Relationship between Salivary Cytokines, and Caries Experience in Children with Different Body Mass Indices

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Abstract

Objective: To determine the relationship between salivary cytokine concentrations and caries experience in children with different body mass indices.

Materials and Methods: Stimulated whole saliva samples were collected from 135 convenient populations of children aged between 4 to 11 years old. The groups should be explained (For example, the children were divided into three groups as obese (O, n=60), non-obese (N-O, n=54) and children At Risk of Becoming Obese (RBO, n=21). Cytokine profiling was obtained using a sandwich immunoassay format with a 10-spot MULTI-SPOT 96-well plate (MSD®).

Age, body mass index (BMI), ethnicity, race and volumes of samples collected were recorded. A chart review of the patients was carried out on the day of salivary sample collection. Total numbers of primary and permanent teeth (N), number of filled surfaces (fs + FS), number of decayed surfaces (ds + DS), mean decayed surface rate (ds + DS/N), mean filled surface rate (fs + FS/N) and mean decayed + filled surfaces rate (dfs + DFS/N) were calculated. Data was analyzed using Microsoft Office Excel (Microsoft Corp., Redmond, WA) and SPSS v 16.0 for Windows (SPSS Inc., Chicago IL).

Results: The study population consisted of 135 subjects (68 females, 67 males), aged 4 -11years with mean age of 8 years. The racial distribution was Hispanic (45.2%), African American (31.8%) and Caucasian (22.9%). All subjects were further stratified into one of three body type categories based on their BMI: Non-Obese (42.2%), At Risk of Becoming Obese (16.3%), and Obese (44.4%). Pearson correlation revealed positive correlations between INfy, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-8, IP-10, TNF-α and number of filled surfaces (fs+FS), filled surface rate (fs+FS/N) and caries + filled surfaces rate (dfs+DFS/N). There was a statistically significant difference between the values of active caries rate for Obese Non-Obese and At Risk of Becoming Obese, (P=.020 p>0,05). The RBO had a significantly higher caries surface rate (P=.029 and .023) than O and N-O groups.

Conclusions: Many salivary cytokines showed significant correlations to caries experience of the children. There was also a significant correlation between salivary flow rate and IL-1β and a significant difference in caries activity within obesity groups.

Keyword: Saliva, Cytokines, Dental Caries, Body Mass Index, Children

Introduction

As of 2005, 41% of US children 2-11 years-old have clinically detectable caries in their primary teeth. By the age of 16-19 years, almost 70% of US children exhibit clinically detectable signs of caries experience [1].This may actually be an underestimation as these data only considered cavitation stage and not the earlier subclinical stages of the disease [2]. Strategies for managing dental caries have increasingly emphasized the concept of risk assessment [3]. Early identification of at-risk patients can allow more effective allocation of resources and encourage greater focus on the medical management of the caries process [4].

The best predictor of caries risk experience in permanent dentition is previous caries experience in the primary dentition, followed by parents' education and socioeconomic status. This information could be obtained from well documented dental records of the patient [5]. However, for children who do not have any past dental records, there is a need for an earlier identification of risk factors which could be used to select out children of high risk of developing the disease- preferably ones that can be utilized by non-dental health care professionals [6,7].

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The quality, quantity, and molecular composition of saliva have been measured in numerous studies with the hopes of relating these markers to oral or systemic disease. These markers include, cytokines, antimicrobial peptides, glycoproteins, growth factors, and immunoglobulins, among others [8-10].

Studies have reported the induction of production of several cytokines production in response to acute pulpal inflammation and dental caries in children. . Most of these studies however have used the cytokines level in the blood or have examined adolescent and adult population [11-13]. Menon, et al. (2016) [14] evaluated the salivary interleukin-6 only in children with early childhood caries after treatment.

Childhood obesity is becoming an epidemic world-wide and several studies have shown the possible link between it and pro inflammatory cytokines [15].

The objective of this study was to assess the salivay levels of several cytokines in relations to caries experience and body mass indices in different racial groups of children.

Methods and Materials

Approval for the study was obtained from the Committee for Protection of Human Subject prior to the start of the project (HSC-DB-07-0175 and HSC-DB- 08-0554).

Subjects

Subjects were convenient sample of patients who attended the University of Texas Health Science Center at Houston graduate pediatric dentistry residency program clinics who attended for routine dental care. Inclusion criteria for participation in prior study consisted of male and female subjects between 3 to 11 years of age who were Hispanic, African American or Caucasian decent. Both obese and non-obese subjects were actively recruited. Subjects were required to have a non-contributory medical history and taking no medications regularly. A completed informed consent was obtained prior to specimen retrieval. When a subject was determined to fit the inclusion criteria specific, height, weight and age (in months) was recorded. This information was used to calculate body mass index (BMI), of each child respectively. Children were placed in one of three body type categories based on the results of BMI calculation and their placement on the percentile tracking curves for boys and girls developed by the Center for disease control and prevention (CDC).

Subjects whose BMI was 95% or greater were considered obese, those with a BMI between 85% and 95% were considered at risk of becoming obese and those whose BMI was less than 85% were considered to have a normal healthy weight.

Stimulated Saliva Collection

A stimulated whole salivary gland secretion was collected in the following manner: A standard piece of paraffin (1.5 g) was placed in subject's mouth, subject was instructed to chew the wax at a regular rate. The subject then expectorated into a disposable, pre-weighed plastic cup at regular intervals for a period of three minutes. The volume and physical characteristics were recorded. After the saliva specimens were collected, the specimen was frozen (-80°C) until assays were performed. All specimens were coded and linked to the subject via a key that only the primary investigators possess.

Cytokines Analysis

The frozen saliva samples were thawed and processed through high-speed ultracentrifugation in order to precipitate cells and mucin for extraction of the cytokine proteins. Cytokine profiling was determined using a sandwich immunoassay format with a 10-spot MULTI-SPOT 96-well plate (MSD®). The specific cytokines that were studied include: Interferon gamma (INFγ), Interleukin 12p70 (IL-12p70), Interleukin 13 (IL-13), Interleukin 1 beta (IL-1B), Interleukin 2 (IL-2), Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 8 (IL-8), Human interferon-inducible protein 10 (IP-10), and Tumor Necrosis Factor alpha (TNFα).

Dental Caries Assessment

The charts of the subjects sampled were reviewed. Data collected from charts on the hard tissue charting for the day of collection included total number of primary and permanent teeth(N), present decayed surfaces of primary and permanent teeth (ds + DS) and filled surfaces of primary and permanent teeth (fs + FS). Mean decayed surface rate (ds + DS/N), filled surface rate (fs +FS/N) and decayed + filled surface rate (dfs + DFS/N) were calculated.

Statistical Analysis

Data was analyzed using Microsoft Office Excel (Microsoft Corp., Redmond, WA) and SPSS v 16.0 for windows (SPSS Inc., Chicago IL). Mean values, standard deviations and descriptive statistics for age, ethnicity, BMI, the salivary flow rate, and cytokine concentrations were calculated.

Pearson correlation and The Spearman rho statistic were utilized to examine any correlation between the concentration of cytokines and dental caries status in the subjects

Post Hoc test, Tukey analysis was used to examine any differences in the salivary flow rate among the races. Two sample test was used for comparison of the variables of the differences between obesity as well as the concentration of cytokines among different races (Watson, et al. 2014) [12].

Result

Demographics

Demographics and Caries Assessment: Of the 135 subjects analyzed, 68 were female and 67 were male with age ranging from 4 - 11 years with a mean age of 8 yrs. The study included persons of Hispanic (45.2%), African American (31.8%) and Caucasian (22.9%) decent (Table 1) The mean number of teeth (primary and permanent teeth) per patient is 22.5 (±2.3); mean filled surfaces per patient is 11.0 (±12.6) and mean decayed

Item	Frequency	Percent %
Hispanic	64	45.7
Black	45	32.1
White	31	22.1
Total	140	100
Male	70	50
Female	70	50
Total	140	100

Table 1: Frequency of gender, ethnicity.

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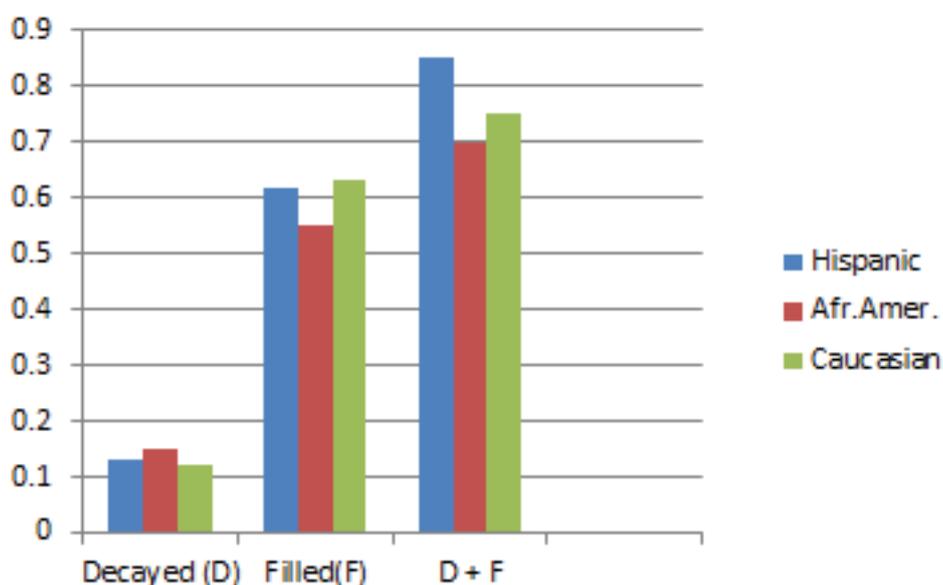
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Figure 1: Mean decayed surfaces, mean filled surfaces and mean decayed and filled surfaces in the different racial groups.

Race	Obesity n (%)	At-Risk n (%)	Normal. n (%)	Total n (%)
Black	18(40)	9(20)	18(40)	45(100)
Hispanics	27(42.2)	9(14.1)	28(43.7)	64(100)
Caucasian	16(51.6)	4(12.9)	11(35.5)	31(100)
Total	61(43.6)	22(15.7)	57(40.7)	140(100)

Table 2: Percentages of Obesity, at-risk of obesity and normal body mass in different racial groups.

surfaces per patient is 2.7 ($\pm$5.1). The mean decayed surfaces rate (ds + DS/N) is 0.13 ($\pm$0.24), filled surface rate (fs + FS/N) is 0.51 ($\pm$0.58) and decayed + filled surface rate (dfs + DFS/N) is 0.63 ($\pm$0.60) (Figure 1).

Race and Obesity

Table 2 shows the body mass indices in the three racial groups. Forty percent 40%0 blacks, 42% Hispanics and 52% Caucasians are classified as being Obese. If these figures are combined with those at risk of being obesed then a significant number in each racial group (60% black, 56% Hispanics and 63% Caucasian) have high BMI values (Table 2).

There is no statistical difference between the different racial groups (p.>0.05)

Cytokines Concentration among Races of different Body Mass Index

One-way ANOVA reveals that the presence of obesity has no significant effect on cytokine concentrations for INFγ, IL-12p70, IL-13, IL-2, IL-4, IL-5, IL-8, IP-10 and TNFα (p>0.05). A one-way ANOVA was used to assess significance of ethnicity within the group of proteins studied, salivary concentration of IL-1B was the only shown to be effected by this factor (p=0.037). Post Hoc Tukey analysis revealed no significance between Hispanics/Whites or Blacks/Whites in salivary concentration of IL-1B (p>0.05), however, there was a significant difference between seen between Hispanics and Blacks (p=0.031)(Table 2).

Cytokine Concentration and Caries

The specific cytokines studied include: Interferon gamma (INFγ), Interleukin 12p70 (IL-12p70), Interleukin 13 (IL-13), Interleukin 1 beta (IL-1β), Interleukin 2 (IL-2), Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 8 (IL-8), Human interferon-inducible protein 10 (IP-10), and Tumor Necrosis Factor alpha (TNFα).

Pearson correlation reveals positive correlations between INFγ, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-8, IP-10, TNFα and filled surface number (fs + FS): (Table 1), filled surface rate (fs + FS/N): (Table 2) and caries + filled surfaces rate (dfs + DFS/N): (Table 2). Pearson correlation reveals positive correlation between INFγ and number of decayed surfaces (r=0.191, p<0,05) and decayed surfaces rate (r=0.198, p<0,05).

Salivary Flow rate, Cytokines concentration and mean caries rates

The mean salivary flow rates among the racial groups are Hispanics 3.39 $\pm$ 2.29, Black 4.53 $\pm$ 3.76 and Caucasian 2.95 $\pm$ 2.02 respectively.. A one way ANOVA showed that

African Americans have a significantly higher salivary flow rate than other groups p<0.05) and the Post Hoc test, showed that there was also a significant difference between African Americans and Caucasians (p<0.05) in salivary flow.

Pearson correlation revealed a negative correlation between salivary flow rate and IL-1β only (p<0.05) and no significant correlation was found between salivary flow rate and mean caries rates in the different racial groups(p<0.05) Significant correlation was found between cytokines concentrations and caries rates (Table 3).

Race and Dental Caries

No significant correlation was found between mean caries

***Cytokines	Black (Mean ± STD)			Hispanic (Mean ± STD)			White (Mean ± STD)		
	0bese.	At Risk	Non-Obese	0bese	At Risk	Non-Obese	0bese.	At Risk	Non-Obese
INF-γ	5.5 ± 8.3	2.9 ± 1.8	4.3 ± 4.8	4.4 ± 4.0	4.8 ± 2.3	5.9 ± 5.9	3.6 ± 2.7	5.0 ± 2.0	4.3 ± 2.5
IL-12p70	5.2 ± 5.2	2.4 ± 3.5	7.7 ± 14.9	4.7 ± 7.3	6.6 ± 11.6	10.7 ± 33.5	18.0 ± 4.3	1.8 ± 1.7	4.4 ± 4.6
IL-13	12.8 ± 7	10.1 ± 5.2	16.4 ± 15.5	14.0 ± 12.0	18.2 ± 13.4	16.0 ± 19.5	11.6 ± 7.6	12.8 ± 4.2	14.2 ± 8.0
IL-1β	99.5 ± 68.6	95.9 ± 62.5	136.1 ± 147.6	165.4 ± 122.	8215.1 ± 100.3	169.4 ± 122.7	119.4 ± 114.9	190.8 ± 66.8	135.6 ± 109.8
IL-2	7.4 ± 7.8	3.2 ± 3.6	8.0 ± 7.9	9.2 ± 9.9	10.4 ± 8.5	12.8 ± 16.8	7.6 ± 7.6	9.8 ± 5.4	8.6 ± 7.9
IL-4	0.8 ± 0.9	0.9 ± 0.6	1.1 ± 1.1	1.3 ± 1.9	1.7 ± 1.1	1.6 ± 2.4	1.8 ± 1.3	1.8 ± 0.5	1.3 ± 0.9
IL-5	2.9 ± 3.0	2.0 ± 1.1	3.1 ± 3.4	3.1 ± 3.6	2.7 ± 2.8	3.4 ± 4.1	2.9 ± 2.7	2.8 ± 1.0	3.1 ± 1.9
IL-8	504.6 ± 514.9	361.9 ± 232.9	691.2 ± 731.9.	650.7 ± 650.7	791.1 ± 783.9	883.9 ± 1370.2	508.9 ± 508.9	669.5 ± 346.9	714.3 ± 863.6
IP-10	9.1 ± 11.6	6.1 ± 3.3	7.4 ± 6.8	8.4 ± 7.6	10.3 ± 6.0	12.6 ± 14.6	7.2 ± 6.2	12.5 ± 3.9	7.3 ± 5.0
TNF-α	16.7 ± 21.7	6.4 ± 3.1	13.1 ± 13.2	15.8 ± 19.5	14.6 ± 6.9	27.3 ± 33.3	11.7 ± 7.2	22.3 ± 8.3	10.1 ± 10.2

* INF-Interferon; IL- Interleukin; IP-Inducible Protien; TNF –Tumor Necrosis Factor
**Cytokines and Body Mass - One Way ANOVA (p>0.05) Not significant
***Cytokines and race - One Way ANOVA .Only IL-1β is significant (p<0.05)

Table 3: Comparison of concentration of cytokines in the different racial groups of different body mass indices.

Cytokines	Correlation (r)	p-value
INFγ	0.274	0.001
IL-12p70	0.189	0.029
IL-13	0.199	0.021
IL-1β	0.303	0.001
IL-2	0.244	0.004
IL-4	0.248	0.004
IL-5	0.214	0.013
IL-8	0.186	0.031
IP-10	0.198	0.022
TNFα	0.184	0.033

Table 4: Correlation of Cytokines concentrations with the mean decayed and filled surfaces.

rates in different racial group. However the trend for higher active caries rate in African American children and higher decayed and filled surface rate in Hispanic children was observed (Figure 1).

Obesity and Caries

There was a significant difference in active caries rate among the three body mass categories (*P*=.020). Post Hoc Tukey analysis revealed At Risk of Becoming Obese group has a significant higher caries surface rate (*P*=.029 and .023) than Obese and Non-Obese groups (Table 4). One-way ANOVA test showed no significant difference in filling rate when compared within the groups of obesity, nor race.

Discussion

Dental caries and obesity are two lifestyle related chronic diseases that are very prevalent world-wide. Dental caries is a multifactorial disease with interplay of many factors such as microorganism, diet, socioeconomic status, genetics and behavior in its pathogenesis [14]. Obesity on the other hand has been shown to elicit pro- inflammatory substances in the body [15]. Cytokines are produced by the activation of monocyte-macrophage cells and act as mediators of infection, inflammation and immunological processes [16].

There are limited prior studies that described the cytokine profiles in saliva of children and their association with the dental caries processes. Many studies have demonstrated the role of cytokines in inflammatory process in especially in oral soft tissue lesions. For example, Fine et al. described macrophage inflammatory protein (MIP)-1a as an early indicator of periodontal disease of children [17]. CXC chemokine ligand 10 (CXCL10) [18], IL-6, IL-8, and IL-18 [19], TNF-a, IL-1b and CXCL8 [20] are linked to pulpitis in children. To our knowledge this is the first study that has demonstrated a correlation between several salivary cytokines (INFγ, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-8, IP-10, TNFα) and caries experience. More importantly, our result also showed a positive correlation between INFγ and the presence of active caries lesions. This is in line with previous studies which showed increased levels of the cytokines IL-6, tumor necrosis factor α, and IL-8 were found in caries-active saliva [21,22]. Our result also shows a correlation between salivary flow rate and IL-1β-a key cytokine that induces inflammatory mediators in periodontal diseases [23,24].

Contrary to our results, Fine, et al. [16] found in their study that dental caries was not significantly correlated with salivary or serum concentrations of IL-1β, IL-1ra, or IL-10.

Although data regarding the possible role of obesity in the pathogenesis of dental caries is confusing, many pointers suggest some linkages. Obesity is regarded as an inflammatory disease and a number of oral bacteria which are involved in disease processes in the oral cavity may also be a factor in the development of obesity by different mechanism such as (1) inducing insulin resistance (2) reducing the concentration of adiponectin and (3) increasing the concentration of some cytokines such as tumor necrosis factor-α (TNF-α) and lipopolysaccharides [25,26].

The preset study found a correlation between obesity and dental caries while there may not be direct causal relationship between obesity and caries, factors such as diet and nutrition, inflammation, changes in salivary glands and saliva composition associated with obesity may modulate caries process. As more research into this area is carried out, better understanding of these processes would become evident [27].

The results of this study should be used with caution as there a few limitations inherent in its design. These include the fact that the caries data was obtained from the charts which have been collected by different uncalibrated clinicians who however work in the same clinic with similar standard of care protocol. Secondly, time of saliva collection was not standardized and it is known that the time of saliva collection during the day may affect its quantity and quality. Finally, for ease of collection, stimulated saliva was used because many young children lack the capacity to produce unstimulated saliva. It is known that differences

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in concentration of proteins and other compositions of saliva do vary depending on whether is stimulated or unstimulated [28,29]. All these notwithstanding, our results show certain trends in the relationships of all the parameters examined and could be a source for further exploration.

Conclusions

Within the limitations of this study, the following conclusions can be reached:

1. There is a positive correlation between salivary cytokines and caries status of children and

2. there are no differences in the concentration of salivary cytokines among different body weights and races

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