SHORT PAPER

Correlation between Salivary Toll Like Receptor-2 Concentration and Early Childhood Caries

Beheshteh Malekafzali[1*], Mandana Sattari[2], Sanaz Keyvanfar[3]

[1]Dental Research Center, Research Institute of Dental Sciences, [2]Department of Immunology, School of Medicine, [3]Department of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Background: Early childhood caries (ECC) is a common health problem in the developing countries. Basic knowledge about the etiology and pathogenesis of ECC plays an important role in its prevention. **Objective:** To determine the relationship between salivary TLR-2 concentration and early childhood caries formation **Methods:** Twenty-Eight children with ages ranging from 36 to 71 months (15 in ECC group and 13 in caries free group) were chosen based on inclusion criteria. Their saliva was aspirated in the volumes of 1-2 ml. Resampling was done for 8 subjects of ECC group 3 months after dental restoration. TLR-2 concentration was measured using ELISA. **Results:** Mean concentrations of TLR-2 in ECC and caries free group were 2.12 and 1.42 ng/ml, respectively. The difference between concentrations was statistically significant ($p=0.008$). Three months after treatment in 8 ECC, the mean concentration of TLR-2 (0.925 ng/ml) significantly decreased compared to the original concentration in ECC ($p<0.001$) and caries free groups ($p<0.001$). **Conclusion:** Elevated concentration of TLR-2 in ECC group compared to caries free group and its decrease after treatment point to the participation of innate immune system and specially TLR-2 in the pathogenesis of early childhood caries.

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*Corresponding author: Dr. Beheshteh Malekafzali , Dental Research Center, Research Institute of Dental Sciences of Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tel/Fax: (+)98 21 22403080, e-mail: beheshtehm@yahoo.com

INTRODUCTION

For a long time, dental caries in infants and young children was recognized as a clinical syndrome (1,2). Early childhood caries (ECC) is usually demonstrated as an early caries formation in maxillary primary teeth, which happen along gingival margin, where plaque accumulation is common and is seen prior to 71 months of age (3-5).
According to Stefano Petti's study in 2010, prevalence of ECC in Iran was estimated to be 26 percent (6). Additionally, in 2008, Bargrizan *et al*. reported that prevalence of ECC in Tehran was 21 percent (7). Pain, acute and chronic abscesses, infections, malnutrition and development of parafunctional habits are among the serious consequences of ECC and some of them are irreversible (2,8-9).
Appropriate treatment for ECC is treatment under anesthesia or sedation, which are the only available dental treatment options for this group of patients despite the risks (2,4,13).
The history of studies performed on ECC microbial flora goes back to 1980 and the main known pathogenic microorganism is *Streptococcus mutans* (14,15).
Saliva has several antimicrobial systems that help remove bacteria (15,16). One of the non-specific immune factors is the Toll like receptor (TLRs) family. TLRs are part of the innate immune system that responds to the pathogen associated molecules such as LPS (17,18). TLR-2 is a key mediator in the innate defense against gram positive bacteria which are the most common cariogenic bacteria (19).
Human odontoblast-like cells produce cytokines in response to ligands that interact with TLR-2. These molecules are important in regulating the intensity of pulp response to gram positive bacteria which are inoculated to dentine during caries formation (20).
Conclusively, cariogenic bacteria can activate immune cells through interaction with TLR-2, thereby triggering immune and inflammatory responses. Regarding available resources, a study on the correlation between salivary TLR-2 concentration and ECC is lacking. Therefore, the aim of this study was to determine the relationship between salivary TLR2 concentration and early childhood caries formation. In addition, we compared the level of TLR-2 in the saliva of ECC patient before and after treatment. Results of this study can help us better understand the immune factors involved in the pathogenesis/immunity of ECC, which can lead to the prevention of subsequent complications.

MATERIALS AND METHODS

Sample Collection. The initial phase of study was designed as both cross-sectional and descriptive-analytical investigations. This part included samples of 28 children (15 in ECC group and 13 in caries free group). The second phase was an interventional phase, which included resampling of 8 subjects with ECC 3 months after completion of therapy. Non-random selection of subjects, ranging from 36 to 71 months old, was done among patients referred to Mofid pediatric hospital (Tehran, Iran) and preschool children of Tehran. Inclusion criteria were the absence of systemic diseases, lack of drug consumption history and absence of gingivitis. Written informed consent was obtained from the parents and then subjects were examined under daylight conditions between 9 and 11 am, using a disposable mirror and catheter. Finally, subjects with

open contact teeth were enrolled in the study. No radiographic investigation was done on the subjects.

As defined by American Academy of Pediatric Dentistry (AAPD), subjects with no dmft (no decayed, lost or restored primary tooth) are classified as healthy subjects and those with some dmft (at least one decayed, lost or restored primary tooth) are known as the patient group. Plaque index was measured in both groups, using simplified oral hygiene index (Greene–Vermillion as defined by two components, debris index and calculus index found on six preselected tooth surfaces (21). Parents were instructed not to let their children eat anything during the one-hour period prior to sampling, in order to obtain non-stimulated saliva. Saliva was sampled in volume of 1-2 ml from buccal or labial vestibuli of each subject in both groups and all samples were stored in sealed micro-tubes within ice-containing boxes to prevent salivary protein hydrolysis during their transportation to the laboratory where they were refrigerated at -20°C.

In the second phase, 3 months after completion of treatment, secondary samples were obtained from 8 subjects of the ECC group.

Laboratory Procedure. Upon completion of all samplings, samples were returned to room temperature in order to defrost. Phosphate buffered saline (PBS, pH = 7.2) was added to each micro-tube till volume of saliva doubled. Then resultant solutions were centrifuged for 30 seconds at 301 ×g. TLR-2 ELISA was conducted according to manufacturer's instructions (Uscn, China). Optical densities (OD) of samples were recorded using ELISA reader at 450 nm wavelength. Eventually, based on the readings of standard samples the optical densities were converted to concentrations, and the standard curve was prepared. TLR2 concentrations in the test samples were calculated in ng/ml according to the manufacturer's instructions.

Statistical Analysis. Statistical analyses was done using Chi-square test, Mann-Whitney test, Fisher's exact test, Kolmogorov Smirnov test, parametric *t-test* and Pearson's correlation coefficient by SPSS 18.0 software (IBM company, USA). In the second phase, paired sample *t-test* was used to compare TLR-2 concentrations before and after treatment.

RESULTS and Discussion

Among 28 subjects studied, there were 15 females and 13 males. No gender difference existed between ECC and caries free group.

Table 1. Statistical indicators of TLR-2 concentrations in studied groups.

Group	n	Mean	SD
ECC	15	2.12	0.643
Caries free	13	1.42	0.652
Total	28	1.79	0.727

Results of Chi-square test showed that there was no statistically significant correlation between gender and TLR-2 concentration ($p>0.05$). Similarly, there was no statistically significant relation between age and TLR2 concentrations in the subjects (*t-test*, $p>0.05$).
Data analysis revealed that the mean concentration of TLR-2 in ECC and caries free group were 2.12 ng/ml and 1.42 ng/ml, respectively, and this difference was significant ($p<0.01$) (Table 1).

Table 2. Statistical indicators of TLR2 concentrations before and after treatment.

Group	n	Mean	SD
ECC after dental treatment	8	0.925	0.291
ECC before dental treatment	8	1.973	0.511

Samples which were obtained 3 months after completion of treatment, had a mean TLR-2 concentration of 0.925 ng/ml which was significantly different compared to the mean TLR-2 concentration before treatment (1.973) according to paired *t-test* ($p<0.001$). Additionally, there was a significant difference between TLR-2 concentrations after treatment and those in caries free group ($p<0.05$, Table 2).
All results are illustrated in Figure 1. Pearson's correlation coefficient revealed a statistically significant correlation between salivary TLR-2 concentration and plaque index ($p<0.001$), which was also seen within the ECC group ($p<0.01$).
Using the same test, we found a significant correlation between TLR-2 concentration and dmft in both groups ($p<0.001$). Parental level of education (using Mann-Whitney test), plaque index (using *t-test*), nocturnal feeding and its type (using Fisher`s exact test) and its duration (using *t-test*) had no correlation with the salivary TLR-2 level in this study.
TLRs proteins are expressed mainly on cells responsible for innate defense by responding to Pathogen Associated Molecule Patterns (PAMPs). TLR-2 is involved in response to gram positive bacterial products (22). Although the information on the correlation between dental caries and TLR levels are scarce, there are few studies performed to address relations between TLRs and periodontal diseases. Kuroishi *et al*. (22) reported that human parotid saliva contains soluble TLR-2. Higher levels of TLR-2 in ECC patients can be assigned to antigenic stimulation, which leads to greater expression of TLR-2 and eventual inflammation. On the other hand, TLR-2 has a positive feedback on its own expression.
This positive feedback is the result of increased inflammation and inflammatory response proteins that tend to deal with germs (22). In 2011, the expression of stimulated salivary TLR-2 and TLR-4 in patients with periodontitis was compared to that of healthy subjects (23). Results showed the stability of salivary stimulated TLR

levels in healthy subjects (in terms of days), and higher average levels of TLR-2 and TLR-4 in patients with periodontitis compared to healthy subjects. Our results are somewhat similar to this study despite the difference in the studied diseases. Therefore we can suggest that with inflammatory stimuli, TLR-2 concentration will rise in significantly higher level than in healthy subjects.

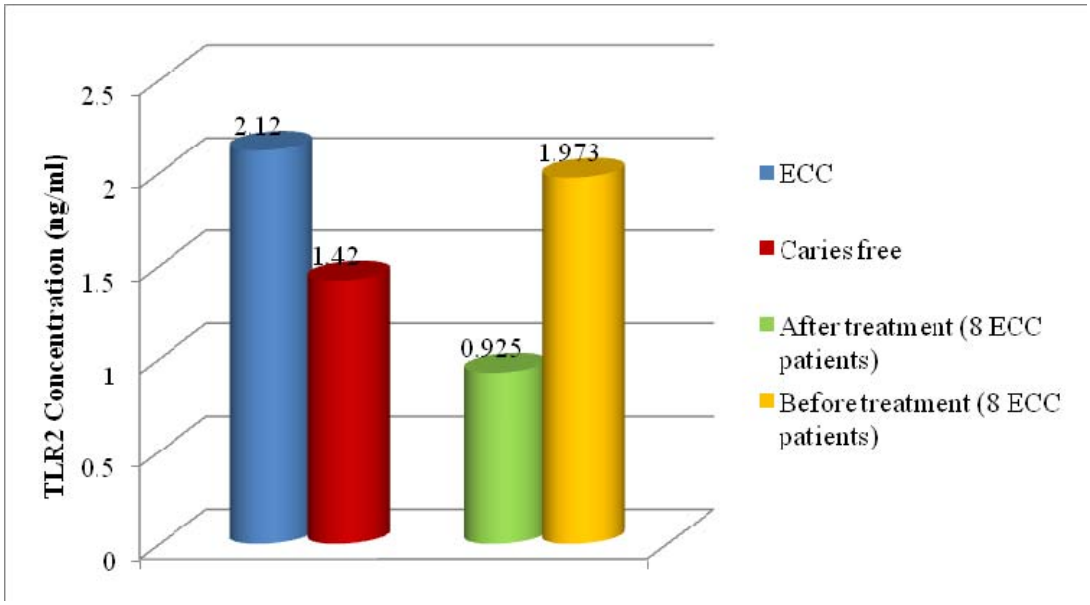

Figure 1. TLR-2 concentration in ECC and caries free groups before treatment and 8 ECC patients after dental treatment.

Less salivary concentration of TLR-2 in subjects under treatment compared to healthy ones may be due to treatment itself or health education, prophylaxis, fluoride therapy, nutritional counseling and maybe one-step completion of surgical treatment. Overall, we conclude that TLR-2 higher concentration in patients with ECC reflects the inflammatory stimulation to provide a better context for the development of an appropriate inflammatory response. On the other hand, soluble TLR-2 binding to surface molecules of gram positive bacteria such as S. mutans do not allow bacteria to affect cells with transmembrane TLR-2 which results in the overproduction of inflammatory cytokines that can be harmful. Additionally, we encountered a reduction in TLR-2 concentrations after completion of dental treatment, which reflects reduced inflammatory stimuli. However, further investigations are recommended to better validate these findings.

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