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FEEDING PATTERN EFFECT ON ORAL HEALTH PARAMETERS AND SALIVARY IL-6 LEVEL AMONG PRESCHOOL CHILDREN

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Abstract

Background: *Interleukin-6 plays a role in both regenerative and anti-inflammatory processes as well as inflammatory reactions. Various cytokines can be produced as a result of caries, which can then trigger oral immune reactions. Different inflammatory states, such as gingivitis, periodontitis, and oral lichen planus, were discovered to have high cytokine levels.This study aimed to evaluate the effect of feeding patterns on oral health parameters (dental caries, gingival health status) and age by measuring the level of salivary IL-6.*

Material and methods: *The study sample included 61 children aged 4-6 years old divided into two groups (breastfeeding group and formula feeding group). The examination of the oral cavity was done on the basic method of oral health surveys of the World Health Organization by using dmfs, plaque, and gingival index according to recording protocols to measure the severity of dental caries and gingivitis. Saliva samples were collected and subjected to an enzyme-linked immunosorbent assay (ELISA) test to measure the level of IL-6.*

Results: *Data of the present study showed a highly significant difference in salivary IL-6 levels between the breastfeeding group and formula-feeding group, and the median level of IL-6 was 5.5 in the breastfeeding group while in the formula-feeding group, it was 32.2. The results were statistically significant in the breastfeeding group between IL-6 and caries rate dmfs (p=0.048), IL-6 and gingival index (p=0.035), and dmfs with age (p=0.049), while for formula feeding group these results were statistically non-significant.*

Conclusion*: In preschool-aged caries rate (dmfs) is strongly correlated to the salivary IL-6 level. IL-6 level has a potent relation to the gingival index with a positive direction so it can be used as an early indicator for the presence of gingivitis and carious lesion. Breastfeeding has a direct effect to reduce the level of salivary IL-6 so it will affect the correlation between IL-6 levels and oral health parameters.*

Keywords: *Interleukin-6, Dental caries, Gingival index, Feeding pattern, ELISA.*

INTRODUCTION

Breastfeeding is a dynamic biological process that involves the transfer of biochemical, physical, psychosocial, and endocrine substances. It is intended to transmit vital nutrients and foster a strong psychosocial connection between moms and babies [1]. According to the World Health Organization (WHO), breastfeeding should start as soon as possible after delivery and continue for the first six months of an infant's life, followed by two years of breastfeeding and complementary foods [2]. Colostrum is frequently referred to as a baby's first immunization due to its high amounts of antibodies, vitamin A, and other protective elements [3]. The antagonistic balance between the oral microbiota and the immune defense system produces the physiological composition of the oral environment. Oral inflammatory diseases are thought to form because of a changed relationship between potential pathogenicity, self-healing capacity, and immune defense [4]. Dental caries has been a widespread illness in the neighborhood for many years. It is recognized as an infectious multifactorial illness that is primarily brought on by complex interactions. By examining the dynamic interaction between pathological factors like acid-producing bacteria, fermentable carbohydrates, host factors, and protective factors like fluoride, calcium, and phosphates, it is possible to comprehend the fundamental process of dental caries. The organic and inorganic components of saliva play a crucial part in bacterial colonization and removal from the oral cavity among pathological factors [5]. The immune system, the inflammatory process, and other variables are modulated by cytokines. They will support the monitoring and diagnosis of numerous illnesses of the oral cavity. Interleukin-6 (IL-6) is one of these cytokines that has a variety of biological impacts and is a key host defense mediator [6].Children's crevicular fluid IL-6 levels can significantly rise as a result of tooth decay, particularly when it is prevalent [7]. An infection of the gingival mucosa called plaque-related gingivitis is brought on by bacteria [8]. Although it can affect anyone at any age, gingivitis is a reversible disease that affects kids and teenagers more frequently than any other age group [9-11]. Gingivitis and periodontal illnesses are risk factors for developing as a result of dental plaque buildup [12].

This study aimed to evaluate the effect of feeding patterns on oral health parameters (dental caries, gingival health status) and age by measuring the level of Interleukin-6 among children aged 4-6 years. The null hypothesis was that there is no effect of feeding type on oral health parameters and salivary Interleukin-6 level

Material and methods

The study sample included 61 children aged 4-6 years old from Baghdad city (Rusafa sector). The study protocol was approved by the Ethics Committee of Al Kut University College, Dentistry Department on 11/6/2022. Informed consent was collected from parents before the examination. After taking the questionnaire from parents the sample was divided into two groups (breastfeeding group and formula feeding group). Children with a history of systemic and autoimmune diseases were excluded from the study. The examination of the oral cavity was done on the basic method of oral health surveys by the World Health Organization [13].

Dental caries index

Dental caries examination was conducted using a plane mouth mirror and an explorer, and dental caries diagnosis was recorded according to WHO criteria (1997). All primary teeth were included in the present study; any erupted permanent teeth were excluded from examination. The assessment was recorded in a special form. The examination was started with all teeth surfaces, criteria and codes of dental caries are:

A) A sound tooth is one that shows no signs of clinical caries, either treated or untreated.

B) Decayed tooth: Caries were noted when a lesion softened the floor, undermined the enamel, or softened the wall of a pit, fissure, or smooth surface. Another tooth that had deterioration was one that had a temporary filling. When the explorer entered the lesion, it was noted that the interproximal caries were already deteriorating. The grades of caries severity are D1, D2, D3, and D4, and they range from grade 1 to grade 4.

C) Filled tooth with no decay: recorded as filling only when there were no primary or secondary caries adjacent to the filling of the same tooth. Otherwise recorded as decay.

D) Caries-related tooth extraction: Only teeth that have been extracted owing to caries were given this score.

The DMF Index When a surface count was performed, caries lesions were noted on all relevant tooth surfaces; lost teeth were counted as having five missing surfaces for posterior teeth and four missing surfaces for anterior teeth. For posterior teeth, retained roots were counted as having five decayed surfaces, while anterior teeth had four decayed surfaces.

The recording guideline for using plaque index was the following, each of the six gingival teeth surfaces was probe-examined in order to determine the amount of plaque present at the gingival teeth margin. Scores ranging from 0 to 3 were recorded. The averages for every tooth determine the score of the sample [14].

The gingival index were used to measure the gingival health, and the ratio-mean score of the teeth/number of teeth examined was used to calculate the score [15].

Saliva collection: Children were instructed to rinse their mouths and stop eating 60 minutes before saliva collection. Saliva was collected with disposable plastic pipettes and placed in sterile Eppendorf tubes.

The sample was kept in storage right away at a frigid 4 C. To eliminate mucosal components, the saliva samples were centrifuged (at 1000 RPM) for 15 minutes. After that, the clear supernatant was transferred to a different Eppendorf tube, labeled, and set up on racks before being tested using an ELISA, an enzyme-linked immunosorbent assay.

The Human IL-6 ELIZA Kit from Elabscince, USA, was used to measure the amount of IL-6 protein in saliva.
This ELISA kit uses the Sandwich-ELISA technique. A human IL-6-specific antibody has been pre-coated on the micro ELISA plate included in this kit. Samples (or standards) are placed in the micro ELISA plate wells along with the specific antibody. After that, a biotinylated detection antibody specific for human IL-6 and an Avidin-Horseradish Peroxidase (HRP) mixture are sequentially added to each microplate well. During washing, free portions are taken out. The substrate solution is poured into each well. The only wells with blue coloration are those that also have human IL-6, a biotinylated detection antibody, and an Avidin-HRP conjugate. The enzyme-substrate reaction stops when a stop solution and a color indicator are added. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Human IL-6 (**Fig 1).**

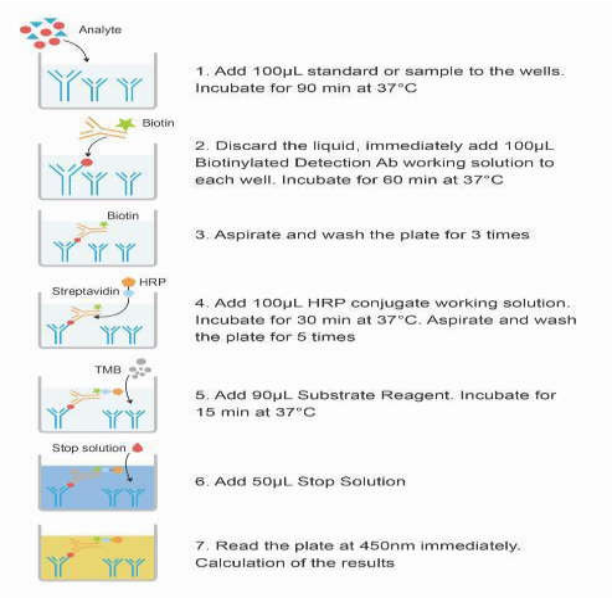

Figure 1 enzyme-linked immunosorbent assay (ELISA) test.

Statistical analysis

Computer software program as Statistical Package for Social Science (SPSS version 25) is used for data description, presentation, and analysis. Statistical comparison between the breastfeeding group and formula feeding group was done by the Mann-Whitney test for numerical parameters. This non-parametric measurement was used because our variables did not present normal distribution as verified by the Kolmogorov-Smirnov test. The Spearman correlation: was used to assess the direction, strength, and statistical significance of the linear correlation between 2 quantitative variables. The level of significance can be tested as the probability of error (P-value): Not significant P>0.05, Significant P ≤ 0.05, and highly significant P ≤ 0.01 (16).

Results

The present study included 61 children (median age of 5 yrs old) divided into two groups (breastfeeding group and formula feeding group) and examined with dmfs, plaque, and gingival index with the level of salivary interleukin- 6. This study shows MWU test and group differences between breastfeeding and formula feeding according to oral health parameters (Table 1). The P-value was non-significant for dmfs, PI and GI while it was highly significant for interleukin 6 level (p-value=0.000).
Also, this study detected that the median level of IL-6=5.5 for breastfeeding children and 32.3 for formula feeding one with a monotonic relationship between the two groups (Figure 2).
The application of Spearman's correlation and p-value between IL-6 and oral health parameters for the breast feeding group and formula feeding group in sequence (Tables 2, 3). The results were statistically significant in the breastfeeding group between IL-6 and dmfs (p=0.048), IL-6 and GI (p=0.035), and dmfs with age (p=0.049). The correlation coefficient between GI and PI for both groups was highly significant. The relation between IL-6 and age for both groups was in a negative direction, the same as between IL-6 and dmfs . For the breastfeeding group, the relation of IL-6 with PI and IL-6 with GI was moderately in the positive direction, while for the formula feeding group, the relation was negative between IL-6 and PI, and positive between IL-6 and GI.

This study shows the relation between oral health parameters and the level of IL-6 for the whole sample. There was a non-significant correlation between IL-6 and dmfs (P= 0.080) but the correlation was significant between age and dmfs (P=0.036).

The relation of dmfs was in a negative direction with IL-6 and GI, while it was in a positive direction with PI and age. This study shows a comparison between the low caries rate group(dmfs 1-10) and high caries rate group(dmfs 11-88) by MWU test, P- values were non-significant for all parameters although the level of IL-6 was slightly higher between low Scaries than high caries group (Table 5).

Table 1: 1MWU test and group differences between breastfeeding and formula feeding

Parameter	**Breastfeeding**			**Formula feeding**			**Group difference**	
	Median	Mean Rank	Sum of Ranks	Median	Mean Rank	Sum of Ranks	MWU test	p-value
Age	5	27.250	763	5	30.690	890	357	0.406
Dmfs	10.5	30.946	866.5	6	27.121	786.5	351.5	0.383
PI	0.8	27.536	771	0.75	30.414	882	365	0.509
GI	0.6	28.143	788	0.6	29.828	865	382	0.701
IL-6	5.597	14.571	408	32.223	42.931	1245	2	**0.000**

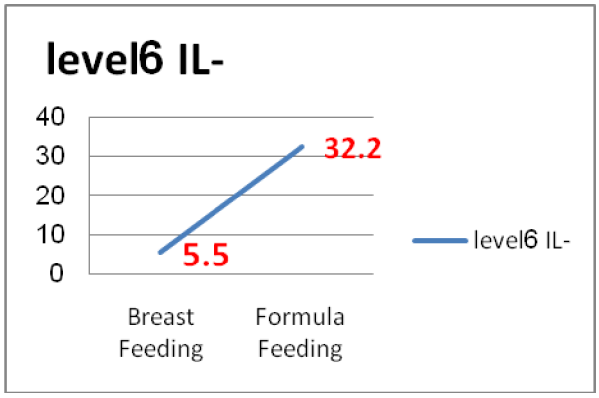

Figure 2: Monotonic relationship between the two groups

Table 2: Spearman's correlation and p-value for the breastfeeding group

Breastfeeding		IL-6	GI	PI	dmfs
Age	r	- 0.136	0.119	0.022	0.376
	P	0.490	0.548	0.911	**0.049**
Dmfs	r	-0.377	- 0.042	0.200	
	P	**0.048**	0.832	0.306	
PI	r	0.333	0.707		
	p	0.083	**0.000**		
GI	R	0.401			
	P	**0.035**			

Table 3 Spearman's correlation and p-value for the formula feeding group.

Formula feeding		IL-6	GI	PI	Dmfs
Age	R	-0.086	-0.053	-0.091	0.238
	P	0.657	0.785	0.640	0.213
Dmfs	R	-0.163	0.028	0.302	
	P	0.399	0.886	0.111	
PI	R	-0.274	0.454		
	P	0.150	**0.013**		
GI	R	0.012			
	P	0.949			

Table 4: shows the relation between oral health parameters and the level of IL-6 for the whole sample

All sample		IL-6	GI	PI	Dmfs
Age	R	0.045	-0.008	-0.049	0.278
	P	0.737	0.954	0.719	**0.036**
Dmfs	R	-0.234	-0.022	0.220	
	P	0.080	0.870	0.100	
PI	R	0.084	0.583		
	P	0.534	**0.000**		
GI	R	0.151			
	P	0.261			

Table 5: Comparison between low caries rate group and the high caries rate group

Parameters	Low caries rate			High caries rate			Comparison	
	Median	Mean Rank	Sum of Ranks	Median	Mean Rank	Sum of Ranks	MWU test	p-value
Age	5	25.6	820.5	5	33.3	832.5	292.5	0.066
PI	0.775	26.2	839.5	1	32.54	813.5	311.5	0.151
GI	0.65	30.3	968.5	0.6	27.38	684.5	359.5	0.513
IL-6	22.93	31.9	1020.5	13.97	25.3	632.5	307.5	0.137

Discussion

The focus of our study was to evaluate the effect of feeding patterns on oral health parameters (dental caries, gingival health status) and age by measuring the level of Interleukin-6 among children aged 4-6 years. In our study saliva collection was used as a diagnostic tool as it is minimally invasive and painless with minimal discomfort as compared to venous blood sampling. Sandwich immunoassay specifically designed for measuring salivary IL-6 level. ELISA has been used previously by a lot of researchers to determine the level of IL-6 [17]. In this study for the whole sample, there was a non-significant correlation between IL-6 and dmfs index while for the breastfeeding group, the correlation was significant (P value =0.035) in agreement with the previous study, especially in primary teeth [18, 19].

The establishment of a healthy oral microbiome in infants through exposure to breastfeeding and contact with skin and breast milk microbiomes may be the mechanism by which breast milk's immunomodulatory factors and rich microbiome are responsible for the initial protection against dental caries. Additionally, when new teeth come in over time, the child's oral microbiome evolves. Sugars, which can come in a variety of forms, are the primary substrate for cariogenic bacteria (e.g. glucose, lactose, sucrose). The risk of dental caries increases the longer these sugars are in contact with teeth. The amount of fermentable carbohydrates present in various milk and formula types may potentially contribute to the explanation of the variations in caries results [20, 21]. That effect increases with time and explains our result which was a significant correlation between age and dmfs index with a positive direction.Microorganisms' mutilation of dental tissues promotes the growth of dental caries, which later becomes the main cause of pulpal inflammation. Foods' steadily rising sugar content causes the bacteria in tooth plaque to adapt, turning them into acid-resistant, acid-forming organisms that enhance the creation of caries [22]. The host immune system, in addition to the microbes, is essential for tissue death. The association between tissue deterioration and IL-6 was shown in a study by Samad et al., 1994, which associated the effect of IL-6 in pulpal inflammation. The extracellular matrix metabolism and the degeneration of the tooth pulp tissue are two processes that may be facilitated by IL-6, which is produced by dental pulp cells [23]. Saliva and gingival crevicular fluid IL-6 concentrations may reveal details about the gingival inflammatory state. Children who perform poor oral hygiene practices may accumulate plaque, which causes gingival inflammation and raises inflammatory biomarker concentrations in oral fluids. It has been extensively discussed how oral hygiene, gingival inflammation, and the inflammatory response are related [24-26]. Although, our sample was aged between 4-6 years GI was strongly correlated to PI as same as a younger child. On another side, there was no significant difference in GI and PI between the breast and formula-feeding group. We can explain these results as the limited age group and not enough time for the effect of both breast and formula feeding to take place locally and systemically. The level of dmfs was in a negative direction with GI and a positive direction with PI , plaque accumulation with time in addition to food stagnation lead to caries lesion more than the effect of marginal gingivitis. The correlation coefficient between IL-6 and GI in breastfeeding children was significantly different while the relationship was in a positive direction for the whole sample. Dental plaque and poor oral hygiene is the most important risk factor in the development of gingival and periodontal diseases [27]. Another possible explanation for this result is that human breast milk contains enzymes, leucocytes, immunoglobulins, and specific anti-inflammatory agents [28, 29]. The release of inflammatory cytokines causes a modification in the host immune responses that links to a higher susceptibility to bacterial infection [30, 31]. According to research by Ebersole et al., patients with periodontal disease had significantly greater salivary IL-6 concentrations than gingivitis patients and healthy individuals [32]. Inflamed gingival tissues had higher IL-6 levels than healthy control tissues, according to Bartold and Haynes [33]. According to Lo Giudice et al., a high level of IL-6 is present when caries is active and is linked to adverse infectious effects [7], while in our study there was no difference for the level of IL-6 between the high caries rate group and low caries group. This dissimilar result was because we didn't use a control group and we couldn't determine whether the caries were active or not during an examination. Furthermore, the median level of salivary IL-6 between breastfeeding and formula feeding groups dramatically differs from 5.5 (breastfeeding) to 32.2 (formula feeding) and the difference was highly significant between them.

The complexity of breast milk, which changes as the baby's needs change, cannot be duplicated by manufactured formula feeding but it is considered an efficient replacement for breast milk that mimics the nutritional profile of breast milk. Infant formula must, among other things, comply with the FDA's (Food and Drug Administration) updated guideline on current good manufacturing practices. This rule specifies that formulas must meet the quality criteria of normal physical growth and a sufficient biological quality of protein components. Adequate amounts of protein in a form that can be used by infants [34].

Conclusions

In preschool-aged caries rate (dmfs) is strongly correlated to the salivary IL-6 level in addition to the direct effect of increasing age and plaque accumulation. Salivary IL-6 level has a potent relation to the gingival index with a positive direction so it can be used as an early indicator for the presence of gingivitis and carious lesion.

Breastfeeding has a direct effect to reduce the level of salivary IL-6 so it will affect the correlation between IL-6 level and oral health parameters, so the type of feeding must be registered when we study the effect of any biomarker on oral health parameters. Further studies are needed with larger sizes and different geographical sites with a longer follow-up and also study the effect of another salivary biomarker on oral health parameters.

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