Salivary Immunoglobulin A in Relation to the Viable Count of Mutans Streptococci in Children with Early Childhood Caries

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Abstract: _Introduction_: _Early childhood caries develops in children due to the unsuitable nocturnal feeding habit, with the presence of mutans streptococci as the caries initiating factor, competing with immunological constituent in saliva, the presence of salivary immunoglobulin A (SIgA) as an antimicrobial peptide could have a defensive role to decrease the amount of the viable count of mutans streptococci._ _Aims of study:_ _this research was done to find the relations between the variables: viable count of mutans streptococci, SIgA level, dental caries index._ _Material and Methods:_ _categorizing the seventy-five children (4-5) years into 3 groups (twenty-five each) mild, moderate and severe. dmfs, dmft measured according to WHO (1987), salivary Immunoglobulin A level measured by ELISA kit, viable count of mutans streptococci measured after culturing it in (Mitis Salivaris Bacitracine) agar and simple identification method._ _Result:_ _the statistical analysis revealed that there was significant correlation in the viable count of MS, dmfs and dmft between (mild and moderate) and between (mild and severe). The Salivary Immunoglobulin A (mean value) showed an increase from the mild to severe as the (dmft, dmfs) increase, in which a significant correlation had been found between (mild and severe) in boys and in the total groups, a significant correlation between the viable count of MS and SIgA in the total groups._ _Conclusion:_ _as the carious (tooth, surface) number increased the viable count of mutans streptococci increased indicating its role in the ECC, SIgA increased as the MS count increased indicating the induction of the immune system to produce salivary immunoglobuline A. that it has no role on the mutans streptococci._

Keywords: early childhood caries, ECC, mutans streptococci, immunoglobulin A (SIgA)

1. Introduction

Early childhood caries (ECC) is an infectious non-threating biofilm dependent disease affecting worldwide , the disease most frequently targets children from poor socioeconomic families and racial/ ethnic minority backgrounds, its etiology doesn't differ much from the caries process in permanent dentition [1], if neglected might affect the child social experience, attitude, general health, also it may possibly exhaust the parents finance till be treated, early diagnosis prevent its progression [2] . EAPD (European Academy of Paediatric Dentistry) uphold the early detection and prevention of the early stages of caries before cavitation [3]. According to the American Academy of Paediatric Dentistry [4] ECC definition is the "presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a 71 month of age or younger. Many nomenclatures had been used to describe the ECC like Rampant caries, Nursing caries syndrome, nursing bottle caries, Bottle mouth caries, milk bottle syndrome, baby bottle caries, and baby bottle tooth decay (these names refer to the feeding habits), labial caries, maxillary anterior caries (refer to its site), rampant infant and early childhood dental decay (refer to the age and type of decay), Severe ECC (S-ECC) [5], [6], [7], [4].

The concept of caries initiation process is the same in adult's dentition, the presence of (cariogenic bact, susceptible host, sugar, time), mutans streptococci (MS) is the considered bacteria to initiate caries with its various strains and many virulent factors competing with other bacteria and antibacterial component in saliva; surviving in a competitive environment to establish its virulence on the enamel [8]. _S. mutans_ was detected significantly more frequently in the severe early childhood caries group than in the caries-free group in both saliva and plaque [9]. Saliva is a pure, to some extent acidic mucoserous exocrine excretion. saliva is a "complicated mixture of liquids" from the major salivay glands (two parotids, two submandibular and two sublingual) and minor salivary glands and also other sources like gingival crevicular fluid, which is (non-glandular), microorganisms, and also from the host diet, "whole saliva" is the term used to describe the non-pure fluid and separate it from the glandular secretion, Saliva have distinct role in the oral cavity in initiation or prevention of: the caries, accumulation of bacteria, entrance of foreign bacteria, virus, fungi, also in the balance between intrinsic and extrinsic factors [10], [11], [12], [13].

The antimicrobial component which is the important immunoglobulin in the salivary secretions is immunoglobulin A (IgA). The secreted form of IgA is called secretory IgA (or S-IgA)**.** The S-IgA prevents the adherence of cariogenic bacteria to enamel [14]. In explaining complicated cariogenic phenomenon, some studies investigated a positive correlation between the viable count of mutans streptococci and the salivary immunoglobulin A level [15], [16], [17], while others revealed a negative one [18] [19]

In Iraq, no previous researches studied the correlation between these two variables in the different stages of the ECC, and the present research was designed to find this subject.

2. Materials and Methods

The current study took 4 months, from December 2016 till the end of March 2017, many public and private kindergartens were visited, 200 children were investigated visually first to categorize them into the wanted groups, seventy-five children participated in the current study aged between 4-5 years the age calculated according to the last birth day [20].

Children were classified into 3 groups 25 each according to Wyne [21]. Selecting the participants from different kindergarten in Baghdad from al-karkh sector, after receiving the child guardian approval, ensuring no systemic disease, for the selected cases their BMI was measured (height and weight to determine BMI) [22] to ensure they are in the same wanted range (15-20), then the teeth of selected children were checked and their dmf was recorded according to the criteria of (WHO, 1987) [23].

The Clinical examination was conducted using the diagnostic tools (disposable mouth mirror and dental explorer, tweezer). The dental caries measurement was achieved, starting from the upper right primary second molar tooth next to the others till reaching the upper left second molar, then moving down to the lower left second molar reaching to the lower right molar. Surface tooth examination begin from the mesial, to the occlusal, distal, buccal and end with the lingual surface for all the teeth [23]. Ensuring that the child was not taking medication in the sample collection day. Saliva collection according to NAVAZESH [24], was done in the morning between 9 – 11 am, transporting the saliva in a cool box to the lab serial dilution from 10^{-1} - 10^{-5} was done and then from 10^{-3} – 10^{-5} were cultured in the Mitis Salivaris Bacitracin agar (HI Media) which was previously prepared as in al mizraqchi [25], simple identification to MS was done by gram stain, colony morphology, catalase test then counting the MS as in. [25] representing the result in cfu/ ml (colony

forming unit). The SIgA was determined by ELISA kit (Demeditec, Germany) by following the manufacture instruction. Statistical analysis relies on SPSS program version 18. Data between the 3 groups were analyzed by Kruskal-Wallis, while between the two groups by Mann-Whitney test because the variables were not normally distributed.

Spearman's test was used to find out the correlation P value (P <0.05), were considered as statistically significant. (P=0.000) highly significant relation, (P> 0.05) non-significant.

3. Result

Table (1) demonstrate the relation between the viable count of MS and the ECC groups and gender, there was highly significant (HS) relation especially between the mild and the moderate and between the mild and severe but between the moderate and severe was not significant relation. The median value of the severe group was the highest and of the mild is the lowest. In the present study, investigating the MS group that had been cultured, in special media as mentioned before, the viable count of MS (mean, ±SD) was (26.542, ±27.029), (110.248, ± 155.419), (185.290 , ±180.152) in mild, moderate, severe respectively, while the median was in the same order (17.000, 66.200, 120.000) so we observe the increase with the MS count, the (mean, ±SD) for (dmft) values was for mild (1.240 , ± .970), moderate (11.440 , ±2.815), severe (12.720 , ± 3.234)

Table (2) illustrates the salivary Immunoglobulin A statistically measured to the ECC groups and gender. in gender, the boys showed significant relation especially between the mild and severe while in the girls the relation was non-significant. The significant relation was between the mild and severe group. There was increase in the concentration of SIgA as the median value for the mild was the lowest and in the severe was the highest. The correlation between the MS count and the SIgA in the different ECC stages (mild, moderate, severe) was demonstrated in table (3), that showed a significant correlation between the viable count of MS and the SIgA in the total ECC groups.

Table 1: The viable count of MS in relation to the ECC groups and gender

Vars.	Cat.	Group	N	%	MS count x10⁴	±SD	Median	*MR	Statistics	*MC
Gender	Boys	Mild	8	20.51	11.949	6.596	15.000	24.38	X^2=2.420 P=0.298	
		Moderate	16	41.03	129.606	186.065	69.450	17.72		
		Severe	15	38.46	124.937	81.814	88.450	20.10		
		Total	39	100	103.675	135.546	72.700			
	Girls	Mild	17	47.22	33.409	30.335	17.800	20.88	X^2=2.103 P=0.349	
		Moderate	9	25.00	75.833	73.961	48.600	16.67		
		Severe	10	27.78	275.820	246.894	192.500	16.10		
		Total	36	100	111.351	168.372	52.675			
Total		Mild	25	33.33	26.542	27.029	17.000	20.52	X^2=29.323 **P=0.000**	***1X2=0.006** **1X3=0.00**0 2X3=0.068
		Moderate	25	33.33	110.248	155.419	66.200	39.72		
		Severe	25	33.33	185.290	180.152	120.000	53.76		
	Total		75	100	107.360	151.189	65.000			

Table 2: Salivary Immunoglobulin A in relation to the ECC groups and gender

Vars.	Cat.	Group	N	%	Mean	±SD	Median	MR	Statistics	MC
Gender	Boys	Mild	8	20.51	42.302	24.367	40.334	9.63	X^2=9.650 **P=0.008**	1X2=0.087 **1X3=0.006** 2X3=0.756
		Moderate	16	41.03	79.730	36.072	80.569	20.41		
		Severe	15	38.46	135.066	98.772	110.537	25.10		
		Total	39	100	93.335	74.370	71.259			
	Girls	Mild	17	47.22	94.905	115.036	66.269	14.29	X^2=5.168 P=0.075	
		Moderate	9	25.00	112.358	74.498	94.550	22.72		
		Severe	10	27.78	123.112	86.368	101.301	21.85		
		Total	36	100	107.104	96.896	78.217			
Total		Mild	25	33.33	78.072	98.094	57.738	26.54	X^2=11.015 **P=0.004**	* 1X2=0.051 **1X3=0.004** 2X3=1.00
		Moderate	25	33.33	91.476	54.025	88.360	41.26		
		Severe	25	33.33	130.284	92.325	101.301	46.20		
Total			75	100	99.944	85.608	74.204			

Table 3: The correlation between the viable count of MS in relation to study variables

Group			MS count
Mild	SIgA	R	.027
		P-value	.900
			NS
Moderate	SIgA	R	.213
		P-value	.306
			NS
Severe	SIgA	R	-.114
		P-value	.589
			NS
Total	SIgA	R	**0.288**
		P-value	**0.012**
			S.

4. Discussion

Early childhood caries considered as a transmissible disease that is widely prevalent in the developed countries, often attributed to "sucrose-civilization- caries trinity", the increased intake of sweetened beverage which is one of the caries etiological factors besides time, susceptible host (teeth), and cariogenic bact., ECC caries development in deciduous teeth doesn't differ that much from the caries development in permanent teeth [26].

Saliva play an important role in the oral cavity by its immunological and non-immunological constituent which reflect a picture of the body secretion, the immunity in children developed with the age and being more susceptible to various microbiota and different antigens [27], [28].

The role of the MS bacteria in the initiation of caries had been studied by many researchers, [9], [25] MS group had the ability to surround itself by forming poly saccharide (PS) layer, which considered one of its virulent factors, that been converted to acids when lack of resources happens in the environment, also the ability of the bacteria in metabolizing the sugar to acid if there was a shift in the balanced environment toward the demineralization process [29]. One of the risk factors for the (ECC) progression is the presence of" Extracellular insoluble polysaccharides" in the plaque on the teeth, those found to be correlated with the increased consumption of sweetened beverage "solid sucrose" [30].

Different values of MS count in plaque and saliva was found in relation to the different stages of the caries, diet, and other types of involved bacteria [31],[32]. The higher count of MS bact.in early childhood will lead to higher DMF index at adulthood [33], so indicating the importance of monitoring the oral hygiene in childhood.

Klinke et al. [34] study the count of MS type and Lactobacillus before and after the treatment of decayed teeth in children with ECC, the result was a significant lower count of the bacteria after the treatment of the decayed teeth.

The present study results demonstrate the viable count of MS increase as the severity of the caries increase, indicating there is more inhabitant of MS species as the dental caries index (dmft) increase, although there was non-significant correlation between the viable count of MS in boys and girls group, but the study revealed a significant relation with the total groups.

When comparing the present study results with other studies, in considering the mild group relatively closer to the caries free groups as their mean dmft was (1.240, SD ± .970), some have the same a findings significant correlation between viable count of MS and dental caries index [35],[36],[31],[32], others have the opposite [37] due to the difference in the criteria been used or methods or the sample collection.

Salivary Immunoglobulin A (SIgA) is the prevalent type in the salivary secretion, SIgA role in the oral cavity against different species of bacteria viruses, other types of microorganisms, by: bacterial adherence inhibition, neutralizing effect, agglutination, IgA presence in breast milk especially colostrum give further defense against microorganisms in the oral cavity in addition to the GIT [38], [13].

A systemic review done by da Silva Fidalgo et al. [38] concluding the higher level of SIgA in caries active groups so referring the cause to "Salivary IgA reflects a previous exposure of the host to cariogenic microorganisms"

The study results revealed the relation between the SIgA and the dental caries index, which is represented in table (2), statistical analyses of the relations between the groups illustrated a highly significant correlation between the mild

and severe type of ECC in the variables: in boys, and between the total groups.

The same concept was found in many studies as in the present article, relating higher levels of SIgA with the higher dental caries index (dmft, dmfs) and ECC cases, **[39], [40], [41], [42], [43].**

A controversy was found in research done by **[44], [45]**. In which Kuriakose et al. **[44]** found lower SIgA level in rampant caries children and higher in caries free children, relating its role in decreasing the caries in children. Also **[45]** Yassin studied the level of SIgA in caries free and caries active groups, stimulated saliva samples had been collected from children aged (7-10) years old, the caries active have lower level than caries free

Table (3) showed that there is a significant correlation ($p<0.05$) between MS count and the SIgA in the total groups (P=0.012) and non-significant correlation in MS count with each group as a variable, and table (1) and (2) showed there is an increase in the mean value between the groups from the lower caries to higher caries subjects, many authors have reached the same conclusion in their experiments like **[15] [16], [17]**. these results didn't relate the SIgA as an important immune component in saliva against SM, associating the higher levels of SIgA in saliva to the immune response, which has been induced by the presence MS species, another explanation could be due to counting the total SIgA not the specific S. mutans SIgA levels.

Thaweboon et al. **[15]** investigated the level of MS count and SIgA, pH, flow rates, and candida in kids (5-10Y) having (rampant caries) and caries free groups, estimating the SIgA level by ELIZA kit, the increased level of SIgA with the increased level of SM count hypothesizing that increased levels of salivary SIgA may reflect a previous exposure of the host to cariogenic bact.

Parisotto et al. **[16]** examine the level of MS, LB microorganism and the level of SIgA, then one year later examining the same variables, in (3-4) years old children revealing, in the first measurement the caries active have higher level of SIgA and MS count than caries free, the one year later examination reveal an increase in the MS count especially in the caries active group, the SIgA level increased with age also the increased number of the MS count may induce the immune system to secrete more immunoglobulins.

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