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Research Article

Salivary Soluble CD40 Ligand Levels and Their Relationship to Periodontal Markers in Patients with Periodontitis and/or Obesity: An Observational Case-Control Study

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Abstract

Background: sCD40L, a co-stimulatory molecule that activates T-helper cells, is one of many mediators that regulate the inflammatory conditions of periodontitis and obesity. Additionally, the association of this biomarker with periodontitis and obesity has not been robustly investigated. *Objective*: Evaluation of salivary levels of sCD40L in periodontitis and obese patients in comparison to healthy controls and their association with different periodontal parameters. *Methods*: 110 subjects were enrolled in this study. Salivary samples were obtained prior to the clinical examination. They were divided into four groups: the first group (20 subjects) was the control group; the second group (30 subjects) consisted of subjects with obesity (BMI$\geq$30 kg/m2); the third group (30 subjects) consisted of subjects with periodontitis; and the fourth group (30 subjects) consisted of subjects with periodontitis and obesity. A periodontal examination was performed to report plaque index (PLI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL). Obesity was assessed using the body mass index (BMI). *Results*: Both periodontitis and obese patients demonstrated elevated salivary sCD40L levels compared to healthy subjects. sCD40L was positively correlated with PLI in periodontitis patients and with PPD in obese periodontitis patients. *Conclusions*: A significant association between sCD40L, periodontitis, and obesity was reported, implicating sCD40L's role in the pathogenesis of these conditions.

Keywords: Inflammation, Obesity, Periodontitis, Saliva, sCD40L.

مستويات CD40 القابلة للذوبان في اللعاب وعلاقتها بعلامات اللثة لدى المرضى الذين يعانون من التهاب دواعم السن و/أو السمنة: دراسة الحالات والشواهد

الخلاصة

الخلفية:sCD40L هو جزيء محفز مشترك ينشط الخلايا التائية المساعدة، هو واحد من العديد من المسببات لحالات التهاب اللثة والسمنة. بالإضافة إلى ذلك، لم يتم التحقيق بقوة في ارتباط هذه العلامة الحيوية بالتهاب دواعم السن والسمنة. **الهدف:** تقييم مستويات اللعاب من sCD40L في مرضى التهاب اللثة والسمنة مقارنة بالضوابط الصحية وارتباطها بمعايير التهاب دواعم السن المختلفة. **الطريقة:** تمت دراسة 110 حالات خلال هذه الدراسة. تم الحصول على عينات لعابية قبل الفحص السريري. تم تقسيمهم إلى أربع مجموعات: المجموعة الأولى (20 شخصا) كانت المجموعة الضابطة. المجموعة الثانية (30 شخصا) تألفت من أشخاص يعانون من السمنة (مؤشر كتلة الجسم $\geq$ 30 كجم / م 2) ؛ المجموعة الثالثة (30 شخصا) تتألف من أشخاص مصابين بالتهاب اللثة. وتألفت المجموعة الرابعة (30 شخصا) من أشخاص يعانون من التهاب اللثة والسمنة. تم إجراء فحص اللثة للإبلاغ عن مؤشر البلاك (PLI) ، والنزيف عند الفحص (BOP) ، وفحص عمق الجيب (PPD) ، وفقدان الارتباط السريري (CAL) و تم تقييم السمنة باستخدام مؤشر كتلة الجسم (BMI). **النتائج:** أظهر كل من مرضى التهاب اللثة والسمنة مستويات مرتفعة من اللعاب sCD40L مقارنة بالأشخاص الأصحاء. كان sCD40L مرتبطا بشكل إيجابي مع PLI في مرضى التهاب اللثة ومع PPD في مرضى التهاب اللثة الذين يعانون من السمنة المفرطة. **الاستنتاجات:** تم الإبلاغ عن ارتباط كبير بين sCD40L والتهاب دواعم السن والسمنة، مما يشير إلى دور sCD40L في التسبب في هذه الحالات.

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INTRODUCTION

Periodontal disease is a multifactorial inflammatory disorder that affects the structures that support the teeth (alveolar bone and connective tissue) [1]. In response to a bacterial infection, the immune system is activated, which leads to the release of cytokines, metalloproteinases, and pro-inflammatory mediators, which destroy tissue [2]. Obesity is defined as a chronic, complex condition characterized by excessive fat deposition and is considered a health risk [3]. It is a major issue and burden because it is an etiological factor in many diseases and ailments, including cardiovascular disease, stroke, diabetes mellitus, and cancer [4]. Obesity and overweight have become major public health issues worldwide [5]. Obesity and overweight were found to be prevalent in Iraq [6]. Periodontal disorders are associated with obesity, according to systematic reviews and meta-analyses [7,8]. Periodontal disease prevalence was found to be greater in obese patients compared to normal-weight individuals, and this link became stronger as BMI levels increased [9]. Obesity may also have an adverse effect on periodontal treatment outcomes [10]. Another study discovered a link between plaque index and BMI [11]. The exact process by which obesity affects periodontal tissue remains unknown. Obesity changes the immune and inflammatory systems, influencing vulnerability in the host [12]. Obese people have larger and more adipocytes, which leads to higher levels of inflammatory adipokines, most notably interleukin-6 and tumor necrosis factor alpha (TNF-α) [13,14]. A recent study on the Iraqi population found a link between obesity and periodontitis severity; however, other risk factors, such as age and smoking, also play a significant role [11]. T-cells are known to either induce microphage production or activate B-cells in response to invading pathogens, both of which are important in influencing the immune response in periodontal disorders. T-helper cells have been demonstrated to aid in the advancement of periodontal disease in vulnerable patients [15]. Co-stimulatory particles are critical in the activation and regulation of T-cell function. CD40 is one of these essential particles. CD40-CD40L ligation has been proposed to govern the production of pro-inflammatory cytokines such as IL-8, IL-12, and TNF-α, all of which are important in the development of T cell responses and the activation process of T cells [16]. The CD40 ligand, also known as CD154, is a transmembrane glycoprotein. It can be found in two forms: membrane-bound or soluble (sCD40L) [17]. It was first discovered on activated CD4+ T-cells and then in considerable numbers on platelets [18]. Understanding the association between sCD40L, periodontitis, and obesity holds immense clinical significance. Firstly, identifying reliable biomarkers for early detection of periodontitis and obesity can aid in timely intervention and preventive measures, which ultimately improve patient outcomes and overall health. Secondly, exploring this association may offer valuable insights into the shared inflammatory pathways between periodontitis and obesity, which could pave the way for novel therapeutic targets aimed at modulating sCD40L. To the author's best knowledge, no previous studies have explored the association of sCD40L in saliva with periodontitis and obesity. This study aims to evaluate salivary levels of sCD40L in periodontitis and obese patients in comparison to healthy controls and their relationship with different periodontal parameters.

METHODS

Study design and patient selection

The design of this study was an observational case-control study. It was undertaken at the Periodontics Department of the dental hospital, the Dental College at the University of Baghdad. Ethical approval was granted from the University of Baghdad (Ref. 454622 on January 19, 2022). Between March 2022 and June 2022, 110 subjects were recruited to take part in this study, which consisted of 30 patients with periodontitis who were systemically healthy with a normal weight (BMI$\leq$24.9), 30 patients with obesity (BMI$\geq$30) (periodontally healthy), 30 patients with periodontitis and obesity (BMI$\geq$30), and 20 subjects who were periodontally and systemically healthy with a normal weight (BMI$\leq$24.9). Informed consent was acquired from every participant before conducting the study, which explained the purpose of the study and the sampling procedure. The inclusion criteria for enrollment in the study comprised subjects with no systemic diseases, not taking any medication in the last 3 months, and at least 20 natural teeth. Subjects who were excluded from the study included smokers and alcohol drinkers, patients affected by systemic diseases, and patients who have had periodontal therapy during the last 3 months.

Saliva collection and outcome measurements

Samples of whole, unstimulated saliva were obtained from all participants prior to any oral examination. The drooling method was used to capture whole saliva in a plastic cup, which was subsequently placed in a test tube in a cooling box. The samples were centrifuged later at 3000 rpm for a period of 10 minutes and stored in a -80º C freezer until the day of analysis. The samples were thawed to room temperature on the day of the laboratory analysis [19]. Levels of sCD40L in saliva were measured using an ELISA kit (catalog number MBS2610637) suitable for the quantitative detection of human concentrations of sCD40L according to the manufacturer's instructions. The double antibody sandwich technique was implemented for this kit. The already-coated antibody was an anti-human sCD40L monoclonal antibody, and a biotinylated polyclonal antibody was used as the detection antibody. Both the

samples and the antibodies were transferred into the plate wells and washed out with a buffered saline solution. Then, avidin-peroxidase conjugates were introduced to the wells. After the enzyme conjugate had been washed out of the wells, TMB (tetramethylbenzidine) substrate was added for coloration. The TMB reaction would lead to the formation of a blue product from the peroxidase activity, and at last it would turn yellow after we had added the stop solution. At 450 nm, the intensity of the color was calculated within 10 minutes on the ELISA reader. The optical density of the standards was plotted against their concentration, and the resulting curve was used to calculate the concentration of sCD40L.

Case definition and data collection

Prior to the periodontal examination, the height and weight of every participant were measured using a scale and a measuring tape. Body mass index (BMI) divided the participants' weight into three categories: normal (BMI: 20-24.9), overweight (BMI: 25-29.9), and obese (BMI: 30). The BMI was calculated according to this formula: BMI = weight (kg)/height^2 (m^2) [20]. A comprehensive periodontal examination was performed by a calibrated examiner with the use of the Michigan O-probe, which included plaque index (PLI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL). All tooth surfaces were examined for all parameters except PLI, where only four surfaces were examined. Subjects were classified as having periodontitis when CAL was detected at ≥2 non-adjacent teeth or buccal or oral CAL≥3mm with a

pocket>3mm at ≥2 teeth [21]. While healthy subjects were classified as having BOP<10%, PPD≤3mm, and intact periodontium (no probing attachment loss) [22].

Statistical analysis

Statistics Package for Social Science (SPSS, version 25) was utilized for statistical analysis, which included both descriptive and inferential statistics. The Shapiro-Wilk test was used to evaluate data normality, and it was discovered that all analyzed variables had a normal distribution among groups. We utilized the chi-square test to assess gender distribution disparities statistically because it is considered categorical data. The remaining parameters were determined to be regularly distributed between healthy and diseased individuals, and because this study comprised four groups, a two-way ANOVA test was performed to evaluate them. Pearson's correlation test was used to assess the relationship between sCD40L and several clinical periodontal markers. The statistical tests in this study employed a significance level of $p<0.05$.

RESULTS

As shown in Table 1, 110 subjects participated in this study; they were allocated into a healthy control group ($n = 20$), an obese group ($n = 30$), a periodontitis group ($n = 30$), and an obese periodontitis group ($n = 30$). The age ranged between 30 and 50 years in all groups; the control group had a mean age of 37.55±7.95 years. The mean age in the obese group was 36.30±5.44 years, and in the periodontitis group it was 37.13±6.46 years, while in the obese periodontitis group it was 40.46±6.64 years.

Table 1: Mean values for all variables and comparisons across all groups

Parameters	Mean values				P values			
	Healthy n=20	Obese n=30	Periodontitis n=30	Obese Periodontitis n=30	Healthy $vs.$ Obese	Healthy $vs.$ Periodontitis	Obese $vs.$ obese periodontitis	Periodontitis $vs.$ obese periodontitis
PI (%)	20.25±7.50	21.7±10.39	40.4±24.39	44.6±21.22	0.78	<0.001	<0.001	0.37
BOP (%)	2.5±2.35	4.53±2.27	36.93±26.29	34.1±20.4	0.68	<0.001	<0.001	0.53
PPD (mm)	-	-	4.62±0.55	4.61±0.49	-	-	-	0.93
CAL (mm)	-	-	3.46±0.98	3.69±1.02	-	-	-	0.40
sCD40L (ng/ml)	3.28±0.75	4.19±1.01	5.15±0.95	4.02±0.95	0.001	<0.001	0.48	<0.001

Regarding sex distribution, 51.82% of the total participants were females, while males constituted 48.18%. For age ($p = 0.083$) and gender ($p = 0.692$), no significant difference was found among all four groups. The highest mean level of sCD40L in saliva was observed in the periodontitis group, followed by the obese group and the obese periodontitis group, while the lowest mean level of sCD40L was found in the control group, with significant differences among groups. The levels of sCD40L and all parameters in this study across all groups have been summarized in Table 1. Regarding plaque index (PLI) and bleeding on probing (BOP), a

significant difference was found between the healthy control group and the periodontitis group, as well as a significant difference between the obese group and the obese periodontitis group; however, no significant difference was found between the periodontitis group and the obese periodontitis group in terms of PLI and BOP. On the other hand, when we compared periodontitis and obese periodontitis groups in terms of probing pocket depth (PPD) and clinical attachment loss (CAL), there was no significant difference noticed between these groups. Regarding the levels of salivary sCD40L, a significant difference was noted between the

healthy control group and the periodontitis group, as well as between the healthy control group and the obese group. Interestingly, no significant difference was found between the obese group and the obese periodontitis group. After looking at how this biomarker correlated with different periodontal parameters, the current study found a positive correlation between sCD40L levels in saliva and PLI in the group with periodontitis (Table 2 and Figure 1).

Table 2: Correlations of various clinical parameters with sCD40L salivary levels in all study groups

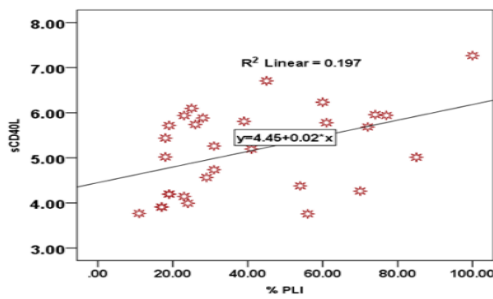| Groups		PLI	BOP	PPD	CAL
Healthy	r value	-0.12	0.02	-	-
	p value	0.61	0.90	-	-
Obese	r value	-0.17	-0.05	-	-
	p value	0.36	0.79	-	-
Periodontitis	r value	0.44	0.23	0.34	0.03
	p value	0.01	0.20[NS]	0.09	0.85
Obese periodontitis	r value	0.21	-0.02	0.45	0.009
	p value	0.24	0.90	0.03	0.96[NS]

Figure 1: Correlation of salivary concentrations of sCD40L with % PLI in periodontitis patients

It also found a positive correlation between the levels of this biomarker and PPD in the obese periodontitis group (Figure 2).
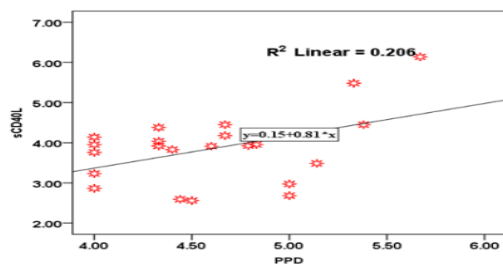

Figure 2: Correlation of salivary concentrations of sCD40L with PPD in obese periodontitis patients

DISCUSSION

The primary aim of this study was to explore the association of salivary sCD40L with periodontitis and obesity, shedding light on the potential role of sCD40L as a shared inflammatory mediator between these two chronic conditions. Our study fills a crucial gap in the current understanding of periodontitis and obesity by investigating salivary sCD40L in these conditions. To the best of our knowledge, there is limited research exploring the association of sCD40L with both periodontitis and obesity. Given the limited studies on sCD40L levels in saliva, we sought to draw comparisons with existing research conducted on other media as well as in saliva. This study showed a significant rise in the salivary levels of sCD40L in patients with periodontitis in comparison to healthy subjects. This result matches a study that found that the levels of sCD40L in gingival crevicular fluid (GCF) were much higher in the group with periodontitis and correlated positively with how bad the disease was. Also, their concentrations dropped a lot after therapy [17]. Other studies evaluated serum levels of sCD40L in periodontitis patients and showed them to be elevated, yet one of them did not reach statistical significance [23, 24]. Our results and the results of previous studies suggest that sCD40L plays an important role in the process of periodontal disease. One of the many functions of sCD40L is to activate and differentiate T-lymphocytes and also stimulate B-cells, which includes producing different types of immunoglobulins in a process called immunoglobulin class switching [25]. sCD40L also plays a big part in regulating the production of pro-inflammatory mediators and facilitates the binding of platelets to leukocytes and endothelial cells. It also facilitates the expression of different chemokines in macrophages and dendritic cells, such as MCP-1 and MIP-1α [26]. The ability of these chemokines to make it easier for monocytes to move to the diseased area [27] means that when the levels of these chemokines go up, they can speed up the destruction of periodontal tissue. The ability of sCD40L to control the expression and activation of MMP, which is an important part of the disease process [28], is another way that it can play a key role in periodontal disease. A few studies have investigated this marker in the periodontal environment. In one of these studies, gingival fibroblasts were shown to release CD40, which, when bound with CD40L, led to the expression of IL-6 and IL-8 in rats that had braces [29]. Another study showed that gingival and periodontal fibroblasts use CD40 to send signals by showing that gingival tissues that are inflamed make more CD40 than gingival tissues that are healthy [30]. Interestingly, another study was inconsistent with the results of this study; it found a strong negative correlation between the severity of periodontitis and the salivary levels of sCD40L [31]. This could be explained by a previous study that showed that the interaction between CD40 and CD40L on gingival fibroblasts led to the downregulation of MMP1 and MMP3, which is contradictory to the expected function of CD40L in mediating MMP function and periodontal tissue degeneration [32]. This controversy invites more research on this matter to investigate and establish the exact role of sCD40L in the periodontal disease process.

This study also noted a significant positive correlation between sCD40L and PLI in the periodontitis group, contrary to a study that found no correlation between sCD40L and PLI [17]. Patients with periodontitis who have more plaque have more microorganisms like Porphyromonas gingivalis and Streptococcus sanguis, which make a protein called platelet aggregation-associated protein (PAAP), which helps platelets stick together [33]. This upregulates and translocates CD40L to the platelet surface, where it splinters and becomes sCD40L [34]. A significant positive correlation was also noted in this study between sCD40L and PPD in the obese periodontitis group, which is consistent with a previous study that also showed a positive correlation between sCD40L and PPD, yet obesity was not investigated in that study [17]. This may be related to the higher number of platelets seen in obese patients compared to normal-weight individuals [35]. Moreover, the number of platelets is increased in periodontitis patients [36] and declines following treatment [37]. On the other hand, this study revealed a significant increase in concentrations of salivary sCD40L in obese patients in comparison to healthy controls. This result agrees with a study that found that sCD40L had significantly higher concentrations in patients with obesity in comparison to normal-weight subjects [35]. Another study found markedly higher sCD40L concentrations in patients with concerning levels of obesity (BMI≥35 kg/m2) in relation to obese patients (BMI 30–34.9 kg/m2) and non-obese subjects (BMI<25 kg/m2) [38]. Moreover, previous studies demonstrated elevated sCD40L concentrations in patients with obesity and decreased them following weight loss [39, 40]. sCD40L was shown to have a positive correlation with BMI as well as waist circumference, which suggests an elevated percentage of visceral fat, which could be a possible mechanism to explain this association through cytokine secretion via adipose tissue [35]. Another potential mechanism is lipid peroxidation. sCD40L was found to be strongly linked to lipid peroxidation [39], which is a measure of oxidative damage. It is well known that obesity is linked to a rise in oxidative stress, which may be the cause of the abnormal production of adipocytokines [41]. Obesity is linked with lipid peroxidation and platelet activation [42]. This is another potential explanation for the elevated levels of sCD40L in obese people, since it was demonstrated that obese individuals had substantially higher platelet counts than normal-weight individuals [35]. However, the process by which platelets stimulate CD40L is still not fully understood. Interestingly, a more recent study investigated numerous inflammatory markers in obese patients and found that all these markers were increased in obese patients compared to normal-weight subjects, apart from sCD40L, which had lower levels in obese patients, which is inconsistent with the results of the current study [43]. Another study showed that sCD40L had a strong negative correlation with BMI [31]. More research is needed to verify the role of sCD40L in relation to obesity.

Study limitations

According to the author's best knowledge, this study has its own limitations: 1) While BMI has been utilized in many previous studies, it still has its drawbacks, especially in muscular individuals, where it overestimates the level of adiposity. 2) The design of this study is cross-sectional case-control, so it is incapable of establishing causation and only identifies associations.

Conclusion

This study found a link between the levels of sCD40L in saliva and both periodontitis and obesity, which shows how important this biomarker is in these conditions. However, the exact mechanism by which sCD40L is involved in these two processes is still not clearly understood. Salivary testing for sCD40L may serve as a non-invasive and potentially cost-effective method for identifying individuals at risk of developing or experiencing disease progression in both periodontitis and obesity. sCD40L could represent a promising therapeutic target for developing novel interventions aimed at mitigating inflammation in periodontitis and obesity. More research needs to be done to find out if targeting sCD40L-related pathways in these conditions is possible and if it works. This could lead to better oral and overall health.

Conflicts of interest

There are no conflicts of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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