The Salivary Inflammatory Biomarkers (Interleukin-6, C - reactive protein) in Relation with Caries-Experience among a Group of 12 Year Old Obese Boys

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ABSTRACT

Background: Obesity and dental caries are multifactorial diseases related to poor eating habits and show a close relationship with the sociodemographic characteristics of individuals presenting these diseases. This research aimed to investigate the severity of dental caries among group of obese boys aged 12 year in relation to salivary interleukin-6 (IL-6) and C-reactive protein (CRP) of unstimulated whole saliva in comparison with normal weighted boys of the same age.

Materials and Methods: The study group included 40 obese boys, with an age of 12 year. The control group included 40 normal weighted boys of the same age. The total sample involved for nutritional status assessment using Body Mass Index specific for age and gender according to CDC growth chart (2000). The diagnosis and recording of dental caries conducted by using (D_{1-4}MFS and d_{1-4}mfs) index according to the criteria of Muhlemann (1976). The collection of unstimulated whole saliva was performed under standardized condition. Salivary samples were chemically analyzed for measuring IL-6 and CRP.

Results: The caries experience among study group was lower than that among control group for both dentitions, with significant differences for D_2, ds and dmfs and highly significant difference for d_4. Salivary inflammatory biomarkers (IL-6, hs-CRP) were slightly higher among study group compared with control group with no significant difference between them. Salivary IL-6 and hs-CRP were negatively correlated with dental caries of both dentitions among study group with significant correlation between IL-6 and D_2, while they were correlated positively with dental caries of both dentitions among control group with highly significant correlation between IL-6 and D_3 and significant correlation between IL-6 and d_4.

Conclusion: Obesity and dental caries are associated with increased levels of salivary interleukine-6 and C-reactive protein, this making both obesity and dental caries as a state of inflammation that exacerbating immune responses in the body.

Key words: Obesity, salivary cytokines, interleukine-6, C-reactive protein. (J Bagh Coll Dentistry 2016; 28(1):138-142).

INTRODUCTION

Obesity in adolescents and children has raised to significant levels globally with serious public health consequences. In addition to cardiovascular, emotional and social issues, it poses a serious hazard to the basic health care delivery system [(1)]. It is associated with an increased risk of morbidity and mortality as well as reduced life expectancy [(2)].

Dental caries is a process that may take place on any tooth surface in the oral cavity where dental plaque is allowed to develop over a period of time [(3)]. According to Mathus-Vliegen et al., [(4)] obesity is related to several aspects of oral health, including dental caries. Dental caries and obesity are multifactorial diseases related to poor eating habits and show a close relationship with the sociodemographic characteristics of individuals presenting these diseases.

Cytokines are small regulatory proteins that are released in a wide variety of cells to modulate cell–cell interaction and other functions especially important for inflammation and immune responses [(5)].

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Some of these cytokines are proinflammatory (make disease worse such as IL-1, TNF, IL-6). IL-6 is produced by adipocytes, monocytes, macrophages, lymphocytes and fibroblasts and stimulates T cell proliferation, activation, apoptosis and cytotoxicity. Besides activating the immune system, the increase in IL-6 induces hepatic synthesis of acute phase proteins and increases the activity of the hypothalamic-pituitary-adrenal axis, altering metabolic responses [(6)]. In obesity, IL-6 secreted in an endocrine manner in proportion to the expansion of fat mass particularly in the abdominal region, with a corresponding increase in hepatic production of CRP [(7)]. Obesity is associated with elevated levels of IL-6 [(8)] and CRP [(9,10)]. As well as, dental caries associated with elevated levels of IL-6 [(11,12)] and CRP [(13)].

MATERIALS AND METHODS:

The study group included 40 obese boys aged 12 years were randomly selected from primary schools in Al-Najaf city, the control group included 40 normal weighted boys were chosen from the same class matching in age with study group. Anthropometric measurements included measurement of weight and height according to Trowbidge [(14)] using Bathroom scale and height

measuring tape. Body Mass Index (BMI) is a number calculated from person`s weight and height, according to this formula

BMI (Kg/m^2) = Body weight (Kg)/ $(height)^2$ (m)

According to specific chart (CDC growth chart) that was used to indicate BMI according to age and gender and identify the obesity at or above 95 percentile while the normal weight between 5-85 percentile [15]. Caries experience was recorded according to decayed, missing and filled index (DMFS, dmfs for permanent and primary teeth respectively) using the criteria of Muhlemann [16] which allow recording decay lesion by severity.

The collection of unstimulated salivary samples was performed under standard condition following instruction cited by Navazesh and Kumar [17]. Salivary inflammatory biomarkers were interleukin-6 and high sensitive C-reactive protein which analyzed by Enzyme Linked Immuno Sorbent Assay (ELISA) test using special kits according to the manufactured instructions. Data analysis was conducted by application of SPSS program (version 16).

RESULTS

The result of present study showed that the mean DMFS for study group was lower than that of control group, but the difference was not significant. Table 1 illustrates DMFs and its components and Ds grades among study and control groups. Table 1 reveals that D_2 was significantly lower among study group compared with control group.

Caries experience for primary dentition showed in table 2, which reveals dmfs, dmfs components and ds grades; dmfs was significantly lower among study group compared with control group with significant difference for ds component, as well as d_4 was highly significantly lower among study group compared with control group.

Table 3 demonstrates salivary inflammatory biomarkers among study and control groups. It reveals that IL-6 was higher among study group compared with control group with no significant difference. It was also found that there is no significant difference between the study and control groups regarding hs-CRP.

Correlation coefficients between salivary inflammatory biomarkers and caries experience among study and control groups are shown in table 4 which reveals that IL-6 and hs-CRP were correlated inversely with dental caries of both dentitions (DMFS/dmfs) among the study group while they were correlated positively with dental caries of both dentitions among the control group; although the differences were not significant. Also IL-6 was negatively significantly correlated with D_2 among the study group, while it was correlated positively highly significantly with D_3 among the control group. Concerning primary dentition, IL-6 was positively significantly correlated with d_4 among the control group.

Table1: Caries Experience of Permanent Dentition (DMFs, DMFs Component and Ds Grades) between Study and Control Groups

Variables	Study group		Control group		Statistical test	
	Mean	± SD	Mean	± SD	t-test	P-value
D_1	0.68	1.35	0.33	0.76	-1.42	0.15
D_2	2.55	2.75	4.10	3.04	2.39	**0.01***
D_3	0.30	0.65	0.58	1.01	1.44	0.15
D_4	0.75	1.98	0.50	1.45	-0.64	0.52
Ds	4.28	3.94	5.50	3.78	1.42	0.15
Ms	0.13	0.79	0.13	0.79	0.00	1.00
Fs	0.05	0.22	0.18	0.68	1.11	0.26
DMFs	4.45	3.95	5.80	4.05	1.51	0.13

***Significant (P<0.05), d.f=78**

Table 2: Caries Experience of Primary Dentition (dmfs, dmfs Component and ds Grades) between Study and Control Groups

Variables	Study group		Control group		Statistical test	
	Mean	± SD	Mean	± SD	t-test	P-value
d_1	0.03	0.16	0.00	0.00	-1.00	0.32
d_2	0.35	1.00	0.40	1.17	0.20	0.83
d_3	0.13	0.65	0.35	0.74	1.45	0.15
d_4	0.33	1.12	1.65	2.78	2.79	**0.00****
Ds	0.83	2.09	2.40	3.82	2.28	**0.02***
ms	0.25	1.10	0.38	1.33	0.45	0.64
fs	0.05	0.32	0.05	0.32	0.00	1.00
dmfs	1.13	2.44	2.83	4.46	2.11	**0.03***

***Significant (P<0.05), **Highly significant (P<0.01), d.f=78**

Table 3: Salivary (IL-6 and hs-CRP) between Study and Control Groups

Variables	Study group		Control group		Statistical value	
	Mean	± SD	Mean	± SD	t-test	P-value
Interleukine-6	56.25	10.54	55.13	13.61	-0.41	0.68
High sensitive C-reactive protein	0.75	0.22	0.75	0.27	-0.02	0.97

d.f=78

Table 4: Correlation Coefficients between Salivary (IL-6 and hs-CRP) and Caries Experiences (DMFs, Ds Grades, dmfs and ds Grades)

Variables	IL-6				hs-CRP			
	Study group		Control group		Study group		Control group	
	R	P	r	P	r	p	r	p
D_1	0.24	0.12	-0.18	0.25	-0.03	0.85	0.00	0.98
D_2	-0.31	**0.04***	0.02	0.90	-0.07	0.64	0.05	0.72
D_3	-0.28	0.07	0.47	**0.00****	-0.00	0.96	-0.02	0.90
D_4	-0.10	0.53	-0.08	0.59	-0.13	0.42	0.21	0.19
DMFs	-0.15	0.33	0.02	0.89	-0.07	0.63	0.12	0.44
d_1	-0.18	0.25	-	-	-0.01	0.93	-	-
d_2	0.00	0.99	-0.08	0.58	0.00	0.98	0.05	0.75
d_3	0.25	0.11	0.02	0.89	-0.10	0.50	-0.02	0.85
d_4	-0.1	0.42	0.04	**0.03***	-0.05	0.71	0.01	0.91
dmfs	-0.04	0.79	0.07	0.66	-0.04	0.78	0.03	0.81

***Significant (P<0.05), **Highly significant (P<0.01)**

DISCUSSION

The present study revealed a low caries experience among obese group compared with normal weight group for both dentitions; this goes in accordance with previous studies [18-20].

The low caries experience among study group could be attributed to that the obese subjects were reported to consume more fast foods [21,22] and these fast foods contain unacceptable high levels of fats.

Fats were found to reduce dental caries through a variety of mechanisms: Fats may form a protective barrier on enamel by coating it and thus making it difficult for penetration of acids. In addition, fats replace the carbohydrate intake thereby reducing its intake; also some fatty acids have antimicrobial effects as it inhibits glycolysis in human dental plaque and it coats the plaque to prevent reduction of fermentable carbohydrate to acids [23]. Additionally, high level of fat in the diet binds to the sugars content of it thus reducing their solubility, resulting in a lower drop in pH and weaker acid attack [24].

Another cause for lower caries experience among study group is that the mean of plaque index in the present study was lower among the study group than that among the control group with no significant difference between them, as the plaque is the primary etiologic factor for the most frequently occurring oral diseases, dental caries and periodontal diseases [25,26]. Also the present study showed that salivary phosphorus was higher among the study group than that among the control group with no significant difference between them, the same result was found in the study of Hartman et al [27], so due to its cariostatic action and its role as a buffer and in

remineralization of the teeth [28], this may lead to low caries experience among the study group.

The results of current study revealed that the concentration of salivary interleukine-6 among study group was higher than that among control group with no significant difference between them; this result was in agreement with Quellet-Murin et al [29]. The explanation for this result could be that the effect of obesity related inflammation which is the characteristic feature of the study group. Results also showed that there was no significant difference between the study and control groups concerning salivary C-reactive protein level, although some studies revealed elevated level of C-reactive protein in obese compared with normal weight subjects [29-31].

The explanation for this result could be the presence of a state of inflammation in both of study and control groups; represented by obesity in the study group where salivary CRP concentration has been found to correlate well with serum concentrations [32], and dental caries in the control group as reported by Gawri et al., [13] who found that there is a significant relation between level of salivary C-reactive protein and dental caries.

Data analysis of the present study found that, in the control group, interleukin-6 (IL-6) was correlated positively with dental caries for both dentitions with no significant difference; this correlation was highly significant with D_3 and significant with d_4. The same also reported by[11, 12]. The explanation for this result was that the cells in the odontoblast layer initiate immunologic responses of the tooth to dental caries through proinflammatory cytokine and chemokine signaling, in the presence of bacteria, odontoblasts secrete various chemotactic cytokines for neutrophils, monocytes/macrophages, immature dendritic cells, and lymphocytes [33], the migratory immune cells, in particular monocytes/ macrophages, release a large amount of pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and IL-12, which regulate inflammatory reactions in the tissue [34].

Studies of Matsushima et al., [35] and Wisithphrom and Windsor [36] suggest that the glycoprotein which was purified from Lactobacillus casei (Gram-positive bacteria frequently isolated from deep carious lesions and suspected to be a pathogen of pulpitis) stimulate IL-6 production in a time- and dose-dependent manner. Data analysis of the present study found that, in the study group, IL-6 was correlated negatively with DMFS and dmfs with no significant differences. This may be attributed to the presence of other factors affecting the caries process like type of diet and oral hygiene, as dental caries is a multifactorial disease, or it may be explained partly by that the functional response of odontoblasts to caries is recognized as extending a barrier to the spread of infection by forming reparative dentine, so that odontoblasts attenuating carious infections thereby limiting the inflammatory changes within odontoblasts and maintaining the pulp in a relatively protected environment [37].

The hs-CRP was negatively correlated with dental caries of both dentitions (DMFS/dmfs) in the study group, and these correlations were not significant.Regarding the control group, hs-CRP was found to be correlated positively with dental caries for both dentitions (DMFS/dmfs). This result was in agreement with the study of Horst et al., [37] and Gawri et al., [13] who found that increasing CRP in the odontoblast of carious teeth. One can explain that by the presence of IL-6 which is secreted by various cell types in response to microbes or cytokines such as IL-1 and TNF-α [38], which stimulates hepatocytes to synthesize two major acute-phase proteins: C-reactive protein (CRP), which increases the rate of bacterial phagocytosis, and serum amyloid A (SAA), which influences cell adhesion, migration, proliferation, and aggregation [39].

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