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Relationship between high CRP and cytokines in Saudi old people with dental caries in alkharij Region, Saudi Arabia



Arwa F. Alanazi^a, Awwad Alenezy^b, Amna Alotiby^c, Talat Bukhari^c, Wael Alturaiki^d,
Abdulkarim S. BinShaya^e, Hisham Ali Waggiallah^e, Harbi W^e, Kahtani Y^e, Majli K^e, Amani F. Alanazi^f,
Faris Q.B. Alenzi^{e,*}

^a Dept of Dentistry, Riyadh Alm University, Riyadh, Saudi Arabia

^b Dept of Family and Community Medicine, College of Medicine, NBU, Arar, Saudi Arabia

^c Dept of Immunology, College of Medicine, Um Qura University, Makka, Saudi Arabia

^d Dept of Med Lab Sci, College of Appl Med Sci, Majmma University, Saudi Arabia

^e Dept of Med Lab Sci, College of Appl Med Sci, Prince Sattam University, Alkharij, Saudi Arabia

^f Dept of RT, Al-Marafa University, Riyadh, Saudi Arabia

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ABSTRACT

Objective: Dental caries is one of the most common problems of the oral cavity which is frequently observed in older people. The aim of this study is to evaluate serum C-reactive proteins (CRP) levels and to identify the correlation between dental caries and CRP levels.

Methodology: The study included 12 aged patients with an average age of 65-years; the patients were diagnosed with dental caries and did not have clinical history of heart diseases, rheumatoid arthritis or any other infection. The control group consisted of 10 healthy donors with an average age of 60-years. The CRP level of positive samples was measured by using CRP Enzyme-linked immunosorbent assay-ELISA Kit.

Results: The current study showed that only 5 out of 12 patients were CRP positive.

Conclusions: Because of study limitations, it is early to conclude of close relationship between serum CRP and dental caries from the findings of this study; however, this study will give a clearer picture to understand the relationship between serum CRP, inflammatory cytokines and dental caries.

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1. Introduction

Dental caries is one of the most common health problems of the oral cavity which is observed most frequently in older people. The main reason behind this situation is that the old-aged individuals usually suffer from chronic disease such as diabetes and they do not take much care about their teeth and, consequently, do not often pay visit to the dentist; brush their teeth inconsistently,

and may consume sugar and frequently do the smoking (Lagerweij and van Loveren, 2020; Razak et al., 2014).

Dental caries is an infectious disease of teeth which is characterized by tooth loss or localized destruction of the teeth caused by bacterial activity. *Streptococcus mutans* is the bacterium which is mainly involved in the process of tooth decay during dental caries (Rathee and Sapra, 2020; Pereira et al., 2010). It is an acid-producing bacterium that usually metabolizes the food in the host (teeth) at under favourable conditions (dryness mouth-reducing saliva) to cause dental caries and this process usually slows within 6 to 24 months (Rathee and Sapra, 2020; Pereira et al., 2010; Forssten et al., 2010; Scully, 1981; Shivakumar et al., 2009). If dental caries advances without the intervention of any treatment, it will cause severe damage to the enamel and dentine of the teeth that ultimately leads to the inflammation of pulp and supporting tissues (Pereira et al., 2010; Scully, 1981). Recently, Kujan and colleagues showed the distribution of dental caries among Saudi healthy adults in a cross-sectional study (Idrees et al., 2017)

* Corresponding author at: Professor of Immunology, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, Saudi Arabia.

E-mail address: f.alenzi@psau.edu.sa (F. Q.B. Alenzi).

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The innate immune response of the host to this bacterial infection and release of inflammatory mediators from the damaged teeth tissues results in increased production of an acute-phase protein known as C-reactive protein (CRP). CRP is synthesized in the liver in response to inflammation or infection and it has a critical role in the host innate immune response that includes activation of the complement system, neutralization of pathogens, initiation of tissue degeneration and repair system (Bansal et al., 2014; Nunes et al., 2011).

The level of CRP usually increases within 1 to 4 days after infection and inflammation and once these two factors subside, the level of CRP goes back to the normal (Bansal et al., 2014). CRP level is considered as a risk factor for some life-threatening diseases such as atherosclerosis, (Moriya, 2019; von Vietinghoff and Koltsova, 2019) cardiovascular diseases (Sarkar et al., 2019; Liu et al., 2019) and hypertension (Liu et al., 2019; Hage, 2014; Xu et al., 2008). This might indicate that caries might be considered as an indirect risk factor of these diseases. Therefore, if dental caries is not treated, it can lead to chronic inflammation triggered by persistent bacterial infection in the teeth and, consequently, the CRP level in patient's serum elevates. Therefore, dental caries can have a negative impact on health and quality of life of the old-aged people (Pereira et al., 2010; Nunes et al., 2011; Petersen and Yamamoto, 2005; Shah, 2019).

Cytokines and chemokines can play an important role in linking DC immunologically with certain accompanying pathologies. This means they may all share certain cellular mechanistic or immunological elements pertaining to their pathophysiology. In inflammation, IL-36, IL-37, IL-38 are found to be pro inflammatory cytokines, promote Th1 and Th17 neutrophil influx and DC (Kuruville et al., 2019; Boutet et al., 2017). However, to the best of our knowledge, no study has been conducted so far to investigate the serum level of CRP in patients who are diagnosed with dental caries. Therefore, this study aims to investigate the correlation between dental caries and serum CRP and cytokines levels in a cohort of older subjects.

2. Methodology

2.1. Patients and healthy donors

The study included 12 patients aged between 60 and 75 years (average age:65 years) who were diagnosed with dental caries and did not have a clinical history of heart diseases, rheumatoid arthritis or any infection. The control group consisted of 10 healthy donors with an average age of 61 years (Table 1). Written informed consent from all patients were obtained.

2.2. Measurement of serum CRP level

The sera were separated from the blood of patients and healthy donors and were used to assess the concentrations of CRP. In the first place, all samples were screened to confirm the presence of CRP by using Card-CRP Single Test (Axis-Shield, Norway) according to the manufacturer's instructions. Afterward, the level of CRP of positive samples was measured by using CRP Enzyme-linked immunosorbent assay-ELISA Kit (Abcam, Massachusetts, USA). The normal range of serum level of CRP is less than 8 mg/ml.

Table 1
Participants Details.

Category	Sex	Frequency	Mean Age
Aged people	Males	6	65-years
	Females	6	
Healthy Controls	Males	10	61- years

2.3. Luminex for IL-36,IL-37,IL-38 inflammatory panel

Plasma from all groups were collected and stored at -20°C for later analysis. During analysis, ELISA kit is used for measuring cytokines. Samples duplicates are utilized and procedure is done according to manufacturer's manual. Luminex kits used to analyze patient's plasma has been purchased from (Merck Millipore, MILLI-PLEX MAP Human) and utilized as per manufacturer's instructions.

3. Results

The study findings indicate that only 5/12 of the dental caries patients were CRP positive, while 7/12 were CRP negative (Table 2). However, the average CRP level of these individuals was very high, (36 mg/ml).

Pro inflammatory cytokines IL-36, IL-37, IL-38 were also measured in the patients with dental caries who were also CRP positive. Table 3 shows the level of pro inflammatory cytokines was significantly elevated in CRP positive patients as compared to those of the control group.

4. Discussion

Dental caries is a low-grade bacterial infection of the oral cavity which could be a risk factor for Cardiovascular Diseases (CVD). CRP is an inflammatory protein which is usually found in high concentration during and inflammation and its high level may increase the incidence of CVD. However, dental caries can be far easily controlled as compared to CVD (Sarkar et al., 2019; Liu et al., 2019).

Several studies have reported that the level of CRP is high in the patients who are suffering from chronic periodontitis, which is an inflammatory condition of the periodontium (von Vietinghoff and Koltsova, 2019; Liu et al., 2019; Alade et al., 2018; Mysak et al., 2017). In addition, other studies have revealed that periodontitis is significantly associated with the development of chronic diseases such as hypertension, atherosclerosis and cardiovascular diseases (Bansal et al., 2014; Beck et al., 2018). The findings of this study will be valuable for improving the health and quality of life of the elderly population as they will enhance knowledge of dentists and will prompt them to evaluate the level of CRP from the patients of dental caries which will indirectly help them to minimize the risk factor of chronic heart diseases and atherosclerosis. Also, the current study will enhance the awareness of older individuals regarding the importance of oral health so that they can properly take care of their teeth and maintain oral hygiene and also visit the dental clinic regularly in case of any problem. In the long run, Saudi Arabia will have a healthy society which is one of the requirements of the vision 2030.

To the best of researchers' knowledge, this is the first study that aims to investigate the relationship between dental caries and serum level of CRP. The samples of the current study comprised people over 65 years of age and the gender factor was not taken into account while selecting the study population. The people included in the study sample did not have any previous history of cardiovascular diseases and also did not suffer from rheumatoid arthritis.

The result of the current study shows that only 30% patients of dental caries were CRP positive and the level of CRP in their sera

Table 2
CRP level of Participants.

Category	CRP	Percentage	Positive/ Negative
Aged People	5/12	41.6%	Positive
Aged People	7/12	58.3%	Negative

Table 3
Levels of Pro-inflammatory Cytokines.

Group investigated	IL-36(M ± SD)	IL-37(M ± SD)	IL-38 (M ± SD)
Healthy donors (n = 10)	1.5 ± 2	2.1 ± 1.3	2.8 ± 1
Aged people (n = 12)	2.8 ± 0.9*	4.8 ± 9.8*	7.1 ± 0.9*

was above the normal range. As this study is the first one in measuring serum level of CRP in the patients of dental caries, no findings of the previous studies were available to compare the outcomes of the current study for better understanding. However, two previous study revealed high level of salivary CRP in the patients of dental caries (Aziz and Mohammed, 2016; Gawri et al., 2012). Nevertheless, the outcome of these studies cannot be taken into account and compared with the result of the current study because the level of CRP was measured in the saliva, not in the serum (which is local, not as a systemic immune response). Moreover, other studies were based on the study sample which includes children and patients less 50 years old respectively (Aziz and Mohammed, 2016; Gawri et al., 2012).

Additionally, the current study shows that 70% patients of dental caries had a normal level of CRP; moreover, it is also not clear whether the patients of the current study were having any antibiotic therapy as antibiotic therapy can reduce the level of serum CRP. This might explain the low parentage of positive CRP in this study. Another limitation in the current study is funding and time restriction which did not allow the researchers to conduct the study on a large study sample and analyse the data accordingly. Because of these limitations, it is early to draw a conclusion about the close relationship between serum CRP and dental caries. However, the findings of this study will surely help future researchers and practitioners gain better picture of understanding of the relationship between serum CRP and dental caries which will help them in their future research.

Cytokines IL-36, IL-37, and IL-38 were found in significantly elevated levels in blood samples obtained from the patients of the study sample. This was verified by the presence of lymphocytosis in all the CRP positive patients and not in others. Indeed, our findings suggest that more attention should be paid to the potential direct regulatory effect of regulatory T cells.

In conclusion, our study is the first one to show a close relationship between high CRP in the presence of inflammatory cytokines correlated with dental caries. However, this study has several limitations including: obtaining more samples, budget restriction and approval from the private clinics, further investigations are required.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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