Mutans Streptococci Count and Salivary Histatin 5 Level in Relation to Early Childhood Caries

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Abstract

Background: ECC is one of the most common chronic infectious diseases of preschool-aged children (3). Mutans Streptococci (MS) is the cheif microorganism that causes ECC Saliva is a significant factor in the development of teeth decay. Saliva have many innate defense molecules that take part in the protection of oral tissues by either direct antimicrobial effect or interference with microbial colonization. These molecules include antimicrobial peptides AMPs among the main natural antimicrobial proteins of saliva . Salivary histatine-5 (HST-5) is one of the protective factors in saliva that possess anti-bacterial properties against several types of oral bacter. This study aimed to measure the viable count of mutans streptococci and salivary histatin 5 level in relation to early childhood caries.

Materials and Method: Sixty three children with ages of 4-5 years old were enrolled in this study. They were divided into two groups; 33 children with severe type of ECC) ategory as the study group and 30 caries free child) as the control group. Unstimulated saliva sample were collected for ms count on mitis bacitracin agar and for evaluation of salivary Histatin 5 level.

Results: Statistical analysis revealed that M.S. count was higher significantly in study group compared to than control group and salivary histatin 5 was significantly higher than the control group.

Keywords: *Early childhood caries, Mutans streptococci, histatin 5.*

Introduction

Early childhood caries (ECC) is one of the most common chronic infectious diseases of preschool-aged children, characterized by the destruction of tooth tissues by synergistic complex effects among acids generated from the fermentation of dietary carbohydrates by bacteria and susceptible host factors, such as teeth and saliva[(1)]. Tooth decay of primary teeth in children 71 months of age or younger was regarded to early childhood caries (ECC). In accordance to American Academy of Pediatric Dentistry, ECC is "the finding of one or more decayed (non cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any decedious tooth in a child below the age of six [(2)]. ECC is a multicausal disease that happened due to host, environment, and microorganism as the causative factors [(3)]. Mutans Streptococci (MS) is the cheif microorganism that causes ECC. In the past decade, numeral researches conducted in the relationship between the occurrence of caries and the founding of salivary MS [(4)]. Saliva may transport the bacteria and have a role as a reservoir for the colonization of the bacteria. If the cariogenic bacteria presedant in saliva and plaque, it will raise the acids, which are made by them through the fermentation process of carbohydrate. Then will rise up the colony of the bacteria and begin creating biofilm on tooth surface[(5)].

One of the fundemantal factors of MS virulence is the potency to give glucan made up by glusyltransferase, which mediated microorganism attachment to the tooth surface along with other protein [(6)].

Numerous of studies previously have based a positive correlation between MS and ECC [(7)]. A positive correlation was also found between severity of ECC as measured by the dmft index and high levels of MS counts, indicating that as the number of colonies increased, the number of teeth and surfaces affected by

decay also increased.Colonization with MS is thus a key event in the pathogenesis of ECC that can be targeted for caries prevention in clinical practice [8].

Increasingly more and more attention is being paid to the potential of saliva as a potent anticaries agent[9]. Saliva is a significant factor in the development of teeth decay. Saliva protects the tooth against missing of calcium and phosphate ions from the enamel by creating a dental pellicle. The salivary pellicle acts as a protective barrier and helps in preventing demineralization, promoting remineralization, keeping the oral cavity pH neutral and cleaning tooth surfaces by washing away remaining food.[1] The development of caries is affected by the physiochemical careteria of saliva, such as pH, salivary flow rate, buffering capacity, varying protein concentrations and other contenant of saliva. Saliva has many innate defense molecules that take part in the protection of oral tissues by either direct antimicrobial effect or interference with microbial colonization. These molecules include antimicrobial peptides AMPs (cathelicidin peptide LL-37, alpha-defensins, beta-defensins, histatins and statherin), major salivary glycoproteins (mucins, proline-rich proteins (PRPs) and immunoglobulins) and minor salivary glycoproteins (agglutinin, LF, cystatins and lysozyme). These proteins play specific functional roles in the first line of defense of the oral cavity. [10]

Histatins are small cationic peptides made up of at least 12 histidine-rich basic peptides . Histatins (HST)-produced in parotid and sublingual salivary glands-are among the main natural antimicrobial proteins of saliva. The HSTgroup includes HST-1, −2, −3, −4, −5 and −6. Salivary histatine-5 (HST-5) is one of the protective factors in saliva; it is a salivary peptide that consists of 24 amino acids produced in parotid and sublingual salivary glands., salivary HST-5 was demonstrated to possess fungicidal and fungistatic properties against Candida albicans and antiviral activity against the human immunodeficiency virus (HIV), in addition to antibacterial properties against several types of oral bacteria [11, 12].

Materials and Method

Sixty three children with ages of 4-5 years old during the period from December 2019 to march 2020 were enrolled in this study. They were divided into two groups; 33 children with severe type of ECC according to Wyne[13] category as the study group and 30 caries free child according to WHO [14] as the control group. They will be selected from pediatric and preventive departments clinics/college of dentistry/Baghdad university Baghdad city.

3-5ml of unstimulated saliva was collected by drooling method [15]. 1 ml from each sample for microbiological investigation and bacterial culturing then the remaining of each salivary sample was centrifuged for 10 minutes at 1000×g at 2.8°C. The supernatant which is about 1-2 ml was transported to eppendroffs tube with the same number of the child for freezing. The sample was stored at -20°C for further biomarkers detection procedure.

Microbiological Analysis: The collected salivary samples were homogenized for 1 minute using vortex mixer and then ten-fold serial dilutions were performed From dilution 10-3 of salivary samples 0.1 ml was spread microbiological on MSB agar plates. The plates were incubated anaerobically by using a gas pack supplied in an anaerobic jar at 37◦C for 48hrs, followed by aerobic incubation for 24hrs, at 37◦C.

Identification was done according to colony morphology, Gram stain reaction, Biochemical test, catalase test. Carbohydrate fermentation test for M.S and bacterial colony count was also done [16]

Biomarker Detection: The level of salivary histatin 5 was estimated by using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit and performed as recommended in leaflet with kit (MyBiosource; USA).

Statically analysis will be carried out using statistical package for social sciences (SPSS) version 21. Descriptive statistics using: frequencies, mean and standard error in addition to the following statically test: Independent sample T test, correlation coefficient (r), chi–square.

Results and Discussion

Table 1: Mutans Streptococci count (CFU/ml×10^3) in early childhood group and caries free group.

Bacterial count	Early childhood Study group n=33	Caries free Control group n=30	T-test	p-value
Minimum	111	31	24.723	0.000**
Maximum	220	74		
Mean	174.545	50.333		
SD	24.102	13.901		
SE	4.195	2.538		

*Highly significant at P≤0.001

Table 1 illustrate the viable count of M.S among E.C.C study group and healthy control group.

The results demonstrated that the viable count of M.S was higher in study group than control group with highly significant differences

The results of this study were in agreement with other researchers who also found that MS.. is associated with E.C.C (E.C.C children had more colonies) [17]

Other researchers were also in agreement with the present study [18][19]

Is well-known that M.S. IS the causative microorganism in the development of dental caries because they drop the plaque pH and produce acids from carbohydrates and survive in the acidic environment.[20]

M.S. constitutes about 60%of cultivable flora of dental plaque obtained from preschool children with ECC[21]. Horizontal and vertical transmission of microbes can be seen. Horizontal transmission occurs between siblings and care givers [22, 23].Vertical transmission is also known as mother to child transmission [24]. Poor maternal oral hygiene and sugar exposure increases chances of vertical transmission[25].

In appropriate feeding practice can prolong the exposure of teeth to fermentable carbohydrates which in turn may aggravate the chances of ECC [26]. Also Bottle feeding during bedtime or sleeping [27] is the most important cause of ECC and hence also known as nursing bottle caries, baby bottle caries. Frequent exposure to sugar, frequent snacking, taking sweetened drinks to bed, sharing foods with adults, as well as maternal caries status, oral hygiene and dietary habits predispose to early *S. mutans* colonization and establishment of its high counts [26].

Table 2: Salivary mean level of histatin 5 in study and control groups

Salivary histatin 5	Early childhood Study group n=33	Caries free Control group n=30	T-test	p-value
Minimum	16.538	5.907	9.378	0.000**
Maximum	43.823	22.303		
Mean	24.514	11.496		
SD	6.542	4.056		
SE	1.138	0.740		

*Highly significant at P≤0.001

Table 2 illustrates salivary levels of histatin 5 in E.C.C. group and healthy control group, the results showed that the levels of histatin5 was higher in E.C.C. group compared with healthy control group and there were highly statistical differences between the two groups

These results are in agreement with the results of other researchers[28],[29] Increased activity of HST-5 was observed in wide pH range. However, in low pH (with presence of caries dental changes) protonation of histidine residues occurs, additionally increasing the antimicrobial force [30].

Histatins are a group of small, cationic, histidine-rich peptides secreted by human

The increased level of histatin 5 may be explained by the fact that high-intensity caries is associated with increased levels of some salivary components (histatin-5) that possess strong bactericidal or bacteriostatic effects resulting in aggregation of oral bacteria and their clearance from the oral cavity, [29].

Conclusion

Salivary histatin 5 level in saliva of children can be used as diagnostic tool or marker for the prediction of caries risk.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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