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Evaluation of salivary calcium, phosphorus and alkaline phosphatase level in children (4-6 years) with nursing caries in Erbil City

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Zana Qadir Omer* Baydaa Adnan Khoshnaw**

Abstract

Background and objective: Nursing caries is a complex and severe form of tooth decay that affects a child's teeth and leads to severe pain, teeth loss, and psychological problems. This type of decay is caused by primary factors (host, cariogenic bacteria, fermentable carbohydrate and the time) and other secondary factors. Besides, the dental caries influence by the role of saliva as a defense system. These defense systems include clearance, buffering, antimicrobial agents, and calcium and phosphate delivery for remineralization. This study aimed to find out the relationship between calcium, inorganic phosphorus and alkaline phosphatase of unstimulated saliva in children with nursing caries.

Methods: The sample included 374 children aged 4-6 years; 324 were the study group (with nursing caries) while 50 were control group (caries-free). The case sheet and questionnaire included the child's feeding habits and oral hygiene. A 1.5-2 ml of saliva was collected from the selected children using spitting technique. The method included dental examination for the recording of the dmft and dmfs indices, and saliva analysis to determine the level of salivary calcium, phosphorus and alkaline phosphatase in control and study groups.

Results: The study group children demonstrated a statistically highly significant ($P \leq 0.01$) higher saliva calcium (5.229 ± 0.963 mg/dl) and alkaline phosphatase concentrations (6.321 ± 1.792 KAU/dl) than the control group (4.968± 0.757mg/dl, 5.384 ± 2.119 KAU/dl), respectively. The same group demonstrated a statistically highly significant ($P \leq 0.01$) lower inorganic phosphorus concentration (10.991 ± 1.376 mg/dl) than the control group (11.961 ± 1.484 mg/dl). The study group showed a lower percentage of breastfeeding (28.4%) in comparison to the control group (42.0%). Concerning mix-feeding, the study group showed the higher percentage (45.4%) in comparison to the control group (32.0%). The bottle-fed found to be equal in both groups, there was a statistically non-significant difference between the study and control group.

Conclusion: Salivary calcium and alkaline phosphatase level have a major role in causing nursing caries in children. On the other hand, salivary inorganic phosphorus level showed a negative correlation with caries activity (dmfs). The type of infant feeding has no effect on the children with nursing caries.

Keywords: Saliva; Nursing caries; Calcium; Alkaline phosphatase; dmft; dmfs.

Introduction

Nursing caries has been described since 1862. It is a serious oral health problem, especially in disadvantaged communities of both developing and industrialized countries, and it continues to have an increased prevalence of dental diseases in infants and children.[1] Dental caries is a pathologic oral environment, where it is an infectious bacterial biofilm disease. Although acid generating bacteria are the etiologic agents, dental caries has been thought of as multifactorial since it is influenced by dietary and host factors as

* Department of Peado, Orthod & Preventive, College of Dentistry, Hawler Medical University, Erbil, Iraq.
** Erbil health Directorate, Erbil, Iraq.

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well. Besides, the dental caries influence by the role of saliva as a defense system. These defense systems include clearance, buffering, antimicrobial agents, and calcium and phosphate delivery for re-mineralization .therefore the caries process is dependent upon the interaction of protective and pathologic factors in saliva and plaque biofilm as well as the balance between the cariogenic and non-cariogenic microbial populations that reside in saliva.[2,3] The early loss of deciduous teeth may create functional and esthetic problems and may lead to the loss in arch length that may interfere with the proper alignment of the permanent teeth. Therefore, preventive measures should be directed to retain primary teeth until their normal shedding time.[4] Iraqi studies were present regarding the correlation between salivary calcium, phosphate and alkaline phosphatase with dental caries.[5] While no previous study about this subject had been done in Erbil city, therefore, this study was carried out as an attempt to determine the relation of calcium, phosphorus and alkaline phosphatase in unstimulated saliva with respect to dmfs index of nursing caries.

Methods

Design and sample collection

The salivary sample collection from children in schools and kindergartens in Erbil city center was done. The total sample of the present study consisted of 1050 children (both genders) aged 4-6 years. After examination, only 374 children participated in the study. All children were healthy and had no history of any systemic disease. Permission was obtained from parents for including their children in the study. The sample was depending on Kurdistan region statistical office in Erbil city, also depending on the last prevalence of nursing caries among children in Erbil city at 2010, where the result was 70%.[6] The following equation used to estimate the sample size: $n= Z^2 P (1-P) / d^2$ where n = sample size, Z= Z statistic for a level of confidence, P= expected prevalence proportion and d= precision.

The samples were divided into two groups: Study group (Nursing group) and Control group (Caries- free). The study group was 324 children (170 boys, 154 girls). They had the following specific criteria of nursing caries:[7] Maxillary incisors were involved by the carious lesion, the child might have one surface at least lingual, facial, or proximal is involved in all four incisors. In addition to the occlusal surface of first primary molars and/or second primary molars without mandibular incisors carious. (Figure 1). The control group was 50 (25 boys, 25 girls) children matched for age and gender chosen from the same kindergarten and school; they were caries-free.

Exclusion criteria: a history of any systemic disease or metabolic diseases will be reported by parents, primary mandibular incisor teeth with caries because this criterion refers to rampant caries, newly erupted permanent mandibular incisor and or molar teeth, and children who are taking medication.

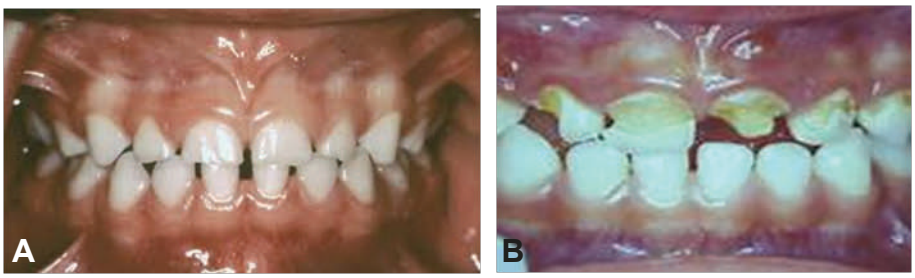

Figure 1: A- Oral cavity of 5 years old child with healthy teeth. **B-** Oral cavity of 5 years old child with the cervical carious lesion and proximal caries of upper primary incisors.

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Dental Examination: selected children with nursing caries were examined by using mouth mirror and dental explorer. The teeth were dried using cotton rolls, then the diagnosis regarding (dmft and dmfs) decayed, filled surfaces, and missing deciduous teeth were gained according to the criteria of (WHO, 1987).[8] Starting with the upper right second primary molar, to the upper left primary molar, then to the lower left second primary, ending with the lower right second primary molar.

Calculation of the dmft and dmfs indices: dmft Information on the decayed, missing, filled surfaces of the deciduous teeth was calculated as follows: d: included all teeth with code B-(decayed surface) and C-(filled surface with decay). m: included all teeth with code E-(teeth missing due to caries). f: included teeth with code D-(filled teeth with no caries).[8]

Saliva Analysis: Saliva samples were collected in the morning between (10-11.30) a.m. at least one hour after breakfast, and then the children were asked to rinse out their mouths with water. The first mouthful of saliva was discarded, and then two ml of unstimulated (resting) whole saliva was collected into small labeled plastic polyethylene tubes using spitting method for collection.[9,10] The supernatant (After saliva collection, each sample was centrifuged at 1800 rpm for 5 minutes at room temperature to remove debris) was stored in the refrigerator at $4C^0$ in polyethylene tubes until biochemical analysis within the maximum period of 24 hours. Biochemical analysis of the saliva done According to Baiolabo Reagents Kit (02160, Maizy, France). Salivary calcium was measured by using CPC (O-CresolPhtalein Complexne) method, while salivary phosphorus was measured by UV method.[11,12] Alkaline phosphatase activity measured by using a Colorimetric method. According to the following:

Phenylphosphate $\xrightarrow{\text{Alkalinephosphatase}}$ phenol+ phosphate

Free phenol liberated by hydrolysis of the substrate reacts then with 4-amino–antipyrine in the presence of alkaline potassium ferricyanide to form a red-colored complex which absorbance measured at 510 nm is directly proportional to the ALKP activity in the specimen.[13,14]

Statistical Analysis: Data processing and analysis were carried out using the SPSS program. Data analysis included calculation and presentation of statistical parameters of means and standard deviation of the clinical and biochemical variables, examination of the significance of the difference between mean values of the examined variables applying Student's t-test and estimation of the significance of difference among percentages using Chi-square test. Pearson's correlation was used for measuring correlation coefficient between caries experience and concentrations of studied salivary elements. A *P* value more than 0.05 was regarded as non-significant, a *P* value less 0.05 and more than 0.01 was regarded as significant, while a *P* value less than 0.01 was regarded as highly significant.

Results

The total sample was 374 children with an age ranging from 4-6 years old; 324 of them were the study group who had nursing caries, and 50 of them were the control group who were caries free. The gender distribution in the study group was 170 (52.5%) male and 154 (47.5%) female as shown in Table 1. Caries experience among study group is shown in Table 2 demonstrates the mean values and SD of dmfs in males and females in the study group. Females showed a slightly lower mean value of dmfs than that of males (8.448 ± 2.58, 8.594 ± 2.84) with the statistically non-significant difference (*P* >0.05). Salivary Calcium concentration was shown in Table 3 and demonstrates the mean values and standard deviation of salivary calcium concentration in study and control group. For the total sample, the results show a higher concentration of salivary calcium in the study group

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(5.229 ± 0.963 mg/dl) than that of the control group (4.968 ± 0.7574 mg/dl) with the statistically highly significant difference.

Table 1: Distribution of the patients according to the age and gender.

Age	Gender	Study group		Control group	
		No.	%	No.	%
4 years	M	49	50.5%	8	47.1%
	F	48	49.5%	9	52.9%
	Total	97	100.0%	17	100.0%
5 years	M	56	51.4%	9	52.9%
	F	53	48.6%	8	47.1%
	Total	109	100.0%	17	100.0%
6 years	M	65	55.1%	8	50.0%
	F	53	44.9%	8	50.0%
	Total	118	100.0%	16	100.0%
Total	M	170	52.5%	25	50.0%
	F	154	47.5%	25	50.0%

M= male, F= female

Table 2: Caries experience (Mean dmfs± S.D) between both genders in different age groups.

Age	Male			Female			t-test	*P* value
	No.	Mean	S.D	No.	Mean	S.D		
4 years	49	8.694	3.077	48	8.521	2.737	0.293	0.770
5 years	56	7.964	2.822	53	8.396	2.405	-0.862	0.391
6 years	65	9.062	2.603	53	8.434	2.664	1.286	0.201
Total	170	8.594	2.84	154	8.448	2.585	0.485	0.628

Table 3: A comparison of salivary calcium concentration (mg/dl) between study and control groups according to age and gender.

Age	gender	Study group			Control group			t-test	*P* value
		No.	Mean	±S.D	No.	Mean	±S.D		
4 years	M	49	5.119	.866	8	5.209	.788	-.306	0.766
	F	48	5.249	.982	9	5.159	.772	.308	0.763
	Total	97	5.184	.922	17	5.182	.755	61.685	< 0.001
5 years	M	56	5.109	1.027	9	5.032	1.026	.209	0.838
	F	53	5.119	.939	8	4.605	.352	2.870	0.0008
	Total	109	5.114	.980	17	4.831	.793	59.364	<0.001
6 years	M	65	5.427	1.004	8	4.505	.496	4.287	0.001
	F	53	5.356	.923	8	5.268	.671	.404	0.694
	Total	118	5.395	.965	16	4.886	.693	64.949	<0.001
Total	M	170	5.218	.980	25	4.920	.833	1.710	0.096
	F	154	5.241	.946	25	5.016	.6721	1.455	0.153
	Total	324	5.229	.963	50	4.968	.750	106.874	< 0.001

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Salivary Inorganic phosphorus concentration was shown in Table 4 and demonstrates the mean values and standard deviation of salivary inorganic phosphorus concentration in study and control groups. For the total sample, the results showed a lower concentration of salivary inorganic phosphorus in the study group (10.991 ± 1.376mg/dl) than that of the control group (11.916 ± 1.484mg/dl) with statistically highly significant differences between them (t = 152.257, *P* ≤0.01). Salivary alkaline phosphatase activity explained in Table 5 demonstrates the mean values and standard deviation of salivary alkaline phosphatase concentration in study and control groups. For the total sample, the results show a higher concentration of salivary Alkaline phosphatase in the study group (6.321 ± 1.792KAU/dl) than that of the control group (5.384±2.119KAU/dl) with statistically highly significant differences (t = 64.446, *P* <0.01).

Table 4: A comparison of salivary inorganic phosphorus concentration((mg/dl) between study and control groups according to age and gender.

Age	Gender	Study group			Control group			t-test	*P* value
		No.	Mean	S.D	No.	Mean	S.D		
4 years	M	49	10.860	1.188	8	11.961	1.373	-2.139	0.062
	F	48	11.066	1.100	9	11.295	1.191	-.537	0.602
	Total	97	10.962	1.144	17	11.608	1.285	99.809	<0.001
5 years	M	56	11.009	1.404	9	11.917	1.747	-1.485	0.169
	F	53	11.140	1.515	8	12.830	1.033	-4.016	0.002
	Total	109	11.073	1.454	17	12.347	1.487	83.212	<0.001
6 years	M	65	10.937	1.525	8	11.621	1.449	-1.252	0.242
	F	53	10.945	1.438	8	11.947	1.347	-1.948	0.081
	Total	118	10.941	1.481	16	11.784	1.362	85.246	<0.001
Total	M	170	10.939	1.388	25	11.836	1.484	-2.846	0.008
	F	154	11.050	1.364	25	11.995	1.315	-3.313	0.002
	Total	324	10.991	1.376	50	11.916	1.390	152.257	< 0.001

Table 5: A comparison of salivary alkaline phosphatase activity (KAU/dl) between study and control groups according to age and gender.

Age	Gender	Study group			Control group			t-test	*P* value
		No.	Mean	S.D	No.	Mean	S.D		
4 years	M	49	6.445	1.586	8	4.630	.732	5.274	<0.001
	F	48	6.298	1.632	9	6.275	3.453	.019	0.986
	Total	97	6.372	1.601	17	5.501	2.629	36.923	<0.001
5 years	M	56	6.112	1.805	9	6.064	2.038	-.766	0.461
	F	53	6.210	1.604	8	6.181	1.679	-.285	0.782
	Total	109	6.161	1.704	17	6.122	1.836	39.887	0.000
6 years	M	65	6.268	1.809	8	4.706	1.361	2.942	0.014
	F	53	6.615	2.219	8	4.371	1.328	3.986	0.001
	Total	118	6.424	2.002	16	4.539	1.311	64.949	<0.001
Total	M	170	6.268	1.741	25	5.133	1.743	2.360	0.025
	F	154	6.374	1.853	25	5.636	2.469	1.297	0.205
	Total	324	6.321	1.792	50	5.384	2.119	64.446	<0.001

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The correlation between salivary calcium concentration and caries experience (dmfs index) is shown in Table 6 of the study group according to the age and gender. The total sample of the study group demonstrated a positive correlation with statistically highly significant. The correlation between salivary inorganic phosphorus concentration and caries experience is shown in Table 7 demonstrates the correlation coefficient between the salivary inorganic phosphorus concentration and dmfs index of the study group according to the age and gender. A total sample of the study group demonstrated a negative correlation with statistically highly significant.

Table 6: Correlation coefficient between calcium concentration in saliva and dmfs index of the study group according to the age and gender.

Age	Gender	No.	R	*P* value
4 years	M	49	.716	<0.001
	F	48	.482	0.001
	Total	97	.592	<0.001
5 years	M	56	.808	<0.001
	F	53	.539	<0.001
	Total	109	.697	<0.001
6 years	M	65	.269	0.030
	F	53	.690	<0.001
	Total	118	.453	<0.001
Total	M	170	.586	<0.001
	F	154	.575	<0.001
	Total	324	.580	<0.001

Table 7: Correlation coefficient between inorganic phosphorus concentration in saliva and dmfs index of the study group according to the age and gender.

Age	Gender	No.	r	*P* value
4 years	M	49	-.465	0.001
	F	48	-.229	0.017
	Total	97	-.357	<0.001
5 years	M	56	-.723	<0.001
	F	53	-.425	0.002
	Total	109	-.580	<0.001
6 years	M	65	-.330	0.007
	F	53	-.494	<0.001
	Total	118	-.399	<0.001
Total	M	170	-.489	<0.001
	F	154	-.409	<0.001
	Total	324	-.454	<0.001

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The correlation coefficient between the salivary alkaline phosphatase concentration and dmfs index of the study group according to the age and gender are shown in Table 8. A total sample of the study group demonstrated a positive correlation with statistically highly significant. Figure 1 demonstrates the comparison between study and control groups concerning the type of infant feeding (breastfeeding, bottle feeding, or mixed feeding). The results found that the study group had a lower percentage of breastfeeding (28.4%) in comparison to the control group (42.0%). Concerning mix -feeding, the study group showed the higher percentage (45.4%) in comparison to the control group (32.0%). The bottle-fed found to be equal in both groups, there was the statistically non-significant difference between the study and control group (*P* = 0.109).

Table 8: Correlation coefficient between Alkaline phosphatase in saliva and dmfs index of the study group according to the age and gender.

Age	Gender	No.	R	*P* value
4 years	M	49	.441	0.002
	F	48	.499	<0.001
	Total	97	.468	<0.001
5 years	M	56	.671	<0.001
	F	53	.556	<0.001
	Total	109	.621	<0.001
6 years	M	65	.342	0.005
	F	53	.554	<0.001
	Total	118	.432	<0.001
Total	M	170	.475	<0.001
	F	154	.539	<0.001
	Total	324	.502	<0.001

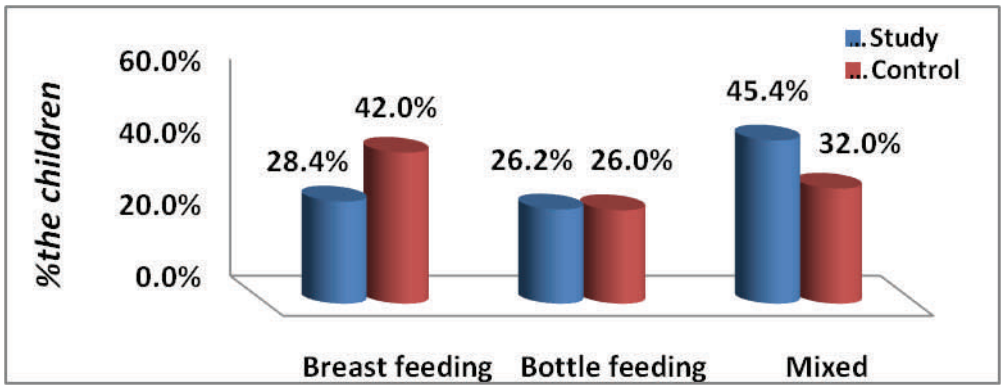

Figure 1: Bar chart graph showing the percentage of the children of the study and control groups, concerning the type of feeding.

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Discussion

Despite advancements in oral disease science, dental caries continues to be a worldwide health concern, affecting humans of all ages, especially children where caries disease is on the rise.[15] Therefore this study conducted at school and kindergartens children to confirm known risk factors and identify the effect of some salivary elements on the nursing caries. Findings of this study showed differences in the results as compared with other studies which may be due to the differences in method of collection of salivary sample whether stimulated or unstimulated type, techniques of biochemical analysis and sample age group. The results of this study showed the higher concentration of salivary calcium in children with nursing caries (5.236±0.963mg/dl) than that of caries-free children (4.968±0.750mg/dl) which was statistically with highly significant differences. This positive correlation of salivary calcium levels with dental caries could be attributed to that the solubility of the mineral phase of enamel depends on, firstly on the composition of the tooth and secondly, the effect of pH of the surrounding environment or may be due to variation in the sample size. Acid produced by bacterial plaque may lead to a fall in the pH of fluid medium to even less than 5.5 (critical pH value) resulting in undersaturation of the fluid medium with respect to hydroxyapatite causing dissolution of hydroxyapatite crystallites to their ionic component in response to the increasing concentration gradient, calcium diffused from the tooth toward the surrounding environment.[16] This finding agrees with many other studies.[17, 18, 19] The results of this study showed that lower salivary inorganic phosphorus concentration of nursing caries children (10.99±1.37mg/dl) than that of the caries free children (11.916±1.390mg/dl) with statistical highly significant differences between them. On the other hand, a negative correlation between the salivary concentration of inorganic phosphorus in children with nursing caries and dmfs index was found, and the statistical difference was at highly significant level. The negative correlation of salivary phosphorus with caries may be due to the cariostatic action of phosphate. Phosphate has a protective role in relation to dental caries due, in part, to its ability to maintain the saturation of saliva with phosphate ions; a state favors the integrity of the mineral phase of the teeth.[20] Phosphate can probably interfere with the adherence of pellicle and plaque bacteria to enamel surface. It can also inhibit bacterial growth.[21] This finding is in agreement with many other researchers.[22,23] While these findings disagree with some studies.[24,25] This study found that higher activity of alkaline phosphatase in the saliva of nursing caries children (6.321±1.792KAU/dl) than that of the caries-free children (5.384±2.11KAU/dl) with highly significant differences. This study found the positive correlation between salivary alkaline phosphatase activity and dmfs index of nursing caries and statistically at high significant differences. This positive correlation of salivary alkaline phosphatase could be attributed to pathogenic flora, when a periodontal tissue becomes diseased or its cells becomes damaged due to edema or destruction of a cellular membrane by bacterial infection, these intracellular enzymes are increasingly released into the GCF (gingival crevicular fluid) and saliva where their activity can be measured.[26] Variation in the level of alkaline phosphatase affects the ionic concentration of phosphate and calcium, which in turn can alter the equilibrium of demineralization and remineralization. [27] Caries –free children showed a higher percentage (42.0%) than that of children with nursing caries (28.4%) who concerning breastfeeding practice, while children with nursing caries who had practice bottle feeding showed an equal percentage (26.2%) with that of caries–free children (26.0%), but mixed practice

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showed a higher percentage of children with nursing caries (45.4%) than that in caries-free children (32.0%), as well as the statistical differences were non-significant between two groups. This result could be attributed to being first, to the mechanics of breastfeeding which make it unlikely for human milk to stay in the baby's mouth for long. During breastfeeding, the nipple is drawn deep into the baby's mouth, and milk is literally squirted into the back of his mouth. The sucking process includes a swallow and the nursing child must swallow before he can go to the next step. In contrast, baby bottles can drip milk, juice, or formula into the baby's mouth even if he is not actively sucking. If the baby does not swallow, the liquid can pool in the front of the mouth around the teeth. The artificial nipple is very short, so the liquid in the bottle is likely to pass over teeth before being swallowed. Secondly, the human milk itself which is protective against dental caries because it contains in addition to lactose a component called lactoferrin that actually kills streptococcus mutans bacteria that cause tooth decay,[28] beside that human milk is not cariogenic.[29] This finding may not agree with other authors,[28,30] who observed that nursing caries was high in children who practicing bottle feeding.

Conclusion

This study concluded that the high level of salivary calcium and alkaline phosphatase have an important role in developing nursing caries and caries activity (dmfs index) when compared with caries-free children. Salivary inorganic phosphorus concentration has a negative effect on the nursing caries and negative correlation with dmfs index, thus the low level of salivary inorganic phosphorus associated with caries activity. The type of infant feeding does not affect the development of the nursing caries and caries activity.

Conflicts of interest

The authors report no conflicts of interest.

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