Immune profiling of saliva in patients with and without dental caries
Ambreen Nawaz1, Husniah Batool2, Muhammad Kashif3, Afia Abbas4, Faheem Shahzad5, Romeeza Tahir6, Nadeem Afzal7

Abstract:
Background: Dental caries is characterized by demineralization of inorganic portion of tooth and destruction of organic substances of tooth, which often leads to cavitation. The immune mechanisms play an important role in the pathogenesis of dental caries. This study was designed to determine the levels of salivary IgA, TGF-β and IL-17 in patients with dental caries.
Methodology: This was a comparative study of 87 individuals (29 healthy controls and 58 patients with dental caries) recruited from the Punjab Dental Hospital, Lahore. Group I included patients with dental caries up to 5 DMFT score (Decayed Missing Filled Teeth), group II had patients with 6 or more DMFT score and group III consisted of healthy individuals without dental caries. Commercially available ELISA kits were used for the detection of salivary IgA, TGF β and IL-17.
Results: Highest mean ± SD level of IL-17 (2.99±1.11ng/L) and TGFβ (127.8±74.0ng/L) were detected in group III. While highest mean ± SD level of salivary IgA (μg/mL) was detected in the group I (34.64±6.37μg/mL)
Conclusion: Level of salivary IgA was increased in patients of dental caries while levels of IL-17 and TGFβ were decreased in patients of dental caries as compared to healthy individuals.
Keywords: Dental caries, Immune response, IL 17, IgA, TGF-β

Introduction
Dental caries is a disease of microbial origin, characterized by demineralization of inorganic portion and destruction of organic substances of tooth, which often leads to cavitation. All carbohydrates can be used by cariogenic bacteria to produce acid that causes tooth destruction. Poor oral hygiene, dental plaque and advanced age have a negative impact on caries.
Innate immunity provides powerful and nonspecific response to prevent infections. The acquired immunity is stimulated by exposure to infectious agents. The cellular immunity is mediated by T-cells and it is involved in resistance to infections caused by viruses and some bacteria. The humoral immunity is associated with circulating antibodies. Cellular and humoral immune responses play an important role in dental caries. Secretory IgA plays a protective role in preventing Streptococcus mutans adherence to the tooth surface.
Gingival crevicular immune mechanism involves both cellular and humoral immunity. Antibodies against many organisms (e.g. Streptococcus mutans) are present in the oral cavity. Before the eruption of teeth (up to 5 months), there is no colonization of Streptococcus mutans, as new born do not have tooth surface to which they can attach but anti-streptococcal antibodies may be present either due to entry of antigen in minor salivary glands or by indirectly swallowing of Streptococcus mutans that

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stimulates gut associated lymphoid tissue (GALT) to mount an immune response. IL-17 induces many mediators of inflammation, including IL-6, G-CSF, GM-CSF and TNFα. IL-17 promotes inflammation by inducing various proinflammatory cytokines and chemokines, recruiting neutrophils, enhancing antibody production and T-cell activation. Anti-inflammatory role of IL-17 in autoimmune uveitis has also been observed. TGF-β is present in both healthy and carious teeth, but their level differs. The objectives of this study were to determine and compare the level of salivary IgA, TGF-β and IL-17 in patients with dental caries and healthy controls.

Materials and methods
This comparative study was conducted in the Department of Immunology, University of Health Sciences (UHS), Lahore, Pakistan. Twenty-nine subjects with up to 5 dental caries (DMFT score) were recruited in Group I and equal number with more than 5 DMFT score were in Group II and control were recruited in Group-III. Patients of both the genders between 18-55 years of age with clinically visible dental caries and healthy individuals without dental caries were recruited. Whereas patients with chronic oral and systemic inflammatory diseases were excluded. Three ml of saliva was collected from each participant by passive drool technique in a sterile container and was transported to the Department of Immunology; UHS, Lahore, in an ice box (4°C) where all the laboratory parameters were performed by the commercially available ELISA kits. The data was entered and analyzed by SPSS 20.0. One way ANOVA and Post HOC Tukey test was applied to observe group mean differences. A p-value of ≤ 0.05 was considered as statistically significant.

Ethical approval: The study was approved by the Ethical Committee of University of Health Sciences Lahore, Pakistan.

Results
Age and gender related data of study subjects are given in table 1 & 2. Highest mean ± SD level of IL-17 ng/L was observed in group-III (2.99±1.11 ng/L), followed by group-I (2.28±5.32 ng/L) and group-II (2.03±3.5 ng/L).

Highest mean ± SD level of TGFβ ng/L was detected in group-III (1.127.8±47.40 ng/L), followed by group-II (48.93±47.27 ng/L) and group-I (41.47±45.61 ng/L). Highest mean ± SD level of IgA μg/mL was detected in group-I (34.64±6.37 μg/mL), followed by group-II (29.69±8.88 μg/mL) and group-III (7.67±8.22 μg/mL). Serum levels of IL17, TGF β and IgA and their comparison are summarized in Table 3.

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<th>Table 1: Number, percentage and comparison of males and females in the study</th>
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<td>Gender</td>
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Group-I=Dental caries up to 5, Group-II=Dental caries 6 or more, Group-III=Control group, n=number, %=percentage, p≤0.05=*statistically significant

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<th>Table 2: Comparison of age between different groups</th>
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<td>Age (years)</td>
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<th>Table 3: Comparison of IL-17, TGFβ and IgA among three groups</th>
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<td>Variables</td>
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<tr>
<td>IL-17 (ng/L)</td>
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<td>TGFβ (ng/L)</td>
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<td>IgA (μg/mL)</td>
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Group I=Dental caries up to 5, Group-II=Dental caries 6 or more, Group-III=Control group, SD: Standard Deviation, IQR: Interquartile Range. *p≤0.05= statistically significant, comparison among the three groups, comparison between group 1 and 3, comparison between group 2 and 3, comparison between group 2 and 1.
Discussion

The current study revealed higher levels of TGFβ in healthy controls (group III) as compared to patients of dental caries (group I and II) suggesting anti-inflammatory role of TGFβ in dental caries. TGFβ has been shown to enhance dental tissue repair. TGF-β inhibits production of pro-inflammatory cytokines from macrophages and T cells. TGF-β derived from non-immune cells also play a protective role in inflammatory conditions. The role of TGF-β in dental caries has not been documented in literature. But increased levels of TGF-β and its anti-inflammatory role has been observed in pulp inflammation as it inhibits TLR2 and TLR4 expression and downregulates odontoblast responses. TGF-β repairs dental tissue after injury.

In the present study, salivary IgA (sIgA) was raised in patients with dental caries compared to healthy controls that suggests its protective mechanism against dental caries to reduce and control the caries severity. sIgA inhibits attachment of oral bacteria to the epithelial and tooth surfaces and it neutralizes viruses, bacterial exotoxins and enzymes that contribute to dental caries. Ranadheer et al. (2011) and Amoundi et al. (2007) have also detected an increased level of IgA in patients of dental caries. However, the current study is not in agreement with Patel et al. (2010), Shetty et al. (2013) and Damle et al. (2011) as they observed decreased levels of slgA in patients of dental caries compared to controls. Similarly, the present study is in disagreement with Shifa et al. (2010), who documented no correlation between dental caries and slgA levels. The difference in the two studies could be due to small sample size, different stages of caries or age group of the subjects included in these studies.

The current study demonstrated high level of IL-17 in controls as compared to patients of dental caries. The role of IL-17 in dental caries is not present in literature. Liu et al. (2009) suggested anti-inflammatory role of IL-17 in autoimmune uveitis. Schenkenin et al. (2010) documented high levels of IL-17 in generalized aggressive periodontitis. The difference in the results of two studies might be due to different mechanisms of diseases.

Xiong et al. (2015), Rohaninasab et al. (2013) and Camille et al. (2016) documented an increase in IL-17 in patients of pulpitis and periodontitis respectively. Differences in the pathogenesis or stages of two different diseases could be the reason for the difference in results of these studies and it could be due to the smaller sample size.

Khuu et al. (2013) observed an increase of IL-17 in tuberculosis, the disagreement from the present study might be due to different mechanisms of diseases, different criteria for patient selection and differences in the sample size.

Conclusion

Levels of TGFβ were higher in healthy controls compared to patients with dental caries and level of salivary IgA was increased in patients with dental caries compared to healthy controls.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Contribution of authors:

Conception of research question: Ambreen Nawaz and Nadeem Afzal
Sample collection, experiments undertaken, data acquisition and analysis: Ambreen Nawaz, Husniah Batool, Muhammad Kashif, Afia Abbas, Faheem Shahzad, Romeeza Tahir, Nadeem Afzal
Paper writing, editing and manuscript submission: Ambreen Nawaz, Muhammad Kashif, Romeeza Tahir, Nadeem Afzal
References: