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Estimation of Salivary sCD14 in Children with Early Childhood Caries in Association with Pneumonia

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Abstract

Background: Dental caries is a chronic disease among children and pneumonia is often seen in young children. Soluble CD14 (sCD14) protein is released by monocytes and changes in periodontal infection. The study aimed to estimate the level of salivary sCD14 in children with early childhood caries in association with pneumonia.

Methods: This case-control study was conducted on 52 children aged between 2 to 5 years. A total of 17 children who were caries free, with no past systemic illness; 17 children with dental caries with no history of systemic illness or dental treatment for caries, and 18 children with caries and pneumonia were included in the control and test groups respectively. Unstimulated saliva of all children was collected. All samples were tested using a commercial available sCD14 ELISA kit.

Results: The sCD14 level was elevated in all three groups. One-way ANOVA was used to compare the mean level of sCD14 values between the groups. Control group had the highest mean sCD14 values (15070.99 ± 4296.44), followed by the caries group (13629.83 ± 5603.76) and pneumonia group (8566.86 ± 4778.81). There is a significant difference between the groups with p=0.001.

Conclusions: Based on the results of the study, it can be concluded that sCD14 can be used as an indicator of the healthy functioning of the oral cavity.

Keywords: Children, CD14, Dental caries, Pneumonia, Saliva.

Introduction

Dental caries is the most prevalent dental disease affecting mankind. The "dental caries" is defined as the localized chemical disintegration of the tooth surface (enamel and dentin) caused by dental plaque and mediated by saliva (1). Approximately 40% of children have dental caries by the age of 5 years, and 8% of the 2-year-old children have some of the decay or previous restorations (2, 3)

The American Academy of Pediatrics stated that dental and oral infections keep on infecting children and, specifically, very young children. In primary teeth, dental caries is a preventable and reversible disease if treated in early stages, but when left untreated it will lead to pain, bacteremia, alteration in growth and development, premature tooth loss, speech disorder, increase in treatment costs, loss of confidence, and negatively affect successor permanent teeth (4). Dental caries in young children have a pattern; diverse terms and terminology have been utilized to express them (5).

Early childhood caries or ECC is multiple carious lesions found in very young children. The expression "ECC" was proposed more than

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20 years ago during a workshop supported by the Centers for Disease Control and Prevention (CDC) trying to scope the consideration upon the various issues, such as financial, socio-psychological, and behavioural, which contributes to the formation of caries at such initial years, instead of attributing its manifestation solely on feeding bottles (6, 7).

The oral cavity may be an important source of bacteria that cause infections of the lungs. Relocalization of the oral flora from dental caries and periodontal disease into the respiratory tract may also influence the initiation and progression of pneumonia.[8, 9] Pneumonia is one of the most common causes of morbidity and mortality in children younger than 5 years in India.(10) The most common cause of bacterial pneumonia is a type of bacteria known as *Streptococcus pneumoniae. Haemophilus influenzae, Chlamydia pneumoniae, Mycoplasma pneumoniae,* and *Legionella pneumophila* are some other major bacteria that cause pneumonia (11, 12). The relationship between oral health and systemic conditions, including the association between poor oral hygiene, periodontal disease, and respiratory disease, has been increasingly debated over recent decades.

Salivary proteins are well known for its role in maintaining the oral health and homeostasis, and any changes of salivary protein composition may play an important role in the etiology of oral disease prevalence and dental caries development the saliva proteome have been associated with frequency and severity of the oral disease (13, 14). Cluster of differentiation 14 (CD14) was first identified on the surface of monocytes and macrophages (15). The innate immune system invades microorganisms through the recognition of pathogen-associated molecules. Salivary soluble (sCD14) is a glycoprotein expressed predominantly on the surface of monocytes, macrophages, and neutrophils (15). It plays a crucial role in the recognition of several microbial products, such as lipopolysaccharides (LPS), endotoxins and peptidoglycans, which are major components of the cell wall of gram-negative and gram-positive (16).

Salivary sCD14 protein is considered as a marker of susceptibility to developing caries and/or as a diagnostic element for the existence of ongoing carious lesions (17, 18). Levels of circulating sCD14 in other body fluids also changes during inflammation and infection (19).

In view of this, we aimed to evaluate the association of the salivary sCD14 children affected by early childhood caries and pneumonia.

Materials and methods

Patients and settings

This prospective case-control study was conducted in children aged between 2 to 5 years in the Department of Pedodontics and Preventive dentistry, Yenepoya Dental College in collaboration with Department of Paediatrics, Yenepoya Medical College, and Yenepoya Research Centre. Ethical clearance for the study was obtained from Yenepoya University Ethics Committee (Reference no. YUEC14/2015).

The ideal sample size to assure adequate power for the study was obtained from G* power software version 3.1 software (20). It was determined that 17 samples per group were necessary to provide 80 % power with an alpha of 0.45. Based on this statistical analysis a minimum sample size of 17 or more per group was used in the study.

After explaining the objectives of the study for the children's parents, informed consent was obtained and a total of 52 children were recruited for three groups. Group 1 (17 children) with early childhood caries (ECC), group 2 (18 children) with pneumonia along with ECC, and group 3 (17 children, control group) were healthy children.

Source of data/sampling method

Unstimulated saliva of healthy children and having early childhood caries were collected from the Department of Pedodontics, Yenepoya Dental College and of children suffering from pneumonia were collected from the Department of Pediatrics, Yenepoya Medical College. A detailed protocol of saliva collection and processing for further investigation is published by Henson and Wong, 2010 (21).

Informed consent was obtained from parents before collecting the saliva samples. Un-stimulated saliva was collected from children by asking them to spit gently into sterile sample containers. It was made sure that the saliva was free from any contaminants. The collected saliva was carefully transferred into coded microtubes. The microtubes were kept on ice in order to prevent hydrolysis of salivary proteins. The samples were stored at -20 °C.

Sample processing and enzyme-linked immunosorbent assay (ELISA)

Phosphate buffered saline (PBS) in an equal volume was added to the microtubes. This was then centrifuged for 30 sec at a speed of 255 G. The supernatants were gently transferred to fresh microtubes and were stored at -70 °C.

The sCD14 levels of the saliva were measured on thawed samples by ELISA method using Human CD14 Quantikine ELISA Kit (Cat# DC140; R&D Systems, Minneapolis, MN, USA) and the data was recorded. All the reagents, working standards and samples were made according to the manufacturer's instructions. All the sample analysis was done by the same kit and from the same lab.

Statistical analysis

All the data was entered into SPSS version 22.0 software package (SPSS Inc, Ill, Chicago, USA). A *p*-value of <0.05 was considered statistically significant. Comparison of mean soluble salivary CD14 concentration was done using ANOVA test, Independent - t-test. Post hoc- Tukey's test was used for multiple comparisons.

Results

The mean levels of salivary sCD14 were estimated and compared in the test and control groups.

Comparison of CD14 levels between genders in different groups

Independent t-test was used to compare sCD14 levels between the males and females in each group (Table 1). There was no significant difference in sCD14 values between males and females in each group.

Table 1. The gender distribution of study groups

	Girls	Boys
Caries	8 (47.1%)	9 (52.9%)
Pneumonia	8 (44.4%)	10 (55.6%)
Control	10 (58.8%)	7 (41.2%)

Comparison of CD14 levels between the age group in different groups

One-way ANOVA test was used to compare the sCD14 levels between the age groups in each study groups (Table 2). There was no statistically significant difference in sCD14 levels between the age groups in all the study groups.

Table 2. The age distribution of study groups

Study Group	Age Group			
	2 years	3 years	4 years	5 years
Caries	-	1 (5.9%)	8 (47.1%)	8 (47.1%)
Pneumonia	6 (33.3%)	1 (5.6%)	4 (22.2%)	7 (38.9%)
Control	-	1 (5.9%)	5 (29.4%)	11 (64.7%)

Comparison of sCD14 levels between pneumonia, ECC, and control groups

ANOVA was used to compare the mean level of sCD14 values between the groups.

There was a significant difference between the groups with p=0.001 (Fig. 1; Table 3). Control group has the highest mean sCD14 values (15070.99 ± 4296.44), followed by Caries group (13629.83 ± 5603.76) and pneumonia group (8566.86 ± 4778.81).

Table 3. Comparison of sCD14 levels (ng/mL) among study groups using ANOVA test

Group	Mean ± SD	Range	ANOVA RESULT	
			F-statistic	P-value
Caries	13629.83 ± 5603.76	2500-21719.1	8.497	0.001*
Pneumonia	8566.86 ± 4778.81	1659.7-18614.4		
Control	15070.99 ± 4296.44	5142.3-21330		

*indicates a significant difference between the groups

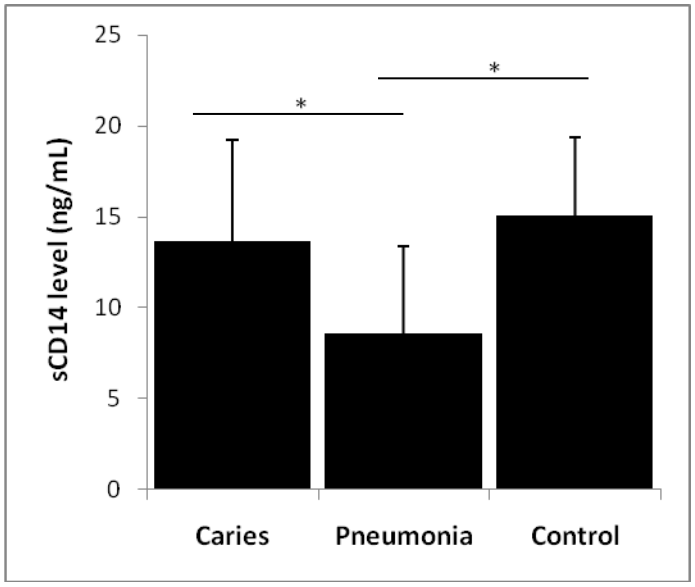

Fig. 1. Mean sCD14 levels in the study groups. The saliva of children with dental caries (n=17), pneumonia along with dental caries (n=18), and caries-free control (n=17) was tested for sCD14. Data shown are mean ± standard deviation (SD). Comparison of sCD14 levels between the study groups using ANOVA test. sCD14, soluble CD14. Star (*) indicates a statistical significance $p < 0.001$.

Discussion

The expression of sCD14 by human salivary glands in a functionally active form has been reported previously. Bas *et al*., said that sCD14 is a glycoprotein which functions in Lipopolysaccharide/cell-wall products signalling, by controlling the immune system level of response (22). ELISA can be used to analyze the sCD14 concentration in saliva (17, 23, 24).

In our study, an inverse relationship between dental caries, pneumonia, and soluble salivary CD14 concentration was observed which was in accordance with the previous study which used western blot method (25). We analyzed sCD14 quantitatively using ELISA test as it is rapid, simple and more sensitive test than western blot test. The results of our study show that the salivary concentration of sCD14 is elevated in control samples that are free from any sort of infectious diseases as compared to the ECC and pneumonia group.

Few researchers have reported that sCD14 can regulate humoral and cellular immune responses through interaction with B and T cells and have a role in natural immunity and it was identified to

have a preventive function against periodontal diseases (22, 25). In our study, a higher concentration of sCD14 might have prevented the individual from caries incidence and other infectious diseases like pneumonia.

Many salivary proteins may have anti-cariogenic roles. This anti-cariogenic characteristic depends on their ability to inhibit the growth of acidogenic bacteria, by binding to these bacteria and preventing the attachment of these bacteria to the tooth surface, and by altering demineralization and re-mineralization processes. In a previous study by Bergandi *et al*. concerning the association between sCD14 and dental caries among children between 6-12 years of age, sCD14 was found to be absent in the saliva of children with dental caries while it appeared a few weeks after treatment; It was also revealed that the sCD14 was present in saliva of CF (caries free) children (4).

To our knowledge, this is the first experimental work showing such a clear-cut difference of a specific salivary protein between pneumonia, caries active and healthy children.

While comparing the salivary sCD14 levels in pneumonia patients it is higher than the normal value but lower than the control group. In a previous study, sCD14 serum levels were specifically increased in serum of children with pneumonia compared to the control group (26). The difference between the results of this study and our findings can be related to the fact that they have estimated the sCD14 levels in serum. They found that the TLR ligands LPS and CpGs induce sCD14 production via two distinct mechanisms and in turn leads to the elevated levels of serum sCD14 in these pneumonia patients. This value also correlates with the fact that sCD14 is a part of the immune system of the body.

The mean concentration of sCD14 was consistent among different age groups and between the girls and boys. The difference in levels of sCD14 in caries experienced and caries-free groups indicates the role of innate immunity in the oral cavity. Pugin *et al*. proposed that sCD14 plays a crucial role in the initiation of immune responses by recognition of several microbial products, such as lipopolysaccharide (LPS), endotoxins and peptidoglycan, which are major components of gram-negative and gram-positive bacteria, respectively (27). Also, Sugawara *et al*., found in their study that saliva contains abundant bio-active CD14 from salivary glands in a soluble form (28). This suggests that sCD14 is important for the maintenance of oral health.

Saliva CD14 is absorbed by bacteria in oral flora, since saliva CD14 is absorbed by a major bacterium in saliva, *S. salivarius*. These findings indicated that the protein portion of saliva CD14 is not truncated and that saliva CD14 is synthesized as a 55-kDa form in salivary glands and secreted as a protein of the same size as in the case of human hepatocytes, the major source of sCD14 in serum. It is possible that saliva CD14 interacts with bacterial components, including LPS and lactoferrin, and prevents the oral mucosa and salivary glands themselves from exacerbating innate immune responses by clearing bacterial components. It also has been reported that sCD14 mediates the aggregation of LPS and that the aggregates undergo internalization by phagocytes, which do not elicit cellular responses to the LPS (29). Thus the salivary sCD14 can also be used as an indicator of the healthy functioning of the oral cavity.

In this study, we did not compare the oral microbial flora and its association with sCD14 levels. We cannot rule out the unrecognized infection that may affect the levels of sCD14. We had a small sample size which was insufficient to make finer observations regarding potential confounder that may influence the results. Furthermore, only the children of visiting our hospital were included in the study. Hence, a larger area should be covered to gain more evidence regarding the possible relation of caries and pneumonia.

The results of the present study suggest that salivary sCD14 is important for the maintenance of oral health and is an important part of the natural immune responses of the body through their interaction with the cellular and humoral immune responses. In view of inconsistent findings on the potential role of sCD14 and its relationship with caries further research is recommended.

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