Salivary vitamins and total proteins, in relation to caries-experience and gingival health, according to nutritional status of a group of five-year old children

Nada J. Radhi B.D.S., M.Sc., Ph.D. [(1)]

ABSTRACT

Background: Malnutrition influences the development of the teeth and the formation, function and secretion of the salivary glands, which in turn influence susceptibility to dental caries and gingival disease. The aims of this study were to assess the salivary antioxidants (vitamin A, C and E) levels as well as total protein and their relation to caries severity and gingival health status among mal- and well-nourished children.

Materials and Methods: The sample consisted of 60 children and they divided according to nutritional status (30 malnourished and 30 well nourished). The 2000 Centers for Disease Control and Prevention (CDC) growth charts was used for assessment of nutritional status (height for age). The age was five years old. Caries severity ($d_{1-4}s$) was assessed according to Muhlemann (1976). Dental plaque recorded following the criteria described by Sillness and Löe (1964). The gingival index (GI) was used according to Löe and Sillness criteria (1963). Stimulated whole saliva samples were collected and chemically analyzed by using colorimetric method to determine the salivary antioxidants (vitamin A, C and E) and total protein. All data were analyzed using SPSS version 18.

Results: Results recorded a higher mean value of dmfs among malnourished in comparison to well nourished with statistically highly significant difference ($P< 0.001$). According to grades of lesion severity, d_4 was significantly the higher among malnourished children ($P< 0.001$). Strong highly significant correlations were noticed between ds, dmfs and PI among malnourished and well nourished children. Significantly lower values of vitamins and total protein were noticed among malnourished children compared to well nourished ($P< 0.001$). Negative highly significant correlations were found with all vitamins among malnourished children regarding caries-experience and GI.

Conclusion: Childhood chronic malnutrition (stunting) is associated with salivary hypofunction. This may act as a risk factor for dental caries and gingival disease in the target group.

Key Words: vitamin A, C, E, total protein, dental caries, gingival disease. (J Bagh Coll Dentistry 2012;24(3):129-136).

INTRODUCTION

Anthropometry can be used to assess nutritional status at both the individual level and the population level and assist to classify person as mal or well nourished in relation to specific level of indicator [(1)]. Height for age indicator provides an excellent index of long term cumulative inadequacies of nutrition (chronic under nutrition: shortness or stunting), which is frequently associated with poor overall economic conditions and/or repeated exposure to adverse conditions [(2)]. Deficiencies of specific nutrients do influence the development of the teeth and the formation, function and secretion of the salivary glands, which in turn influence susceptibility to dental caries and gingival disease [(3)].

The index age (5 years) is considered as a critical human life stage which has recorded the past and present history of malnutrition and oral health conditions [(4, 5)]. Several studies reported that malnourished children were more susceptible to be affected by dental caries and gingival disease [(6-12)].

Saliva is important for the health of oral soft and hard tissues. The complexity of physical and chemical composition of salivary secretions performs a considerable number of protective functions through buffer capacity, minerals, total protein and antioxidants (proteins and vitamins) [(13-15)]. Salivary antioxidants were found to reduce the susceptibility to dental caries and periodontal disease [(16-19)]. As far as it is known, no Iraqi study was found to determine the relation between salivary vitamins, total proteins and dental caries as well as gingival disease among a group of mal and well nourished children aged 5 years old. Therefore, this study was conducted.

SUBJECTS AND METHODS

The sample consisted of 60 children with age 5 years old from kindergartens in Baghdad. The selected sample divided according to nutritional status (30 malnourished and 30 well nourished children). All childs had deciduous teeth, no permanent teeth erupted. Carious tooth was diagnosed according to criteria suggested by Muhlemann, this allowed recording decayed lesion of primary teeth by severity (d_{1-4}mfs) [(20)]. Plane mouth mirror and sickle-shaped dental explorer were used. Dental plaque (PI) recorded [(21)] following the criteria described by Sillness and Löe. For the assessment of gingival health

(1) Lecturer, Department of Pediatric and Preventive dentistry, College of Dentistry, University of Baghdad.

condition, the gingival index (GI) of Löe and Sillness used [22], the whole teeth were examined and four surfaces of each tooth were scored. Nutritional status was assessed by the indicator: height for age (HA) [23] by using height measuring board concerning the date of birth. The value of nutritional indicators was compared with the international reference values that defined by the 2000 Centers for Disease Control and Prevention (CDC) growth charts [24]. The cut-off point (HA) to define malnutrition was -2 SD [25].

Each child was asked to chew a piece of Arabic gum (0.5-0.7 gm) until 5 ml of whole stimulated saliva was collected in a sterile capped bottle using a standardized method [26]. Salivary vitamin E (mg/dl) was determined by using a colorimetric method [27]. This method depends on Emmerie-Engel reaction (oxidation-reduction reaction). Level of vitamin C (mg/dl) in saliva was determined photometrically with 2, 4-dinitrophenyl hydrazine (DNPH) to form red bis-hydrazone [28]. The optical density differences between irradiated and non-irradiated saliva extracts can therefore be used to estimate vitamin A concentration in saliva [29]. Total protein (mg/dl) determined by colorimetric method. A ready kit was used by Labkit, Nau J. Protein react in acid solution with pirogallol red and molybdate to form a colored complex. The intensity of the color formed is proportional to the protein concentration in the sample [30].

SPSS version 18 (Statistical Package for Social Sciences) was used for statistical analyses. The variables were described by mean and SD and the parametric statistical tests of significance were used. The independent samples t-test was used to test the statistical significance of difference in mean between groups of study. The linear correlation between two quantitative variables is measured by Spearman's rank linear correlation coefficient, while multiple linear regressions were used to assess independent effect of explanatory variables on dependent quantitative variable. The confidence limit was accepted at 95%.

RESULTS

The relative frequency of caries free children among well nourished and malnourished groups is seen in Table (1). A higher percentage of caries-free children were recorded among well nourished compared to malnourished children.

Table (2) represents the caries-experience (dmfs) of primary teeth among malnourished and well nourished children. A higher mean value of decayed surface (ds) was noticed among malnourished children compared to well nourished with statistically highly significant difference (t= 5.497, df= 58, P< 0.001). A statistically significant difference was recorded between two groups regarding missing surface (ms), as a higher value was noticed among malnourished group (t= 2.175, df= 58, P< 0.05). A higher mean value of filling surface (fs) was recorded among malnourished with no statistical significant difference (P> 0.05). A higher mean value of dmfs was reported among malnourished in comparison to well nourished children with statistically highly significant difference (t= 6.349, df= 58, P< 0.001).

Table (3) illustrates the grades of caries severity for primary teeth among study groups. A higher mean values of d_1, d_2, d_3 and d_4 were recorded among malnourished children in comparison to well nourished, a statistically significant and highly significant differences were noticed regarding d_2, d_3 and d_4 (d_2: t= 2.336, df= 58, P< 0.05; d_3: t= 2.473, df= 58, P< 0.05; d_4: t= 4.104, df= 58, P< 0.001).

Table (4) demonstrates the mean values of plaque and gingival indices among malnourished and well nourished children. A higher mean values of plaque and gingival indices were recorded among malnourished children in comparison to well nourished, differences were statistically highly significant (PI: t= 7.340, df= 58, P< 0.001; GI: t= 7.826, df= 58, P< 0.001).

The correlation coefficient between caries – experience of primary teeth and dental plaque in both groups is seen in Table (5). Positive strong highly significant correlations were noticed between ds, dmfs and PI among malnourished and well nourished children. A positive strong highly significant correlation was recorded between plaque and gingival indices in both groups (r = 0.99, P< 0.001; r = 0.97, P< 0.001) respectively.

The mean values of salivary vitamins and total protein among malnourished and well nourished children are seen in Table (6). A lower mean values of salivary vitamins (A, C and E) and total protein were recorded among malnourished children in comparison to well nourished children with statistically highly significant differences (vitamin A: t= 11.658, df= 58, P< 0.001; vitamin C: t= 5.197, df= 58, P< 0.001; vitamin E: t= 21.460, df= 58, P< 0.001; total protein: t= 14.391, df= 58, P< 0.001).

Table (7) illustrates the correlation coefficient between caries-experience (ds, dmfs) and salivary vitamins and total protein in both groups. Results revealed strong negative highly significant correlations between salivary

vitamins and caries-experience in malnourished children. Regarding total protein, a positive weak significant correlation was noticed with ds, while it was not significant with dmfs. In well nourished children the direction of correlation was varied between negative and positive with higher significance regarding ds, while it was non significant correlation with dmfs.

Table (8) illustrates the correlation coefficient between grades of caries severity and salivary vitamins and total protein among both groups. In malnourished children a positive highly significant correlations were recorded between d_1 and vitamin A, d_4 and total protein. While negative highly significant correlations were noticed for vitamins A, C, E with d_4. Among well nourished children a positive highly significant correlations were recorded between d_3, d_4 and vitamin A, C while negative correlation with vitamin E and total protein.

Table (9) demonstrates the correlation between PI, GI and salivary vitamins and total protein among both groups. In mal nourished children a positive highly significant correlations were seen between PI, GI and total protein, while negative highly significant correlations were found with all vitamins. In well nourished children a positive highly significant correlations were seen between GI and vitamins A and C, while negative highly significant correlations were found with vitamin E and total protein.

Table (10) demonstrates the multiple linear regressions of dmfs with salivary constituents and plaque index. Vitamin A and PI were the most important factors in predicting dmfs, for each one unit increased in vitamin A the dmfs is expected to significantly decrease by 447.116, after adjusting other explanatory variables included in the model. While for each one unit increased in PI the dmfs is expected to significantly increase by mean of 12.260. The model was statistically significant and being able to explain 87 % of observed variation in the outcome variable (dmfs).

Table (11) represents the multiple linear regressions of GI with salivary constituents and plaque index. For each one unit increased in vitamin A and PI, the GI increase by 10.7 and 0.94 respectively. The model was statistically significant and being able to explain 99% of observed variation in the outcome variable (GI).

DISCUSSION

Protein-energy malnutrition occurs when there are deficiencies in protein, energy foods or both, relative to a body's needs. Studies suggest that caries of the primary dentition is associated with early childhood malnutrition [7-9, 31]. In the present study a higher mean value of dmfs was recorded among malnourished in comparison to well nourished children with statistically highly significant difference. Mal- nutrition may influence the development of the teeth (organic and inorganic composition) [3, 32] and this may explain the higher mean values of d_1, d_2, d_3 and d_4 among malnourished children.

Protein-energy malnutrition (PEM) appears to have multiple effects on the oral tissues and subsequent oral disease development. During childhood, malnutrition may restrict organ development as salivary glands leading to a diminished metabolic capacity involving concomitant deficiencies of antioxidant nutrients [32-35]. This may explain the finding of present study as a lower mean values of salivary vitamins (A, C and E) and total protein were recorded among malnourished children compared to well nourished with highly significant differences. This was also found by other limited studies [33, 36-38] and animal study concerning vitamin A [39]. Although lower mean values of salivary vitamins were noticed among malnourished children, strong negative highly significant correlations were recorded between vitamins A, C, E and caries-experience (d_4, ds, dmfs) of primary teeth. While a positive highly significant correlations was seen with caries-experience (d_3, d_4, ds) among well nourished. Salivary antioxidant system serves as an important ingredient in building resistance and might reduce the susceptibility to dental caries because of its free radical scavenging action [40]. These vitamins were found adversely affect the oxidative carbohydrate metabolism within the plaque and this will affect the oxidation-reduction balance within the cell of microorganism thereby affecting bacteria metabolism and energy generation leading to cell death [41]. These findings were also recorded by previous Iraqi studies among healthy adult and elderly individuals concerning vitamin A, C and E [18, 19]. The present study revealed a positive non significant correlation between d_1 and vitamins among malnourished children; this may be explaining the protective role of these vitamins against oxidative stress [42]. Salivary glandular hypofunction and saliva compositional changes may be mechanisms through which malnutrition is associated with caries. Regarding salivary total protein, a positive significant correlation with ds and d_4 was seen among malnourished while it was negative highly significant correlation among well nourished children and this was found in other studies [18, 43,

[44]. This may explain the anticaries effect of total protein as increased concentration may give a protective role. In humans, after eruption of teeth there is no direct effect of protein on tooth susceptibility to caries, theoretically protein adsorbs on tooth surface and could decreases dental caries risk, but precise evidence is lacking [45].

Dental plaque is a complex microbial community growing as a biofilm on enamel surfaces. The etiology of both dental caries (tooth decay) and various forms of periodontal disease has long been recognized to be related to bacterial accumulations and plaque composition [32] and this finding was confirmed by studies [46, 47] as well as this present study; as a positive strong highly significant correlation was recorded among both groups.

Gingival index showed a higher mean value among malnourished children in the present study and this was also reported by other studies [48, 49]. Vitamin requirements vary from species to species and are influenced by age, gender, and physiological conditions such as pregnancy, breast-feeding, physical exercise, and nutrition [50]. Periodontal disease progresses more rapidly in undernourished populations, the important role of nutrition in maintaining an adequate host immune response may explain this observation [51]. Although a lower mean of plaque and gingival index was seen among malnourished children, strong negative highly significant correlations were noticed with salivary vitamins. Other studies reported the same findings [38, 52, 53]. The contents of saliva are likely influenced by nutrients consumed daily, with consequences to oral health [50, 54, 55]. Low salivary antioxidant levels could be the result of periodontal inflammation or could be a risk factor for periodontitis, Hypothetical associations between gingivitis and vitamins are supported by the observations that additional vitamins are required during infectious diseases, due to increased oxidative stress. It appears that water soluble antioxidant nutrients reduced vitamin C may be initially consumed, followed by lipid soluble antioxidants E. Also, it has been reported that vitamin C regenerates vitamin E by non-enzymatic mechanisms [56]. These results can also explained as the periodontal disease is associated with reduced levels of salivary antioxidant and this may lead to increased oxidative damage within the oral cavity [57]. protective effects of vitamin C in maintaining tissue homeostasis include its key function in collagen synthesis and therefore maintenance of the structural integrity of the connective tissues as well as its role as a radical scavenger, so lower vitamin C level is associated primarily with defective collagen synthesis, causing tissue dysfunction such as impaired wound healing and ruptured capillaries because of insufficient support of the capillary walls by the connective tissues [58]. Total protein was found to correlate positively and strongly highly significant with plaque index and gingival index among malnourished children and only with GI among well nourished. This was also found in other study [18]. This may provide protection against Reactive Oxygen Species (ROS) induced damage of periodontal tissue [51, 59].

This study suggests that malnutrition act as a risk factor for dental caries and gingival disease.

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Table 1: The Relative Frequency of Caries Free Children by Gender

	Malnourished		Well nourished	
Caries free	**No.**	**%**	**No.**	**%**
Males	(0/15)	0.0	(4/15)	26.7
Females	(0/15)	0.0	(3/15)	20.0
Total	(0/30)	0.0	(7/30)	23.3

Table 2: Caries – Experience (dmfs) of Primary Teeth among Malnourished and Well nourished Children

Groups	ds	ms	fs	dmfs
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Malnourished	20.36±12.79	1.43±3.19	0.27±0.87	22.06±12.08
Well nourished	6.13±6.12	0.13±0.73	0.13±0.51	6.39±6.05

Table 3: Decayed Surfaces of Primary Teeth by Grades of Lesion Severity (d_{1-4}) of Malnourished and Well nourished Children

Groups	d_1	d_2	d_3	d_4
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Malnourished	1.23±1.85	1.80±1.86	4.36±3.77	12.96±13.69
Well nourished	0.90±1.77	0.83±1.29	2.07±3.42	2.33±3.73

Table 4: Plaque and Gingival Indices of Malnourished and Well nourished Children

Groups	Mal nourished	Well nourished
	Mean ± SD	**Mean ± SD**
PI	1.29 ± 0.67	0.31 ± 0.29
GI	1.24 ± 0.67	0.23 ± 0.22

Table 5: Correlation Coefficient between Plaque Index and Caries – Experience of Primary Teeth in Malnourished and Well nourished Children

Groups	ds		dmfs	
	r	**P**	**r**	**P**
Mal nourished	0.91	<0.001*	0.90	<0.001*
Well nourished	0.87	<0.001*	0.86	<0.001*

*** Highly Significant**

Table 6: Mean Values of Salivary Vitamins (mg/dl) and Total protein (mg/dl) among Malnourished and Well nourished Children

Groups	Vitamin A	Vitamin C	Vitamin E	Total protein
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Malnourished	0.013±0.003	0.020±0.005	0.025±0.036	0.199±0.175
Well nourished	0.028±0.006	0.039±0.004	0.045±0.004	0.398±0.074

Table 7: Correlation Coefficient between Caries – Experience (ds, dmfs) of Primary Teeth and Salivary Vitamins (mg/dl) and Total protein (mg/dl) in Malnourished and Well nourished Children

Groups	Variables	ds		dmfs	
		r	P	r	P
Malnourished	Vitamin A	-0.74	<0.001**	-0.76	<0.001**
	Vitamin C	-0.80	<0.001**	-0.85	<0.001**
	Vitamin E	-0.80	<0.001**	-0.79	<0.001**
	Total protein	0.37	0.04*	0.32	0.08
Well nourished	Vitamin A	0.87	<0.001**	-0.03	0.87
	Vitamin C	0.91	<0.001**	-0.02	0.88
	Vitamin E	-0.86	<0.001**	-0.08	0.64
	Total protein	-0.81	<0.001**	0.006	0.97

*** Significant, ** Highly Significant**

Table 8: Correlation Coefficient between Caries – Experience (Grades of Caries Severity) of Primary Teeth and Salivary Vitamins (mg/dl) and Total protein (mg/dl) among Malnourished and Well nourished Children

Groups	Variables	d_1		d_2		d_3		d_4	
		r	P	r	P	r	P	r	P
Malnourished	Vitamin A	0.50	0.004**	-0.13	0.44	-0.25	0.18	-0.67	<0.001**
	Vitamin C	0.29	0.11	-0.20	0.91	0.01	0.93	-0.76	<0.001**
	Vitamin E	0.23	0.20	-0.14	0.43	-0.03	0.86	-0.73	<0.001**
	Total protein	-0.03	0.86	-0.14	0.46	-0.10	0.56	0.40	0.02*
Well nourished	Vitamin A	0.10	0.58	0.14	0.43	0.56	0.001**	0.81	<0.001**
	Vitamin C	0.11	0.55	0.13	0.46	0.62	<0.001**	0.82	<0.001**
	Vitamin E	-0.20	0.28	-0.20	0.28	-0.63	<0.001**	-0.64	<0.001**
	Total protein	-0.17	0.35	-0.28	0.13	-0.53	0.002**	-0.67	<0.001**

*** Significant, ** Highly Significant**

Table 9: Correlation Coefficient between Plaque Index, Gingival Index and Salivary Vitamins and Total protein In Malnourished and Well nourished Children

Groups	Variables	PI		GI	
		r	P	r	P
Malnourished	Vitamin A	-0.63	<0.001*	-0.62	<0.001*
	Vitamin C	-0.81	<0.001*	-0.79	<0.001*
	Vitamin E	-0.77	<0.001*	-0. 77	<0.001*
	Total protein	0.49	0.005*	0.48	0.006*
Well nourished	Vitamin A	-0.08	0.67	0.88	<0.001*
	Vitamin C	-0.06	0.73	0.91	<0.001*
	Vitamin E	-0.12	0.51	-0.89	<0.001*
	Total protein	0.07	0.69	-0.82	<0.001*

*** Highly Significant**

Table 10: Multiple Linear Regressions of dmfs with Salivary Constituents and Plaque Index

Variables	Partial Regression Coefficient	Standardized Coefficient	P-Value
Vitamin A	-447.116	-0.301	0.022*
Vitamin C	-288.393	-0.243	0.190
Vitamin E	249.187	0.219	0.164
Total protein	10.197	0.094	0.307
PI	12.260	0.705	0.000**

P (model) < 0.001 R^2= 0.87 *Significant, **Highly significant

Table 11: Multiple Linear Regressions of Gingival Index with Salivary Vriables and Plaque Index

Variables	Partial Regression Coefficient	Standardized Coefficient	P-Value
Vitamin A	10.752	0.126	0.000*
Vitamin C	-2.564	-0.038	0.408
Vitamin E	-10.147	-0.155	0.000*
Total protein	0.108	0.017	0.444
PI	0.947	0.946	0.000*

P (model) < 0.001 R^2= 0.99 *Highly significant