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The Effect of Type of Feeding on Salivary Immunoglobulin A (S-IgA) and Total Protein in Relation to Caries Severity among Children Aged 4-5 Years Old

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Abstract: ***Human milk is the best source for infant feeding and nutrition and has many short- and long-term medical advantages. Oral immunity as a part of body immunity may be affected by type of feeding. This study was conducted to determine the accumulative effects of type of feeding on concentration of salivary S-IgA, total salivary proteins and to determine the effect of type of feeding on caries severity. Seventy five children aged 4 and 5 years selected from children attending the Preventive clinic of Dental College in Baghdad University. They classified into three groups (breastfed group, bottle-fed group and mixed-fed group). The diagnosis of dental caries was done according to (d_{1-4} mfs) criteria for primary teeth. Stimulated saliva was collected from children under standardized conditions and chemically analyzed to determine the concentration of S-IgA by using ELISA kit and to determine concentration of salivary total proteins calorimetrically by using ready-made kits and spectrophotometer machine. Results showed that salivary S-IgA and Total proteins were normally distributed among those children according to Shapiro-Wilk test. Concentration of S-IgA was highest among breastfed children followed mixed-fed and finally by bottle-fed children (15.25mg/dl ± 0.63, 13.40mg/dl ± 0.38, 12.44mg/dl ± 0.32) respectively. Differences in concentrations were statistically highly significant according to analysis of variance. S-IgA was highly significant higher among breast and mixed fed children than that of bottle-fed children according to LSD test (p<0.01.). Concentration of total salivary proteins was highest among breastfed followed bottle-fed and finally mixed-fed (77.75 mg/dl ± 20.98, 67.25 mg/dl ± 26.50, 63.65 mg/dl± 17.29) respectively. Differences in these concentrations were statistically significant according to analysis of variance. Total proteins were significantly higher among breastfed than that of bottle-fed groups (p<0.05) according to LSD test. Fraction of caries severity (not normally distributed according to Shapiro-Wilk test) showed statistically no significant differences among the study groups according to Kruskal-Wallis test. Regarding effect of S-IgA on caries severity, statistically significant negative correlations were recorded between S-IgA and d_1 (p<0.05) and highly significant negative correlations were recorded with ds, dmfs d_2 and d_3) (p<0.01). In conclusion breastfeeding enhance oral immune system by increasing concentration of salivary S-IgA and salivary total protiens. Salivary S-IgA protects teeth against initiation and progression of dental caries. Type of feeding may has no effect on caries severity due to multifactorial nature of caries.***

Keywords: Type of feeding among preschool children; salivary S-IgA; salivary total proteins; caries severity

1. Introduction

The health benefits of breastfeeding are well recognized all around the world. Breast milk is considered to be a live substance with unparalleled immunological and anti-inflammatory properties that protect against illnesses and diseases for both mothers and children [1, 2]. Although immune system disregulations is multifactorial in origin but type of feeding still the most important factor affecting immune system development [3, 4]. One of the important immunologic factors in breast milk is secretory immunoglobulin A (S-IgA) that formed against mother's previous exposure to infectious pathogens. These antibodies transmitted to infant and adapted to respiratory and gastrointestinal mucous membrane to remain there throughout the life, they bind to potential pathogens and prevent their attachment to infant cells [5, 6]. In addition to S-IgA that adapt to mucous membrane of oral cavity, other S-IgA formed inside major and minor salivary glands and continuously secreted with saliva that paths oral cavity. These antibodies bind to cariogenic bacteria (especially *streptococcus mutans*) and thus reduce accumulation of these bacteria on enamel surface [7, 8, 9]. Previous studies focused on the effect of type of feeding on dental caries and cariogenic bacteria [10, 11]. There is a limitation in Iraqi studies regarding the effect of infant feeding on oral immunity. On the other hand salivary total proteins are considered as important protective factors in saliva. These proteins include (lysozyme, lactoferrin, mucin, agglutinin and histatin) in addition to several polypeptides, protect teeth against decay by their bacteria killing activity and buffering capacity of some of them [12, 13]. No previous study determine whether type of feeding affect concentrations of these proteins. For all of the above and in order to understand whether type of feeding affects concentrations of salivary S-IgA and total proteins in saliva and whether it affects caries severity (as accumulative effect) this study was conducted.

2. Materials and Methods

The sample: The study group consisted of 75 children who were selected from those attending the Preventive Clinic, College of Dentistry, University of Baghdad, with ages of 4 and 5 years of both genders. Collection of data extended during the period from 1/3/2015 to 1/5/2015. Each child's mother was asked about type of child feeding during the first year of infancy and according to type of feeding those children were classified into three groups (breastfed group, bottle-fed group and mixed-fed group), each group was consist of 25 child to compare between them. For each volunteer's parents the objectives of the study were

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explained to, and they approved to participate. This work had been approved by the ethical committee in college of dentistry/ University of Baghdad.

Collection of saliva and recoding of caries: Stimulated saliva was collected from children. Each child was sited in relaxed position without any heavy physical stress and was asked to chew a small piece of Arabic gum then to remove all saliva by expectoration and saliva collected in sterile screw capped bottle [(14)]. Each salivary sample was then centrifuged by centrifugater at 3000 r.p.m. (revolution per minute) for 10 minutes. Salivary supernatant was stored at (-20ºC) in polyethylene tubes for subsequent chemical analysis. Clinical examination of teeth was conducted by using plain mouth mirror and dental explorer. Assessment and recording of caries experience was done by the application of decayed, missing, filled index (dmfs for primary teeth). The diagnosis of dental caries was according to (d_{1-4} mfs) criteria for primary teeth [(15)].

Analysis of salivary sample and data:
Each salivary sample was classified into two tubes one for analysis of S-IgA and the other for analysis of total salivary proteins after one thawing for both analysis. Total salivary proteins were analyzed by using readymade kit (Protein.U.S, Pyrogallol method, Syrbio diagnostic reagent, Paris-France) where protein modify spectrum of absorption of the complex Pyrogallol red molybdate. Globlins together with albumin react. The optimal density read at 598nm is proportional to the concentration of proteins. Each sample was analyzed by UV visible recording Spectrophotometry (Cecil CE 7200 UK) machine. On the other hand analysis of salivary S-IgA was carried out by using Abcam's IgA Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab 137980, USA) that designed for the quantitative measurement of IgA in plasma, serum, urine, saliva, milk, and cell culture supernatants. The principle of the procedure is that, An IgA specific antibody has been precoated onto microwells plates. When test samples are added to the microwells, antigen from the specimen is captured by antibodies coated onto the microwells surface and subsequently an IgA specific biotinylated detection antibody is added, then unbounded material is removed by washing procedure. Second antibody Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away. TMB (Tetra Methyl Benzidin) is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of IgA captured in plate (photometry at 450nm). Data were analyzed by using SPSS software version 19 (Statistical Package for Social Sciences) by application of both descriptive statistic including (number, percentage, mean, median, mean rank and standard deviation) and interferential statistic including (Shapiro-Wilk test, Analysis of Variance (One Way ANOVA test), Spearman correlation coefficient and Kruskal-Wallis test). The confidence limit was accepted at 95% ($P < 0.05$).

3. Results

By distribution of total sample according to age and gender it had been found that boys aged 5 years were the highest among those children (42.67%), while girls aged 4 years were the lowest among them (16.00%) as recorded in Table (1). Normality distribution of sample was tested by application of Shapiro-Wilk test. Results showed that concentration of salivary S-IgA and Total protiens were normally distributed, while grads of caries severity were not normally distributed so non-parametric testes were applied. Concentration of salivary S-IgA was highest among children of breast feeding followed by that of mixed feeding and finally by that of bottle feeding according to results recorded in Table (2). Differences of means of salivary S-IgA concentration were statistically highly significant among these three groups according to ANOVA test (analysis of variance). By application of LSD, difference was found to be between breastfed and bottle- fed groups on one hand (mean difference= 1.63, P= 0.00) and between mixed-fed and bottle-fed groups on the other hand (mean difference= 1.07, P= 0.00). Concentration of salivary total proteins was highest among children of breast feeding, followed by that of bottle feeding and finally by that of mixed feeding. Differences of means of salivary total proteins concentration were statistically significant between these three groups according to ANOVA test (analysis of variance). By application of LSD, difference was found to be between breastfed and bottle-fed children (mean difference= 13.32, P= 0.06). Caries severity among children of each feeding group was recorded in Table (3). Differences in results of all fraction of caries severity among feeding groups were statistically not significant. According to the results recorded in Table (4); negative, weak and statistically highly significant correlations were recorded between ds, dmfs d_2 and d_3 and S-IgA; furthermore weak, negative and statistically significant correlations were recorded between d_1 and S-IgA. While all correlations recorded between fraction of caries severity and salivary total proteins, were not significant.

4. Discussion

Mother of each child attending the Preventive clinic during two months period was asked about type of feeding of her child. Seventy five of those children were selected to be involved in this study, in order to classify in to three groups according to their feeding type. Results revealed that concentration of salivary S-IgA was highest among children who were breastfed, followed by that of children who were mixed-fed, and finally followed by that of children who depended on bottle in their feeding. Concentrations among breastfed and mixed-fed children were statistically highly significant higher than those of bottle-fed children. S-IgA is an essential biomarker for the local defense of the mouth [(16)]. Major amount of S-IgA that found in oral cavity is formed in major salivary glands (especially parotid and submandibular glands) and some of minor salivary glands and then secreted with saliva that continuously paths oral cavity [(7, 8)]. Secretion of S-IgA in the mouth depends on the general health of the organism and its immunity that found to be improved by breast feeding [(17)]. Results achieved by this study give an indication that breast feeding may enhance tissues and

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organs responsible for formation of S-IgA in saliva [16, 18]. In addition to that by breastfeeding S-IgA transmit to child from mother's breast milk and adapted to oral mucous membrane that remain throughout the life [5, 6]. This may explain that why children (involved in this study) depended on pure breastfeeding or depended on breastfeeding mixed with formula milk during infancy recorded higher concentrations of salivary S-IgA than those depend on formula milk only.

Regarding total salivary proteins, results showed that concentrations of these proteins were highest among breastfed children, followed by that of bottle-fed and finally by that of mixed-fed children. But difference in concentration was statistically significant between breastfed and bottle-fed groups only. These results may indicate that mother milk would enhance growth and development of salivary glands and their surrounding tissues that responsible for building of oral immune system that considered as a part of body immune system [6, 7, 17]. This could explain why these proteins were higher among breastfed children than that of bottle-fed children.

Although type of feeding had effect on caries experience as mentioned by many studies [19, 20, 21], results of this study recorded differences in fractions of caries severity, but all of these differences were statistically not significant, the same results were recorded by other Iraqi studies [10, 11]. Breastfeeding may improve teeth composition and oral immune system [22], but dental caries is multifactorial in its nature and its initiation may be largely depend on composition of outer enamel surface that undergo demineralization and remineralization process, so this surface is continuously affected by factors of oral environment rather than accumulative effect of type of feeding [23, 24]. This could be the cause of results achieved in this study.

When Spearman correlations between salivary S-IgA concentration and caries severity were tested, results showed that, when salivary S-IgA concentration increased, caries severity decreased. One could expect that these results represent the effect of S-IgA in separation of the effect of other factors. However, these results may indicate that salivary S-IgA protects teeth against initiation of caries represented by negative correlations with d**1** (enamel caries) by fighting bacteria responsible for initiation of caries (*streptococcus mutans*) [25]. Furthermore, salivary S-IgA protected teeth against progression of caries represented by negative correlations with d_3 (dentine caries) by fighting bacteria responsible for progression of caries (*Lactobacilli*) [25]. All of these results could be responsible for negative correlation recorded between concentration of S-IgA in saliva and caries-experience (dmfs). While total salivary proteins showed statistically no significant correlations with all fractions of caries severity, this could be due to multifactorial nature of caries as mentioned previously.

5. Conclusion

Breastfeeding enhance oral immune system by increasing concentration of salivary S-IgA and salivary total protiens. Salivary S-IgA protects teeth against initiation and progression of dental caries. Type of feeding may has no effect on caries severity due to multifactorial nature of caries.

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Table 1: Description of sample according to age and gender

Age of group in years	Boys		Girls		Both	
	No	%	No	%	No	%
4 years	14	18.67	12	16.00	26	34.67
5 years	32	42.66	17	22.67	49	65.33
Both	46	61.33	29	38.67	75	100

Table 2: Concentrations of salivary IgA and Total protein among children according to type of feeding

Type of feeding Concentrations	Breast	Bottle	Mixed	Anova test	
	Mean ± SD	Mean ± SD	Mean ± SD	F	Sig.
IgA (mg/dl)	15.25 ± 0.63	12.44 ± 0.32	13.40 ± 0.38	229.57	0.00**
Total protein (mg/dl)	77.75 ± 20.98	67.25 ± 26.50	63.65 ± 17.29	2.85	0.06*

*SD=Standard deviation, * significant, ** highly significant.*

Table 3: Caries severity among children according to types of feeding

Type of feeding Fraction of caries	Breast			Bottle			Mixed			Kruskal-Wallis test	
	Mean ± SD	Median	Mean rank	Mean ± SD	Median	Mean rank	Mean ± SD	Median	Mean rank	Chi-square	Sig.
ds	5.68 ± 6.12	3.00	37.34	4.68 ± 3.09	4.00	38.50	5.68 ± 5.89	5.00	38.16	0.038	0.98
ms	0.92 ± 2.21	0.00	39.66	0.40 ± 1.38	0.00	36.50	0.56 ± 1.55	0.00	37.84	0.833	0.65
fs	0.92 ± 2.49	0.00	37.36	1.00 ± 1.97	0.00	41.26	0.44 ± 1.63	0.00	35.38	2.040	0.36
dmfs	7.52 ± 8.27	6.00	37.68	6.04 ± 3.58	7.00	39.02	6.68 ± 6.53	5.00	37.30	0.087	0.95
d_1	1.00 ± 1.44	0.00	33.10	1.60 ± 1.73	1.00	40.28	1.52 ± 1.63	1.00	40.62	2.112	0.34
d_2	2.88 ± 4.05	2.00	37.30	2.56 ± 2.16	2.00	40.30	3.04 ± 4.64	1.00	36.40	0.463	0.79
d_3	0.32 ± 0.85	0.00	34.14	0.40 ± 0.76	0.00	37.08	0.84 ± 1.34	0.00	42.78	3.166	0.20
d_4	0.84 ± 2.15	0.00	39.82	0.12 ± 0.44	0.00	36.32	0.28 ± 0.89	0.00	37.86	1.017	0.60

Table 4: Correlations between caries severity and salivary immunoglobulin (IgA) and total proteins

Fraction	Salivary constituents			
	IgA		Total proteins	
	r	Sig	r	Sig
ds	- 0.44**	0.00	0.00	0.95
ms	- 0.05	0.62	- 0.06	0.59
fs	- 0.20	0.08	- 0.03	0.75
dmfs	- 0.46**	0.00	0.00	0.99
d_1	- 0.26*	0.02	- 0.13	0.24
d_2	- 0.39**	0.00	0.15	0.19
d_3	- 0.34**	0.00	- 0.19	0.31
d_4	- 0.11	0.33	- 0.08	0.48

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