significantly, that is they showed comparatively positive or better behavior during the morning as compared to evening where the behavior worsened.

However behavior and cortisol levels in healthy children could not be correlated. They showed comparatively positive or better behavior during evening as compared to morning. This can be due to various extrinsic factors like sensitization to dental treatment, alleviation of the fear of unknown etc. Further research is required to explain as to why children with autism exhibited a different behavioral pattern, however variable circadian rhythm can be one of the reasons[4]. One of the greatest challenge a pediatric dentist confronts is to provide a comprehensive treatment effectively and efficiently to a child with special health care needs like autism. The present study can help the pediatric dentists in treatment planning and scheduling appointments preferably during morning hours for this group of special In addition, this may also give us an insight into selecting the behavior modification techniques in this group of special children.

But it must be kept in mind that like any other neurodevelopmental disorder autism is also multifactorial in nature, and efficient dental management in this group of differently abled individuals might require multiple behavior modification approaches and further research can be done in this direction. Also in future studies could be directed to explore the variation of cortisol following extensive dental procedures or any stressful situation.

CONCLUSION

- Healthy children followed normal diurnal variation of salivary cortisol, that is higher levels of salivary cortisol in the morning and lower levels of salivary cortisol in the evenings.
- Children with autism showed lower salivary cortisol levels in the morning and higher levels in the evenings.
- Significant difference was seen on comparing the salivary cortisol levels between healthy and children with autism.
- In children with autism as well as healthy children the behavior differed in the morning and evening.
- As cortisol levels increased behavior of children with autism worsened and vice versa.
- No significant correlation was seen between behavior and cortisol levels during the day and in the evening in case of healthy children.
- Significant correlation was seen between behavior and cortisol levels during the day and in the evening in case of children with autism.

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