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## SHORT PAPER

# Association of Salivary sCD14 Concentration Levels with Early Childhood Caries

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### ABSTRACT

**Background:** Early childhood caries (ECC) is a severe type of dental caries affecting infants and pre-school children. Because of the infectious nature of the disease, the immunologic response by the host plays an essential role in its development. **Objective:** This study investigated the association between the presence of salivary sCD14 and ECC. **Methods:** This study was carried out on 40 healthy children, of whom 20 were caries-free (CF) and 20 had ECC, within the ages of 36 to 71 months. Unstimulated saliva of the children was collected with disposable needle-less syringe from buccal and labial vestibules. Seven children with ECC received complete treatments. Saliva was collected for a second time after 3 months from this group. The sCD14 levels in salivary samples were analyzed by ELISA method. **Results:** Mean concentrations of sCD14 in ECC and CF groups were 57.82 and 31.92 ng/ml respectively ( $p=0.008$ ). After three months, the mean concentration of sCD14 among the treated children decreased to 11.38 ng/ml, which was significantly lower compared to that of ECC children before intervention ( $p<0.001$ ), and also CF children ( $p<0.05$ ). **Conclusion:** The increased levels of sCD14 can be considered as a marker of inflammation and innate immune response during ECC.

**Keywords:** Dental Caries, Saliva, sCD14

### INTRODUCTION

Early Childhood Caries (ECC) is a severe type of dental caries affecting infants and pre-school children (1-3). In addition to its long-term adverse effects on the child's health and development, this disease has also a number of immediate outcomes, the most common of which is pain (4,5). ECC-related pain may last for weeks and interfere with the child's daily activities (6,7). Although the etiology of ECC seems to be similar to other types of dental caries, there is no clear understanding of its predisposing factors (8).

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However, there is no doubt that the disease has an infectious nature, and the immunologic response by the host is effective in its development, as a recent study reported higher concentrations of secretory IgA, and IgG among children with ECC compared to caries-free controls (9). CD14 is a 55-KDa cell membrane glycoprotein which mainly recognizes such bacterial products as LPS, endotoxins, and peptidoglycans (10). This glycoprotein is often represented through a glycosyl phosphatidyl inositol anchor on the surface of monocytes, macrophages, and neutrophils (10). In addition to this cell-bound state, it can also be found as a circulating soluble component called sCD<sub>14</sub> (9). sCD14 is secreted into saliva by the main salivary glands and mediates the function of CD14-lacking cells (10). CD14 exists in human whole saliva and parotid saliva is 10 fold more concentrated in CD14 than whole saliva and its concentration is comparable to that of serum CD14 concentration. Because it mediates the activation of CD14-lacking intestinal epithelial cells by LPS in a Toll-like receptor 4-dependent manner, the saliva CD14 is considered functionally active. It is important in the maintenance of oral health and even in intestinal homeostasis (11).

It has been shown that sCD14 is a regulating agent with the power to adjust humoral and cellular immune responses through interaction with B and T cells (12). Thus, it plays an important role in natural immunity of the oral cavity (10). Some studies have identified a preventive function for sCD14 against periodontal diseases (13), and some others have recognized an increase in sCD14 levels as a part of immune response seen upon clinical manifestations of these diseases (14). However, very few studies exist concerning the association of this cytokine with dental caries prevention (10).

This study investigates the relation between the presence of sCD14 in saliva and ECC as well as the effect of restorative intervention on the concentration of sCD14 in saliva.

## MATERIALS AND METHODS

This cross-sectional, longitudinal study was performed on 40 healthy children, of whom 20 were caries-free (CF) and 20 had early childhood caries (ECC), ranging in age from 36 to 71 months. These children were specifically selected from kindergartens in northern Tehran, and the children who were referred to Pediatric Dentistry Department, Shahid Beheshti Dental School, Tehran, from 2008 to 2009 through a convenient sampling method. The inclusion criteria were absence of any infectious or congenital diseases, periodontal diseases, and dental abscess. Children with the history of prior medication and tooth extraction were excluded. According to AAPD, children with more than one decayed surface in deciduous teeth (cavitated or non-cavitated) were put in ECC group, and those lacking any kind of dental caries upon examination with a dental mirror under sufficient light were categorized as caries-free (CF) group. Parents received detailed information regarding the research, and all accepted to participate in the research and signed the informed consent form.

The parents were instructed to prevent their children from eating one hour prior to saliva collection. Unstimulated saliva of the children was collected with disposable needle-less syringe from buccal and labial vestibules in the morning between 8 AM to 11 AM. One to two ml of the collected saliva of each subject was poured into coded capped microtubes. The microtubes were kept on ice in order to prevent hydrolysis of salivary proteins, and were sent immediately to the immunology laboratory. The samples were

stored at  $-20^{\circ}\text{C}$ . Parents of ten patients in ECC group looked for dental treatment for their children. Two of these cases failed to complete follow-ups, and one was excluded from the study because of the development of a dental abscess. The other seven children received complete treatments and saliva from this group was collected again 3 months after the first collection. In the laboratory, phosphate buffered saline (PBS) in an equal volume to the saliva was added to the micro-tubes. The micro-tubes were then centrifuged (Eppendorf centrifuge, 5415, Germany) for 30 seconds at a speed of 1800 RPM. The supernatants were gently transferred to fresh micro-tubes and stored at  $-70^{\circ}\text{C}$ . The sCD14 levels of the saliva were measured on thawed samples by ELISA method using an sCD14 kit (Diacclone Atepnel Company, France). The optical density was read at 450 nm and the sCD14 concentration (ng/ml) was determined according to the manufacturer's instructions. Chi-square, Student's t-test, Mann-Whitney, and Wilcoxon ranked tests were used for statistical analysis.

## RESULTS

Among the 40 children, 19 were boys. No gender difference existed between ECC and CF groups ( $p < 0.05$ , Chi-square test). Mean concentrations of salivary sCD14 in ECC and CF groups were 57.82 and 31.92 ng/ml, respectively. The difference between the two groups was found to be statistically significant ( $p = 0.008$ ) using Student's t-test (Table 1).

**Table 1. Salivary concentration of sCD14 (mean  $\pm$  SD) in children with Early Childhood Caries (n = 20) and Caries Free children (n = 20).**

Study groups	Number of children	sCD <sub>14</sub> concentration (mean $\pm$ SD)	p-value*
Early Childhood Caries	20	57.82 $\pm$ 37.675	0.008
Caries Free	20	31.92 $\pm$ 26.276	

\*T-test

After three months, the mean concentration of sCD14 among the treated children was 11.38 ng/ml, which was significantly lower compared to that of the ECC group prior to the intervention ( $p < 0.001$ , Wilcoxon signed rank test) (Table 2). Besides, a statistically significant difference was observed between the mean concentration of salivary sCD14 of the treated and that of CF group ( $p < 0.05$ , Mann-Whitney test).

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**Table 2. Salivary concentration of sCD14 (mean  $\pm$  SD) in children with treated Early Childhood Caries (n = 7) and those with Early Childhood Caries prior to the treatment (n = 20).**

Study groups	Number of children	sCD <sub>14</sub> concentration (mean $\pm$ SD)	P-value*
Treated Early Childhood Caries	7	11.3834 $\pm$ 8.32	< 0.001
Early Childhood Caries before treatment	20	57.82 $\pm$ 37.675	

\* Wilcoxon signed rank test

## DISCUSSION

This study examines the relation between the concentration of sCD14 in saliva and ECC and also the effect of restorative intervention on the concentration of sCD14 in saliva. The results showed that this protein was present in the salivary samples of both ECC and CF groups, and its concentration was significantly higher among ECC group compared to the CF subjects ( $p=0.008$ ). Moreover, the concentration of sCD<sub>14</sub> in ECC group decreased significantly after complete treatment ( $p<0.001$ ) and reached a level which was significantly lower than those of the untreated children and the CF children ( $p<0.05$ ). Many salivary proteins may have carcinogenic or anti-carcinogenic roles. This anti-carcinogenic 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 (10). In a previous study by Bergandi et al. concerning the association between sCD<sub>14</sub> and dental caries among children between 6-12 years of age, sCD<sub>14</sub> was found to be absent in the saliva of children with dental caries (10) while it appeared a few weeks after treatment (10). It was also revealed that the sCD<sub>14</sub> is present in saliva of CF children. The difference between the results of this study and our findings can be related to the age of the subjects, which was 36 to 71 months (average: 4.5 years) in our study, and 6 to 12 years in Bergandi et al. study (10). Since specific immune responses have not yet been developed completely in 3-5 year old children, the increase in sCD<sub>14</sub> level in saliva may be a reparative response of immune system to the inadequacy of such immunoglobulins as IgA and IgG. IgA and IgG reach their maximum levels at ages of 7 and 12, respectively. This concept is reinforced by the results of another study which has identified sCD<sub>14</sub> as an inflammatory protein covering the gap between innate and specific immune responses (12). It seems that this inflammatory protein is developed in response to the bacterial stimulus of ECC. This might be the reason why we observed a decrease in sCD<sub>14</sub> level upon the completion of treatment. While Bergandi et al. states that restorative treatment increases sCD<sub>14</sub> in the saliva of children with dental caries, the observations in our study showed a decrease in the concentration of sCD<sub>14</sub> after the treatment. If considered as an inflammatory protein, sCD<sub>14</sub> will be produced after infection and would decrease after intervention which is in line with the findings of this study.

The treatment protocol in our study included oral health education and dietary counsel-

ing. Moreover, professional prophylaxis and fluoride therapy were performed at the end of the treatment. It seems that these procedures lead to a significant decrease in microbial plaque accumulation and a decrease in the level of sCD14 compared to its level in CF children. It can be hypothesized that sCD14, as an inflammatory protein, may be effective in innate-immune response to bacterial invasion in ECC. After treatment and the reduction of bacterial load, the level of this protein also decreases. Proof of this hypothesis requires further research especially on the effect of other important mediators contributing to inflammation.

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