Salivary Physicochemical Characteristics in Relation to Oral Health Status Among Institutionalized Autistic Adolescents in Baghdad/Iraq

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ABSTRACT

Background: Autism spectrum disorder (ASD) is characterized by impairments in social interaction and communication, restricted patterns of behavior, and unusual sensory sensitivities. Saliva may provide an easily accessible sample for analysis. Some salivary constituents levels altered in adolescents with ASD including antioxidants . This study aimed to investigate salivary physicochemical characteristic in relation to oral health status among adolescents with ASD.

Materials and methods: Two groups were included in this study: 40 institutionalized autistic adolescents and 40 apparently healthy school adolescents control group with age range (12-15 years old, only males) selected randomly from Baghdad. Each group subdivided into two groups according to the severity of dental caries: caries free group (20 child, DMFT=0) and high caries group (20 child, DMFT≥6). Decayed, missing and filled surfaces (DMFS), plaque (PII), Gingival (GI) and calculus (CI) indices were used to measure oral health status for both groups. Copper (Cu), zinc (Zn) and thiocyanate (SCN) in saliva measured by atomic absorption spectrophotometer. Salivary alpha amylase (sAA) and glutathione (GSH) assessed by enzyme-linked immunosorbent assay (ELISA). Salivary pH and flow rate were measured directly. The data of current study was analyzed using SPSS version 21.

Results: A higher value of salivary pH, flow rate, sAA, SCN, Cu and Zn were found among study group than control group with significant difference, also higher in caries free subgroup than high caries subgroup. While GSH was significantly higher in control group than study group. Moderate negative correlations between sAA, Cu, Zn and PII, CI, GI with highly significant and salivary pH correlate moderately with PII and CI with highly significant.

Conclusion: There was alteration in salivary constituents levels which related to oral health status in adolescents with ASD and can act as adjunctive diagnostic aid for diagnosing autism.

Keywords: Autism spectrum disorder; sAA; SCN; GSH. **(J Bagh Coll Dentistry 2017; 29(3):68-74)**

INTRODUCTION

Autism spectrum disorders (ASD) is a neurodevelopmental disorder in which social interaction, language, behavior and cognitive functions are impaired severely [1]. It is characterized by a complex, behaviorally defined, static immature brain disorder that is of great concern to various professions [2]. Individuals with ASD may have less learning abilities than healthy individuals. This may affect their oral hygiene [3]. Various studies in the past have shown a higher prevalence of periodontal disease and a lower caries in autistics compared to unaffected children [2,4,5].

Oral fluid or whole saliva is an important complex chemical milieu of teeth and oral soft tissues, which maintains the homeostasis of the oral cavity [6]. The most abundant protein in saliva is alpha amylase (sAA), accounting for approximately 20% of salivary proteins.
Thiocyanate (SCN) is an important physiological anion involved in innate defense of mucosal surfaces.

There are indispensable parts of host defense system that act as a substrate for lactoperoxidase that oxidizes airway surface lipid thiocyanate thereby generating antimicrobial agent hypothiocyanate [7]. Copper (Cu) ions have been reported to have an antibacterial effect. It was suggested that there is an altered regulation of ceruloplasmin in a subset of children with autism. Such alterations may lead to abnormal copper metabolism that may play a pathological role in autism [8]. Zinc (Zn) can reduce dental calculus formation, control plaque and reduce the solubility of enamel. In fact, some preliminary studies suggested altered serum Cu/Zn ratios in autism [8,9]. The antioxidant defense system of saliva has several components. One of these antioxidants is glutathione (GSH), salivary GSH levels decrease in periodontal diseases and dental caries [10]. Individuals with ASD showed elevated levels protein, SCN, and sAA when compared to normal children. Antioxidant enzymes, cholesterol and GSH levels were found to be decreased in ASD than in normal children [7].

As far as, no previous study was conducted among institutionalized autistic adolescents in Baghdad regarding the relation between physicochemical characteristic of saliva, dental

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caries and gingival health. For all these explanation, this study was designed.

MATERIALS AND METHODS

The study included two male groups: 40 institutionalized autistic children and 40 apparently healthy schoolchildren with age range (12-15 years old, only males). The sample selected according to the severity of dental caries. Each group subdivided into two groups: caries free group (20 child, DMFT=0) and high caries group (20 child, DMFT≥6).

For all children intra-oral assessment of caries experience was measured according to criteria of WHO (1997) [11], Oral hygiene status was evaluated by application plaque index (PlI) of Silness and Löe [12], and calculus index (CI) of Ramfjord [13]. Gingival inflammation assessed by using Gingival Index (GI) of Löe and Silness [14]. For salivary samples each child was asked to sit down and relaxes as much as possible and was asked to chew a piece of Arabic gum for one minute before all the saliva was removed by expectoration; chewing was then continued for ten minutes with the same piece of gum and the collection of saliva by spitting was done during this time [15]. The collected saliva was centrifuged at 1000 rpm for 10 minutes; this done after 1 hour after collection to eliminate debris and cellular matter. The centrifuged supernatants stored frozen at (-20°C) in polyethylene tube until assayed. Salivary flow rate assessed by dividing the total volume of saliva collected in milliliter over the time of collection in minute. pH of saliva was measured by using pH meter.

Copper and zinc in saliva were measured by atomic absorption spectrophotometer using commercial kits (Spectrum, Germany). Salivary thiocyanate assessed according to the method described by Slowinski et al. using spectrophotometer [16]. Salivary alpha amylase and glutathione measured by enzyme-linked immunosorbent assay (ELISA) using special commercial kits (Cloud-Clone Corp., USA). The mean values with SD were measured for all tested salivary variables. The statistical significance, direction and strength of linear correlation between two quantitative normally distributed variables, by Pearson's rank linear correlation coefficient. P value less than the 0.05 level of significance was considered statistically significant. A receiver operating characteristic (ROC), or simply ROC curve, is a graphical plot which illustrates the performance of a binary classifier system as its discrimination threshold is varied. All analyzed tests were bilateral.

RESULTS

Table 1 shows mean values of pH and flow rate (ml/min) in stimulated saliva among study and control groups by caries severity. A higher value of salivary pH and flow rate were found among study compared to the control group, which was statistically high significant with pH ($P<0.001$) and flow rate ($P=0.003$). A higher value of salivary pH and flow rate were found among caries free subgroup than high caries subgroup, which was statistically high significant with pH ($P<0.01$) and not significant difference with flow rate ($P>0.05$).

The correlation coefficients of salivary pH and flow rate with PlI, GI and CI among study and control groups are illustrated in table 2. In study group, data of present study shows that a moderate negative correlations between salivary pH and PlI, CI. In control group, a strong negative correlation between salivary pH and PlI, GI, CI. A moderate negative correlation between salivary flow rate and PlI, CI among control group.

The mean values of all salivary constituents were found higher in caries free than high caries subgroup among study group with high significant differences for sAA, Cu and Zn. As shown in Table 3.

Table 4 demonstrates the mean concentration of salivary constituents in (µg/dl) among study and control groups with statistical differences. A higher mean value of salivary sAA, SCN, Cu, Zn, and Cu/Zn were found among study compared to the control group, with high statistical significant difference for sAA, SCN and Cu, while significant difference with Zn. Mean value of GSH was lower in study group than control group with high significant difference.

Figure 1 and Table 5 demonstrate the areas under ROC curve for sAA (0.838), Cu (0.813) and Zn (0.763) were significantly from 0.5 value of an equivocal test ($P<0.001$ for sAA, Cu and $P= 0.005$ for Zn), whereas the areas under ROC curve for Cu/Zn (0.621), SCN (0.616) and GSH (0.501) were not significantly different from 0.5 value of an equivocal test.

Figure 2 and Table 6 show the areas under ROC curve for salivary GSH (0.858), SCN (0.821), sAA (0.786), Cu (0.652) and Zn (0.648) were significantly from 0.5 value of an equivocal test ($P<0.001$ for GSH, SCN, sAA; $P= 0.019$ for Cu; $P= 0.023$ for Zn), whereas the areas under ROC curve for Cu/Zn (0.538) was not significantly different from 0.5 value of an equivocal test.

Table 7 demonstrates the correlation coefficients of salivary constituents with PlI, GI and CI among study and control groups. In study group, a moderate negative correlations between sAA, Cu, Zn and PlI, GI, CI. A very weak negative correlation between GSH, SCN, Cu/Zn and PlI, GI, CI. In control group, a moderate negative correlation between Zn and PlI as well as CI, while a very weak negative correlation was seen regarding sAA, GSH, SCN. A weak negative correlation between Cu and PlI, CI.

DISSCUSION

Autism spectrum disorder is a neurodevelopment disorder characterized by impaired social interaction and behavior, which can have adverse influence on dental care and oral health in the affected individuals. Studies on oral health in individuals with ASD have conflicting results. In a population-based sample of autistic children and adolescents, parents reported poorer condition of their children's teeth and gingiva compared to other children [17,18,19].

This study recorded an increasing in the flow rate and pH of stimulated saliva in the study group which was statistically significant compared to control group. Individuals with ASD report more stress in everyday life and they also report a higher level of dental anxiety, both factors that may negatively affect salivary secretion. These results disagreement with other studies [5,18,20].

Alpha-amylase is a calcium-dependent metalloenzyme which can hydrolyze starch to glucose and maltose; consequently, having an important role in binding to bacteria [21]. In the present study, sAA was higher in study group compared to control group with highly significant difference. In addition, sAA was higher in caries free subgroup than high caries subgroup with highly significant difference.

sAA activity is an increasingly investigated biomarker for the activation of the autonomic nervous system. Hence, the high activity of α-amylase in autistic children shows that there is hyperstimulation of autonomic nerves, which may be associated with hyperactive nature of autistic children. This result in line with other studies [7,22]. Glutathione is a family of multifunctional enzymes that plays an important role in the detoxification of xenobiotics including carcinogens. In addition, GSH plays a critical role in cellular protection against oxidative stress and toxic foreign chemicals. The level of salivary GSH was lower in ASD group compared to control group with highly significant differences. In addition, there is a slight increase of GSH level in high caries subgroup in relative to caries free subgroup among ASD group with non-statistical differences, and this might be due to lack of the substrate GSH and hence may be accounted for the reduced detoxifying capacity of GSH in autistic children. The activity of the enzyme may possibly be affected, if there is any genetic polymorphism in GST [23]. This result was supported by other studies [24,25,26].

Thiocyanate is an important physiological anion involved in innate defense of mucosal surfaces. It is a metabolite of cyanide and the product of detoxification of compounds containing cyanide through a reaction catalyzed by the enzyme rhodanese [27]. In the current study, the concentration of salivary SCN was higher in study group than control group with highly significant differences. In addition, the level of salivary SCN was slightly higher in caries free subgroup than high caries subgroup among ASD group, but with non-significant differences. The elevated SCN level in ASD group may be attributed to low rhodanese activity as supported by Waring and Klovrza [28], who stated that the raised ratio of thiosulfate and SCN might be due to reduced rhodanese activity.

Scientists interested the relationship between salivary trace elements (Zn and Cu) and dental caries activity for many years. Conflicting reports from researchers who have investigated Cu and Zn in saliva indicate that the nature of the role that these elements play in the carious process remains undefined [29]. Elevated level of Cu and depressed Zn have been associated with ASD, attention deficit disorders and depression [30]. In this study, the concentration of Cu and Zn were higher in study group than control group with significant differences, in addition, the level of Cu and Zn were higher in caries free subgroup than high caries subgroup among study group with highly significant differences. This may be attributed to the antimicrobial properties of Cu and Zn, which include acute loss of bacterial intracellular K^+ and inhibition of H⁻ATP synthase, inhibition of various bacterial metabolic enzymes through oxidation of key thiol groups, and formation of insoluble salts on the tooth surface, thereby increasing its acid resistance [31].

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Table 1: Mean values of salivary pH and flow rate among study and control groups by caries severity with statistical difference.

Salivary parameters	Subgroups	Study	Control	Statistical difference	
		(Mean ± SD)	(Mean ± SD)	t-value	P-value
pH	Caries Free	7.71 ± 0.36	7.29 ± 0.36	3.718	< 0.001**
	High Caries	7.19 ± 0.44	6.51 ± 0.46	4.757	< 0.001**
	Total	7.45 ± 0.48	6.90 ± 0.57	4.689	< 0.001**
Flow Rate (ml/min)	Caries Free	1.75 ± 0.21	1.61 ± 0.22	2.062	0.046*
	High Caries	1.65 ± 0.29	1.41 ± 0.34	2.476	0.018*
	Total	1.70 ± 0.25	1.51 ± 0.30	3.103	0.003**

*** Significant, ** Highly Significant**

Table 2: Correlation coefficients of salivary pH and flow rate with plaque, gingival and calculus indices among study and control groups.

Groups	Salivary parameters	PII		GI		CI	
		r	P	r	P	r	P
Study	pH	-0.491	< 0.001**	-0.281	0.079	-0.421	0.007**
	Flow Rate	-0.182	0.262	-0.277	0.084	-0.219	0.175
Control	pH	-0.675	< 0.001**	-0.527	< 0.001**	-0.556	< 0.001**
	Flow Rate	-0.321	0.043*	-0.246	0.126	-0.337	0.033*

*** Significant, ** Highly Significant**

Table 3: Mean values of salivary constituents (µg/dl) among study group by caries experience with statistical differences.

Salivary Variable	Caries free		High caries		t-value	P-value
	Mean	SD	Mean	SD		
α-Amylase	10.39	0.80	9.24	0.87	4.311	< 0.001**
Glutathione	0.91	0.61	0.87	0.56	0.197	0.845
Thiocyanate	105.39	13.25	100.02	10.75	1.408	0.167
Copper	37.92	11.57	25.24	8.03	4.026	<0.001**
Zinc	82.18	14.40	65.15	16.75	3.448	<0.001**
Cu/Zn	0.48	0.19	0.40	0.14	1.428	0.162

**** Highly Significant**

Table 4: Mean values of salivary constituents (µg/dl) among study and control subgroups with statistical differences.

Groups	Study	Control	Statistical difference	
	(Mean ± SD)	(Mean ± SD)	t-value	P-value
α-Amylase	9.81 1.01	8.63 1.07	5.113	<0.001**
Glutathione	0.89 ± 0.58	2.20 ± 1.10	6.626	<0.001**
Thiocyanate	102.70 ± 12.21	87.21 ± 11.14	5.929	<0.001**
Copper	31.58 ± 11.74	25.21 ± 9.15	2.708	0.008**
Zinc	73.66 ± 17.67	64.05 ± 16.64	2.505	0.014*
Cu/Zn	0.44 ± 0.17	0.41 ± 0.16	0.729	0.468

*** Significant, ** Highly Significant**

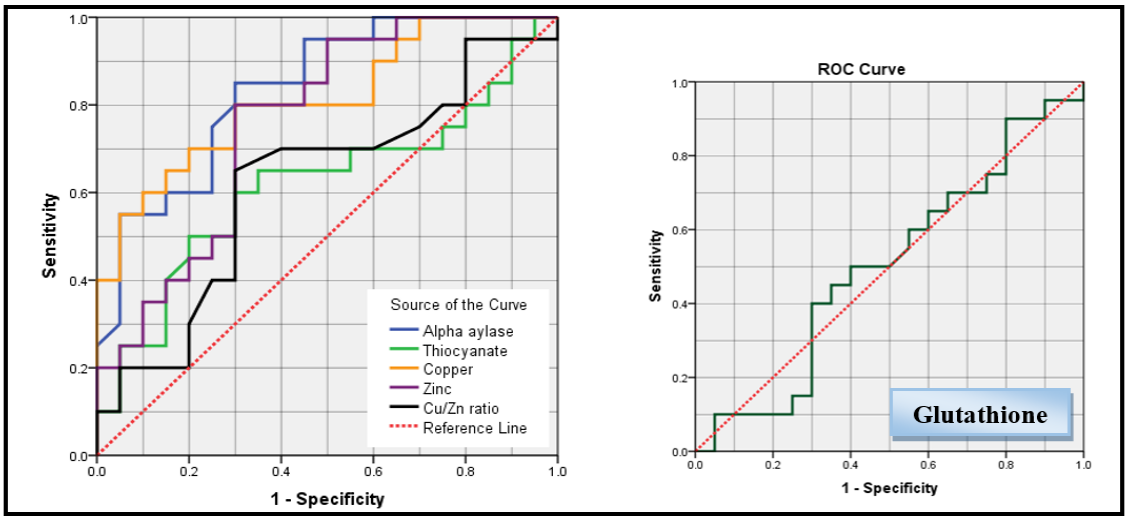

Figure 1: ROC curve for selected quantitative measurements when used to differentiate caries free subgroup from high caries subgroup among study group.

Table 5: Area under ROC curve selected quantitative measurements when used to differentiate caries free subgroup from high caries subgroup among study group.

Tested variable	Area under the curve	P-value
Alpha amylase	0.838	< 0.001**
Copper	0.813	< 0.001**
Zinc	0.763	0.005**
Cu/Zn ratio	0.621	0.190
Thiocyanate	0.616	0.199
Glutathione	0.501	0.989

**** Highly Significant**

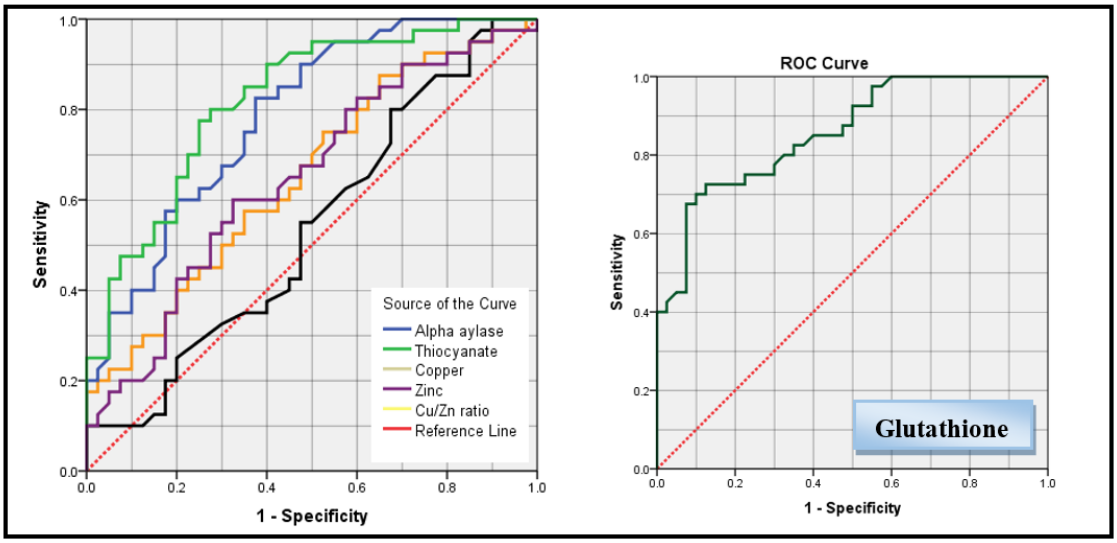

Figure 2: ROC curve for selected quantitative measurements when used to differentiate study group from control group.

Table 6: Area under ROC curve selected quantitative measurements when used to differentiate study group from control group.

Tested variable	Area under the curve	P-value
Glutathione	0.858	< 0.001**
Thiocyanate	0.821	< 0.001**
Alpha amylase	0.786	< 0.001**
Copper	0.652	0.019*
Zinc	0.648	0.023*
Cu/Zn ratio	0.538	0.564

*** Significant, ** Highly Significant**

Table 7: Correlation coefficient between salivary constituents and plaque, gingival, calculus indices among study and control groups.

Groups	Salivary constituents	PlI		GI		CI	
		r	P	r	P	r	P
Study	Amylase	-0.537	< 0.001**	-0.460	0.003**	-0.491	< 0.001**
	Glutathione	-0.016	0.924	-0.028	0.866	-0.023	0.890
	Thiocyanate	-0.043	0.790	-0.101	0.534	-0.225	0.162
	Copper	-0.577	< 0.001**	-0.493	< 0.001**	-0.539	< 0.001**
	Zinc	-0.526	< 0.001**	-0.546	< 0.001**	-0.560	< 0.001**
	Cu/Zn	-0.278	0.082	-0.209	0.195	-0.181	0.263
Control	Amylase	-0.089	0.587	-0.078	0.631	-0.245	0.127
	Glutathione	-0.097	0.553	-0.094	0.564	-0.162	0.317
	Thiocyanate	-0.120	0.460	-0.194	0.230	-0.097	0.551
	Copper	-0.221	0.170	-0.089	0.583	-0.263	0.101
	Zinc	-0.415	0.008**	-0.163	0.315	-0.500	< 0.001**
	Cu/Zn	0.069	0.670	-0.025	0.877	0.118	0.467

**** Highly Significant**

الخلاصة

المقدمة: يتميز اضطراب طيف التوحد (ASD) بضعف في التفاعل الاجتماعي والتواصل، أنماط محدودة من السلوك وتحسس حسي غير عادي. امراض الفم تكون غير محددة للغاية لدى الأفراد الذين يعانون من اضطراب التوحد الا انها تشكل تحد كبير لأطباء الاسنان وذلك بسبب المظاهر السريرية المعقدة والمتنوعة الخاصة بهم. تهدف الدراسة لبحث الخواص الفيزيائية-الكيميائية للعاب فيما يتعلق بصحة الفم للمراهقين الذين يعانون من اضطراب التوحد في بغداد.

المواد والطرق: تضمنت الدراسة مجموعتين: مجموعة الدراسة وتتكون من 40 مراهق يعانون من اضطراب التوحد تم اختيارهم من معاهد رعاية التوحد ومجموعة السيطرة وتتكون من 40 مراهق سليم من المدارس الثانوية القريبة لمعاهد رعاية التوحد وبعمر (12-15) سنة ومن الذكور فقط. كل مجموعة قسمت الى مجموعتين وفقا لشدة التسوس: مجموعة خالية التسوس (DMFT=0) ومجوعة عالية التسوس (DMFT≥6). استخدام مقاييس تسوس الاسنان (DMFS)، اللويحة الجرثومية (PlI)، التهاب اللثة (GI) والقلح (CI) لتقييم صحة الفم والاسنان للمجموعتين. كلا من النحاس (Cu)، الزنك (Zn) والثايوسيانيت (SCN) تم قياسهم باستخدام جهاز معامل الامتصاص الذري (Spectrophotometer). بينما الفا اميليز والجلوتاثيون تم قياسهم باستخدام تقنية قياس الانزيم المناعي المرتبط (ELISA). تم تحليل بيانات الدراسة الحالية باستخدام برنامج SPSS النسخة 21.

النتائج: أظهرت الدراسة ان قيم كلا من الاس الهيدروجيني للعاب، سرعة تدفق اللعاب، الفا اميليز، ثايوسيانيت، النحاس والزنك في مجموعة الدراسة اعلى من مجموعة السيطرة وبفارق معنوي، كذلك هذه القيم في المجموعة خالية التسوس اعلى من المجموعة عالية التسوس وبفارق معنوي. بينما مستوى الجلوتاثيون في مجموعة الدراسة كان اقل من مجموعة السيطرة. وجود علاقة ارتباط متوسطة عكسية بين الفا اميليز، النحاس والزنك مع مقياس اللويحة الجرثومية (PlI)، مقياس القلح (CI) ومقياس صحة اللثة (GI) وبفارق معنوي عالي، وكذلك الاس الهيدروجيني للعاب يرتبط بعلاقة متوسطة بمقياسي اللويحة الجرثومية (PlI) والقلح (CI) وبفارق معنوي عالي.

الاستنتاج: ان قيم كلا من الاس الهيدروجيني للعاب، سرعة تدفق اللعاب، الفا اميليز، ثايوسيانيت، النحاس والزنك في مجموعة اضطراب التوحد اعلى من مجموعة السيطرة، وجميع هذه القيم في المجموعة خالية التسوس اعلى من المجموعة عالية التسوس. بينما الجلوتاثيون كان اقل في مجموعة اضطراب التوحد مقارنة بمجموعة السيطرة.