International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Hormonal and Physiological Salivary Changes in Sample of Children with Autism Spectrum Disorder

Mohanad Ali B. D. S[1], Shatha Q. Jawad BDS, MSc, PhD[2]

[1]MSc student in Oral Physiology, College of Dentistry, Kufa University, Iraq

[2]Assistant Professor Physiology, College of Dentistry, Baghdad University, Iraq

Abstract: ***Autism, which considered as a severe neurological disorder, presents in early child's life. There is severe defect in contact and behavior. It considered as a multi-factorial disorder, which influenced by genetic, environmental and immunological factors, which linked by oxidative stress. Diagnosis of oral manifestations; measurement of stressbiomarker in saliva has to be evaluated and measured in to be used as a diagnostic aid because saliva considered as ultra-filtrate of serum and inexpensive, noninvasive and accessible diagnostic methodology. Aim of the study: To assess any oral manifestations associated with autism and the value of saliva as a diagnostic tool, by measuring some biochemical markers; to provide a greater mechanistic insight into autism spectrum disorder pathology. Subjects, Materials and Methods: Fifty autistic children and thirty, sex and age-matched healthy control, aged between (6-12) years were enrolled in this study. Dental health status and salivalevel of cortisol measured for all participants. Results: Current study revealed that caries prevalence and severity of permanent teeth in autistics (DMFT: mean=1.680) were significantly lower than in healthy children(DMFT: mean=2.367) with P=0.003;while for deciduous teeth, the prevalence and severity of caries (dmft: mean=1.420) were lower than that of healthy children (dmft:mean=2.033,) but the difference was statistically not significant (p = 0.057).There is significant increased production of stress biomarker cortisol(mean=5.043)in autistics than in healthy ones (mean=1.750). Conclusions: The study results revealed that autistic children sample in Iraq was nearly caries-free. Saliva can considered as accompanying diagnostic aid for measurement of stress markers.***

Keywords: Autism, cortisol, saliva

1. Introduction

Autism spectrum disorders (ASDs) are prevalent neurodevelopmental disorders characterized by mental retardation, abnormal emotional, social, linguistic development, poor muscle tone, poor coordination, as well as visual and hearing impairment.Researchers reported that males are four to five times affected more than females, but more severe symptoms are exhibited in females**(1).** The word autism derived from the Greek word autos, which means self **(2).**The number of reported cases of autism increased dramatically, and the most recent reviews tend to estimate a prevalence of 1–2 per 1, 000. The etiology of is not clear and the pathophysiology is not completely understood, there are several evidences for involvement of neurotransmitter system in the pathogenesis, but available data implicate a dysregulation of the catecholaminergic system, and this malfunction might be effected by polymorphisms of several genes, such brain abnormalities might be due to early prenatal insult such as chromosomal abnormalities, intrauterine viral infections, and metabolic disorders suspected to play a role in the pathogenesis of this syndrome **(1).**It is thought to be caused by complex combination of genetic and environmental changes.

Cortisol, steroid hormone produced in adrenal gland, is released in response to stress and low blood _glucose concentration. Although this enhanced responsibility may help prepare the individual to adapt to increased demands and new challenges, it may also mark a time of increased vulnerability in populations already prone to enhanced physiological arousal and poor adaption to change, such as autism.

2. Aims of study

1) Assessment of DMFT index and plaque indexin autistic children and sample of primary school children as control group.
2) Assessment salivary flow rate of autistic children and sample of primary school children
3) Assessment of salivary cortisol in autistic and control groups.

3. Subjects, Materials and Methods

The study was carried out during the period between December 2015 and April 2016 in Najaf city; the samples collected from Al –Hussein center for autism. Approval was obtained from the Ministry of Health for children examining and laboratory work.

3.1 Subjects

The subjects of this study were divided into two groups:

1) Autistic group

Fifty children were enrolled in this study after obtaining the consent form from their families. Al-Hussein Center for autism and slow learning staff was contacted and the purpose of the study was explained to them to assure full cooperation. The names of children, who fulfilled the requirements of the research, were recorded and given to the principle of the center, together with a special consent, form which had been prepared to be given to the parents of each child who was selected as candidate for participation in the research.

Healthy control group: Thirty healthy children were selected from the schools at same area and privates. All subjects should not suffer from any systemic disease or periodontitis after examination, taking history from their families and clinical examination by pediatric specialists.

2) Materials and Equipments :

A: Equipments

1. Centrifuge machine / Hettich universal (D-7200) W. Germany.
2. Disposable test tubes.
3. Elisa reader, Biotek – USA.
4. Elisa washer, Biotek - USA.
5. Glass Pasteur Pipette & tips.
6. Mouth dental mirror.
7. Sickle shaped explorer.
8. Kidney dishes.
9. Disposable cups for saliva collection.
10. Cotton and gauze.

B: Materials

The kits and their companies that were used in this study listed in following table.

Table 1: kits and their companies and origin

Kit	Company supplied	Country
"Salivary Cortisol ELISA kit"	"DIAMETRA S.r.l"	"Italy"

C. Methods

Clinical examination:

The children have been previously examined and diagnosed medically as autistics by the hospital's medical reports; their ages ranged between 6 to 12years.

Extra-Oral Examination

Any scars, trauma in head and neck, hands or fingers.

Intra-Oral Examination

Examination and oral health assessments of hard and soft tissues were performed according to the basic method of the WHO for the year1997. Individuals were examined in a suitable room under good light condition. Sterilization of instrument was done by an autoclave.

3) Dentition status

Clinical examination was conducted using plane mouth mirror and dental explorer. Radiographic examinations were not used due to difficulties. The systematic approach used in examination for dental caries was performed starting from the upper right second molar preceded from one tooth to the adjacent tooth or tooth space passing the midline to the upper left second molar, then going to the lower left second molar passing the midline to the lower right second molar. The examination started with mesial surface, and this was followed by occlusal, distal, buccal and lingual surfaces of all the examined teeth.

An alphabetical coding system was applied for mention crown status of decidous teeth, while anumerical coding was used for permanent teeth (3). If permanent and decidous teeth occupied the same tooth space, just the status of the permanent was considered. The assessments of caries experience were done through the application of Decayed, Missing and Filled teeth Index (DMFT) for permanent teeth and (dmft) index for primary teeth.

4) Plaque index condition

The state of oral hygiene measured by "Silness-Löe", 1964. Both soft and mineralized depositions were recorded on the teeth. A score from 0-3 was given for each of the four surfaces of the teeth (buccal, lingual, mesial and distal). In order to give the plaque index for the tooth, the scores from the four areas of the tooth are added and divided by four

5) Sample collection and preparation

Stimulated salivary samples were collected from the individuals under standardized conditions; (4, 5). Each child was asked to sit down and relax-as much as possible-and asked to chew a piece of gum for one minute before all the saliva was removed; chewing was then continuous for ten minutes with the same piece of gum and the collection of saliva by spitting was done during this time in a small plastic polyethylene tube.

Saliva had been collected at morning, one hour after center morning meal and prior to teeth brushing. Then the saliva allowed flowing down into the glass tube of centrifuge. Then the sample centrifuged for fifteen minutes at three thousands round per minute. The sample then stored at-20°C for one h. Another centrifuge had been done for sample for fifteen minutes at 3000 round per minutes. The sample now is ready for use and stored at 2-8°C for one week until all samples are collected and prepared.

4. Determination of Salivary Cortisol

Procedure

All reagents allowed reaching room temperature (22-28°C). At the end of the assay, the reagents stored immediately at 2-8°C.

1) Two wells were prepared for each point of the calibration curve (C0-C6), two for each Control, two for each sample, one for Blank.
2) A blank well was set without any solution.
3) Twenty five μL 0f calibrator C0-C6 was added.
4) Then 25μL of sample was added into each well.
5) Two hundred μL of the diluted Conjugate was added then.
6) Mixed and Incubated at 37°C for 1 hour.
7) The contents were removed from each well. Washed the wells by automatic washer in six steps with 300μL of diluted wash solution.
8) One hundred μL of TMB Substrate was added and Incubated at room temperature (22-28°C) for 15 minute in the dark.
9) One hundred μL of Stop Solution was added and then the micro plate was shaking gently.
10) The absorbance (E) read at 450 nm against a reference wavelength of 620-630 nm within 5 minutes.

Table 2: Reagents for each well

Reagent	Calibrator	Samples	Blank
Calibrator Co-C6	25μL		
Samples		25μL	
Diluted Conjugate	200 μL	200 μL	

5. Results

1. Clinical Findings
Demographics Parameters

2. Autistic Children (Cases)
Fiftyautistic children included in this study, their age ranged from 6-12 years with mean 8.88.

3. Healthy Group Children (Controls)
Thirty healthy children were included in this study, their age ranged from 6-12 years with mean 8.73.
According to the demographic data, autistics and their controls showed homogeneity and there were no significant difference between the two groups as shown in table (3.1).

Table (3.1): Demographics of the Autistic and Healthy Participants included in the present study

Statistics	Group		Statistics	
	Control	Study	T	P value
N	30	50		
Mean	8.733	8.880		
±SD	1.818	1.913	0.338	0.736(NS)
Range	6.00	6.00		

Extra-Oral Examination, medical and Dental history
According to center' staff reports there was no history of trauma and there was no signs of self injurious habits, but they expressed their anger with peculiar repetitive hand movements and hyperactivity without hurting themselves or the others. Parents' responses to the questionnaire regarding dental visits indicated that only 4 (8 %) child have visited a dental clinic and had history of treatment and follow up while 46(92%) other children did not make any previous dental visits.

Dental Health Status

1. DMFT\dmft:
The caries severity for DMFT in autistic children group (mean=1.680, ±SD=1.019) was lower than that of unaffected group(mean=2.367, ±SD=0.890) with highly significant difference (P-value=0.003)as shown in table(3-2). While for primary dentition, the caries severity of children in the ASD group (mean=1.420, ±SD=1.341) was lower than that in the healthy control group (mean=2.033, ±SD=1.426) but the difference was statistically not significant (p = 0.057) as shown in table (3-3)

Table (3-2): DMFT in autistic and healthy children

	Group	N	Mean	±SD	T	Sig.
DMFT	Control	30	2.367	.890	3.065	0.003 (HS)
	Study	50	1.680	1.019		

Table (3-3): dmft in autistic and healthy children.

	Group	N	Mean	±SD	T	Sig.
dmft	Control	30	2.033	1.426	1.934	0.057 (NS)
	Study	50	1.420	1.341		

P = 0.057 → not significant

2. Plaque index:
In present study there is a highly significant increase in plaque index in autistic group (mean =1.783, ±SD=0.149) than that of healthy control group (mean =1.117, ±SD=0.420) with P value =0.000 as shown in table (3-3).

Table (3-4): Plaque Index in autistic and healthy groups.

Variables	Group	N	Mean	±SD	T	Sig.
PLI	Control	30	1.117	.420	8.369	0.000 (HS)
	Study	50	1.783	.149		

P = 0.000 → highly significant.

3. Salivary flow rate:
Clinical findings of this study showed that there was highly significant decrease in salivary flow rate in autistic group (mean =0.982, ±SD=0.129) than that of healthy group (mean =1.067, ±SD=0.137) with P value = 0.008 as shown in table (3-4).

Table (3-5): Salivary flow rate in autistic and healthy groups.

Variables	Group	N	Mean	±SD	T	Sig.
Flow rate	Control	30	1.067ml/min.	0.137	2.732	0.008 (HS)
	Study	50	0.982 ml/min.	0.129		

P = 0.008 → highly significant.

Assessment of salivary cortisol level:
Laboratory results in present study showed highly significant increase in salivary cortisol in autistic group (mean=5.043 ng/mL, ±SD=5.392) than that of healthy control group(mean=1.750 ng/mL, ±SD=1.747) with P value =0.000 as shown in table(3-5).

Table (3-6): salivary cortisol level in autistic and control groups

Group	N	Mean	±SD	T	Sig.
Control	30	1.750 ng/ml	1.747	3.984	0.000 (HS)
Study	50	5.043 ng/ml	5.392		

P=0.000 → Highly significant

6. Discussion

DMFT/dmft
The dental history of children with autism reflects incapability of cooperation in the dentalsetting owing to their impaired social interaction and communicationskills. In addition to cognitive dysfunction, aggression and other associated psychiatric symptoms also may impede theprovision of dental care. This could explain the results of the present study in which only four children (8%) had a history of previous dental treatment, meanwhile the rest 46 children (92%) had never received dental treatment and follow up. This result was in good agreement with Lowe & Lindemann, (2000), (6); Klein & Nowak, (1999), (7); Pilebro & Backman, (2005), (8); Barbaresi *et al*., (2006), (9); Friedlander *et al*., (2006), (10); Marshall *et al*., (2007), (11).

DMFT/dmft

In the current study, DMFT/dmft showed that caries prevalence and severity in autistics were lower than that in unaffected children. This could be due to good home care by the autistics' parentsor caregivers and a less cariogenic diet. Also autistic children have ritualistic behaviorwhich characterized by unvarying pattern of daily activities, such as an unchanging menu (Lam &Aman, 2007), (12), so they are moreregular in their behavior at meals than are unaffected children. Therefore, a lower frequency of snacking between meals and lowerintake of carbohydrates could have contributed to the lowercaries rate observed.

This result was in good agreement with the results of previous studies of, Cheen*et al*., (2008), (13). Also Morinushi *et al*., (2001), (14) observed lower carries prevalence and severity in Japanese children with autism compared with those of unaffected children.

Plaque Index:

Regarding to oral hygiene status, this study showed a higher plaque index in autistic children than that of healthy ones. This result was in good agreement with previous study by Al-Omar *et al*., (2005), (15) in Kuwait which reported that all autistic children in their study had visible plaque on their maxillary incisors and canines and also the mandibular incisors with signs of gingival inflammation.

Also; Hafez M Diab *et al*., (2016)**, (16),** who evaluated PI among autistic children compared to normal children in Riyadh City, showed that children with autism appear to have higher gingival inflammation, higher plaque index and poor oral hygiene as compared to healthy control group.

Salivary flow rate:

The present study revealed that there is insignificant difference in salivary flow rate of autistics and that of healthy children. This result was in good agreement with previous study by Ivy *et al* (2009), (17), which showed that there is no significant statistical difference in salivary flow rate in autistic and healthy children. But these findings were not in good agreement with the results of previous study by Susanne Bejerot and Göran Dahllöf (2015), (18), which showed that the stimulated saliva secretion was significantly lower in the autism group than that of healthy group.

Salivary cortisol

This study measured the salivary cortisol level in fifty autistic children and compared it with that of thirty healthy ones, and it revealed that there is significant difference in salivary cortisol level with a higher level in autistics.

This result was in good agreement with previous study by Abdulla AM (2015), (19), which showed that there is a significant increase of salivary cortisol level in autistic children. Also Eve G. *et al*., (2012), (20), showed enhanced cortisol response to stress in children with autism.

References

[1] Geier, D. A., Kern, J. K., Garver, C. R., Adams, J. B., Audhya, T., & Geier, M. R. (2009): A prospective study of transsulfuration biomarkers in autistic disorders. Neurochemical Research, 34, 386-393.
[2] Kuhn R; tr. Cahn CH. (2004): Eugen Bleuler's concepts of psychopathology. Hist Psychiatry. 15(3):361–6.
[3] WHO (1997): oral health surveys basic methods 4th ed. World Health Organization. Geneva, Switzerland.
[4] Tenovuo J, Lagerlf F. (1996): Saliva In: Thylstrup A A. and Fejerskove. O. edt. Textbook of Clinical Cariology.2ndMunksgaard.Copenhagen, 17-43.
[5] Farsi NMA. (2007): Signs of oral dryness in relation to salivary flow rate, Ph, buffering capacity and dry mouth complaints. BMC Oral Health, 7:15.
[6] Lowe Q, Lindemann R. (2000): Assessment of the autistic patient's dental needs and ability to undergo dental examination. ASDC J Dent Child 1985; 3:29-35.
[7] Nowak, (1999).
[8] Pilebro C, Backman B. (2005): Teaching oral hygiene to children with autism. Int J Paediatr Dent; 15 (1):1–9.
[9] Barbaresi WJ, Katusic SK, Voigt RG. (2006): Autism: a review of the state of the science for pediatric primary health care clinicians. Arch Pediatr Adolesc Med; 160(11):1167–1175.
[10] Friedlander AH, Yagiela JA, Paterno VI, Mahler ME. (2006): The neuropathology, medical management and dental implications of autism. JADA 137(11):1517–1527.
[11] Marshall J, Sheller B, Williams BJ, Mancl L, Cowan C. (2007): Cooperation predictors for dental patients with autism (published correction appears in Pediatr Dent 2008;30[2]:97). Pediatr Dent. 29(5): 369–376.
[12] Lam KSL, Aman MG. (2007): The Repetitive Behavior Scale-Revised: independent validation in individualswith autism spectrum disorders. J Autism Dev Disord. 37(5):855–66.
[13] Cheen Y. Loo, Richard M. Graham, and Christopher V. Hughes. (2008): The Caries Experience and Behavior of Dental Patients With Autism Spectrum Disorder. J Am Dent Assoc, Vol 139, No 11, 1518-1524.
[14] Morinushi *et al*., (2001).
[15] Al-Omar H, Ebrahim F, Joseph B, and Al-Saad S, (2005): Dental Care of Autistic Children in Kuwait Autism Centre; The Preliminary Program for First African and Middle-East IADR Federation Conference ;27-29.
[16] Hafez MD, Suha SM, Amal A, Ashwaq S, Nadia S, Shabnam G, Altaf H (2016). Journal of Clinical and Diagnostic Research, volume 10.
[17] Ivy Haralambos Bassoukou, José Nicolau and Maria Teresa dos Santos (2009): Saliva flow rate, buffer capacity, and pH of autistic individuals. Journal of Investigations; March, Volume 13, Number 1.
[18] Susanne Bejerot and Göran Dahll öf (2015).
[19] Abdulla AM(2015). J Clin Pediatr Dent. 2015 Winter;39(2):128-32.
[20] Eve G., Joyce S., Kathleen T., Laura A., Charles R., Kirk A., Richard W., and Jane M. (2012). J Autism DevDisord. 2012 January; 42(1): 75–81. doi:10.1007/s10803-011-1214-0.