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Assessment of sCD14 levels in patients with endodontic pathology requiring root canal treatment

Avaliação dos níveis de sCD14 em pacientes que necessitam de tratamento endodôntico

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ABSTRACT

Objective: Dental caries is one of the most common microbial diseases. Because of the infectious nature of the disease, the immunologic response by the host plays an essential role in its development. Therefore, the aim of this study was to evaluate the sCD14 levels in patients exhibiting two to three teeth with caries involving pulp along with apical periodontitis requiring root canal treatment. **Material and Methods:** This study was carried out on 20 participants, of whom 10 were caries-free (Control) and 10 had two to three teeth with symptomatic irreversible pulpitis along with apical periodontitis requiring root canal treatment, within the ages of 20- 30 years. Unstimulated saliva of the participants was collected with disposable needle-less syringe from buccal and labial vestibules. The sCD14 levels in salivary samples were assessed before and following endodontic treatment. The results were analyzed by ELISA. **Results:** The obtained levels of sCD14 were analyzed statistically. Paired T test was performed to assess the significance. The results revealed that there was a significant difference in sCD14 levels with a P=0.0005, as it had drastically reduced once the inflammation has subsided. **Conclusion:** Higher values of sCD14 levels were seen in patients with symptomatic irreversible pulpitis along with apical periodontitis than in caries free group. The study also showed that sCD levels were significantly reduced following post endodontic treatment. Therefore, increased levels of sCD14 can be considered as a marker of inflammation.

KEYWORDS

Dental caries; Apical periodontitis; Dental caries; ELISA; sCD14.

RESUMO

Objetivo: A cárie dentária é uma das doenças microbianas mais comuns. Devido à natureza infecciosa da doença, a resposta imunológica do hospedeiro desempenha um papel essencial no seu desenvolvimento. Portanto, o objetivo deste estudo foi avaliar os níveis de sCD14 em pacientes que possuiam dois a três dentes com necessidade de tratamento endodôntico por apresentarem lesão de cárie envolvendo polpa e periodontite periapical. **Material e Métodos:** Este estudo foi realizado em 20 participantes, dos quais 10 estavam livres de cárie (controle) e 10 tinham dois a três dentes com pulpite irreversível sintomática e periodontite periapical com necessidade de tratamento endodôntico, nas idades de 20 a 30 anos. A saliva não estimulada das crianças foi coletada com seringa descartável sem agulha dos vestíbulos bucal e labial. Os níveis de sCD14 em amostras salivares foram avaliados antes e após o tratamento endodôntico. Os resultados foram analisados por ELISA. Resultados: Os níveis de sCD14 obtidos foram analisados estatisticamente. O teste T pareado foi realizado para avaliar a significância. Os resultados revelaram que houve uma diferença significativa nos níveis de sCD14 com um P = 0,0005, uma vez que reduziu drasticamente uma vez

que a inflamação diminuiu. **Resultados:** Os níveis de sCD14 obtidos foram analisados estatisticamente. O teste T pareado foi realizado para avaliar a significância. Os resultados revelaram que houve uma diferença significativa nos níveis de sCD14 com um P = 0,0005, uma vez que reduziu drasticamente uma vez que a inflamação diminuiu. **Conclusão:** Valores mais elevados de níveis de sCD14 foram observados em pacientes com pulpite irreversível sintomática junto com periodontite periapical do que no grupo livre de cárie. O estudo também mostrou que os níveis de sCD foram significativamente reduzidos após o tratamento endodôntico. Portanto, níveis aumentados de sCD14 podem ser considerados um marcador de inflamação.

PALAVRAS-CHAVE

Cárie dental; Periodontite periapical; ELISA; sCD14.

INTRODUCTION

Salivary proteins play an important role in the caries initiation [1]. In progression of caries, the role of salivary protein remains unanswered. These proteins share a common function or may have an opposite function [2,3]. In endodontic infections, the common virulence factor for gram positive bacteria it is the lipoteichoic acid and for gram negative bacteria it is the endotoxins, whose biologically active components are lipopolysaccharide (LPS) [4,5].

CD14 is a 55-KDa cell membrane glycoprotein which mainly recognizes such bacterial products as LPS, endotoxins, and peptidoglycans [6]. LPS, lipoteichoic acid and other peptidoglycans binds to CD 14 co receptor and gets neutralized by CD 14 [7,8]. One of the forms of CD14 receptor is a type of sCD 14. The other CD14 receptor is membrane bound CD14 (mCD14). Expression of mCD14 was seen on the surface of macrophage-like cells, gingival fibroblasts, numerous non-myeloid cells and activated neutrophils [7,9,10]. These cells release sCD14, circulate in serum and interacts with the LPS-binding protein (LBP) [11].

Interaction occurs between Toll like Receptors (TLR) and CD14/LPS/LPB ternary complex leads to cell activation [12]. TLR-2 and TLR-4 gets activated by the bacteria trigger the synthesis of inflammatory cytokine and chemokine [13,14]. Hence this is mechanism which leads to development of caries [15].

Therefore, this study aims to assess the level of sCD14 in patients with two to three teeth with caries involving pulp along with apical periodontitis requiring root canal treatment. To the best of our knowledge this is the first report comparing the sCD14 levels using ELISA in caries free group and in group with caries involving pulp along with apical periodontitis.

MATERIALS AND METHODS

Ethical consideration

The protocol of this investigation was approved by institutional ethics committee. The research was performed in full accordance with the ethical principles of world medical association declaration of Helsinki after obtaining informed consent was obtained from all the participants.

Study population

In this prospective clinical study twenty participants between 20 and 30 years of age reporting to the department of conservative dentistry and endodontics were randomly recruited in the study after obtaining approval by the Institutional ethical committee. A sample size of 20 participants were required which was based on the calculation with 95% power and an alpha error of 0.05 for an effect size of 0.9. Sample size calculation was done based on the findings of Biria et al. [16].

Inclusion and exclusion criteria

Two groups with twenty participants were enrolled in the study.

Group 1- caries free group (n=10);

Group 2- caries active exhibiting two to three teeth with caries involving pulp along with apical periodontitis requiring root canal treatment (n=10).

Patients with systemic disease such as diabetes, autoimmune disease, under any medical therapy, deep periodontal pockets and malignancy were excluded from the study.

Dental examination

Caries status was assessed according to WHO criteria and the endodontic pathology as diagnosed according to current suggestion [17,18].

The clinical examinations were performed by principle investigator. The detection of caries was based on clinically visible caries cavity and radiographically confirmed by radiolucency involving enamel and dentin. The detection of apical periodontitis was carried out clinically by tenderness on percussion and radiographically by the presence of widening of periodontal ligament space using periapical radiograph with long cone paralleling technique. The reliability of examination was checked by an intra examiner which indicates a very level of agreement.

Saliva sample collection

One hour prior to saliva collection, the participants were asked to restrict themselves from drinking and eating. Unstimulated saliva was collected by passive drooling method in the morning after rinsing their mouth [19]. Saliva was collected in sterilized containers and samples were immediately transported on ice to laboratory for processing.

ELISA test CD14

Salivary sCD14 was determined by ELISA technique (Commercial Enzo Life Science kit Farmingdale, NY, USA; catalogue No ALX-850-302- KI01). Monoclonal antibody specific for CD14 was added to a 96 well microplate and then 100 μL of the standard and samples were pipette into the wells. After removing any unbound substance, a biotin – conjugated antibody specific for CD14 was added into the wells. After washing three times with phosphate buffer solution (PBS), avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin – enzyme reagent, a tetramethyl benzidine) TMB substrate solution was added to the wells and then stop reagent was added to stop the reaction. The color developed in proportion to the amount of sCD14 bound to the initial step. The intensity of the color was measured using a microplate reader. The sCD14 were calculated and expressed in μg/mL.

RESULTS

T test was performed to analyze the paired samples. sCD14 levels were evaluated before and following root canal treatment. The results revealed highly statistically significant difference with a P=0.0005 (Table I).

DISCUSSION

Increase in CD 14 in an individual can be due associated with infectious diseases and also can be evident in traumatic conditions and other systemic complication [20-24]. In short it can be justified as whenever there is increase in inflammatory response the level of sCD14 levels are said to increase. This mechanism is due to host innate immune response. Therefore, the present study was performed to assess the sCD levels before and after root canal treatment in patients presenting with symptomatic apical periodontitis. There have been two previous reports which reported the correlation between CD14 levels and ECC. Both the studies were performed on children of age group 3 to 5 years. The novelty in the present study is that, this is the first study which investigates the correlation between caries involving pulp along with apical periodontitis and CD14. In the present study ELISA was performed to quantitatively analyze the CD 14 as it highly sensitive and can detect presence of sCD14 even when there is slight modification.

Previous study reported that in caries active group, sCD14 concentrations was found to be significantly higher [16]. On the contrary a study by Bergandi et al. [6] reported absence of sCD14 in the saliva of children aged 6-12 years. The reason for absence of sCD14 in the saliva children below 12 years may be due to absence of development of specific immune response. The contradictory results in the previous studies could be due to confounding factors such as age and immune response. As reparative immune response, the level of cd14 in saliva increase due to deficiency of IgA and IgG immunoglobulins.

Table I - Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Before - After	123.700	.57850	.18294	.82316	165.084	6.762	9	0.0005

The results of the present study reported that there was a significant difference between sCD14 levels between the study group and the control group. The sCD14 level was high in group with caries involving pulp along with apical periodontitis when compared to control group. This result was in corroboration with the previous study [25]. The reason for difference in the sCD14 level is because of the innate immune response by recognition of lipopolysaccharide (LPS), endotoxins and peptidoglycan [26]. Increased bioactive sCD14 that is found in saliva can be used as a tool for assessing oral health.

CONCLUSION

This study highlights the diagnostic potential of saliva. Salivary sCD14 concentrations were significantly lower in caries free group than caries involving pulp along with apical periodontitis Future research should confirm the role of sCD14 in dental caries development and focus on its clinical application as a predictor of future caries events.

Authors' Contributions

KJ: concept and design of the study, data acquisition. PA: analysis and interpretation of data, revising the manuscript critically for intellectual content. KVT: analysis and interpretation of data, revising the manuscript critically for intellectual content. RS: data acquisition,analysis, revising the manuscript critically for intellectual content.

Conflict of Interest

The author declare no conflict of interest.

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Regulatory Statement

Nil.

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