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Relationship between NLRC4 and TNF-α Level as a Diagnostic marker to Differentiate Periodontal disease from Healthy status

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Abstract

The nod-like receptor family CARD domain-including protein 4 (NLRC4) inflammasome controls the development and production of inflammtory molecules and the triggering of a reaction to threat signs of pathogen, impairment to tissues, and altered metabolism. These alterations play a part in the development of many diseases, including periodontitis. The aim of the present investigation was to estimate the salivary concentrations of NLRC4 and tumor necrosis factor-alpha (TNF-α) in discriminating periodontal disese patients from individuals have a healthy periodontium and detects NLRC4 and TNF-α possible linking. Materials and Methods, this case-control study, 88 participants were split into two groups: the control group (C) consisted of healthy subjects with a healthy periodontium, while the patient group (P) consisted of subjects with periodontitis. The plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL) were the markers used to assess the periodontal health. All subjects' unstimulated saliva was collected, and ELISA technique was used to clarify the quantity of NLRC4 and TNF-α in the salivary samples. Results the salivary levels of NLRC4 and TNF-α were high significantly of periodontitis disease participants to health individuals and have significantly linked periodontal indicators. Likewise, a noteworthy connectionof NLRC4 and TNF-α was observed. Conclusion in the present study findings that the salivary NLRC4 and TNF- α levels are noticeably greater in patients with periodontitis. Furthermore, there was a possible association between NLRC4 and TNF- α. Therefore, it was found that salivary NLRC4 and TNF- α play a part in the pathophysiology of periodontitis and can be used to diagnose the disease and distinguish it from periodontal health.

Keywords NLRC4; Periodontitis; Saliva; TNF-α; Periodontal parameters

1. Introduction

periodontitis is the damaging inflammatory disease; it is thought to have a multifaceted aetiopathogenesis. It destroys tooth-supporting tissues in people worldwide and is brought on by an imbalance between the defenses of the host and the quantity of harmful microbes in the subgingival microenvironment. [1-3].

Pathogenic bacteria trigger the human immune response[4,5], through their nucleotide binding oligomerization domain (NOD)-like receptors (NLRs), which are pattern recognition receptors (PRRs)[6]. Inflammasomes, in particular, are intracellular receptors for pattern recognition that activate after numerous signs are identified as pathogenic microbes-associating molecules pattern (PAMPs)[7]. The mainly complex is reacted PAMP and (NLRP3) inflammasomes [8]. Greater production of developed interleukins, which are essential for the host defenses when periodontitis is present [8.9].

NLRC4 (NLR family CARD domain including 4) inflammasomae, similar NLRP3, revealed triggering thru microbiome eliciting, such as DAMPs and gram-negative bacteria, which causes the release of physiologically active IL-1β [10-12]. In

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vitro experiments found that P. gingivalis alone could not trigger NLRC4 concentrations at fibroblast and a monocyte cells of individual tissue of gingiva samples[13].

The periodontal soft and hard tissues play a role in the process of inflammation, that is regulated by NF-kB [14]. Induction of NF-kB can produce and activate several bio-molecular inflammtory molecules, like TNF-α. When a pathogenic or detrimental effect is recognized, inflammatory caspase activation was required TNF--α release during periodontal disease[15,16].

Mainly, TNF-α, is the principal inflammatory molecule in innate immune response, and essential of the exacerbation tissue deficit and bone damage associated with periodontitis, since it promotes the induction, proliferating, and differentiating of osteoclasts, leading to bone loss[17,18].

presently, nevertheless, no research examined the NLRC4 concentrations at disorders of periodontium and correlate with TNF- α. Therefore, the initial research is done to evaluate NLRC4 inflammasome concentrations in saliva of participants had periodontitis illness. Added, to investigate NLRC4 and TNF-α assembly of saliva in discriminating participants had periodontitis illness than health periodontal participants and determine relationship between NLRC4 and TNF- α for exploring NLRC4 possible effects on TNF- α in periodontitis.

2. Materials and Methods

The current inquiry was intended as a study of case control that took place in Karabla, Iraq, between May and September 2023. The committee of ethics of the University of Al-Ameed, College of Dentistry, accepted the study (reference number: 125). Every subject was told about the study and how it would be done, and they all signed a paper saying they understood and agreed to take part. All participants with age range (35-52years) males and females, attending the Department of Periodontics, College of Dentistry, University of Al-Ameed. Patients were first screened to determine whether they were suitable for recruiting. After implementing inclusion/exclusion criteria, participants were generally well and had the ordinary weight less than 30 kg/m^2 according to BMI, had 20 teeth or more, wanted to sign a written consent form, were cooperative, did not smoke, had not received periodontal treatment within the previous six months, had not taken any medications within the previous three months, had no symptoms of an acute illness recently, and had no oral lesions unrelated to periodontitis.

This study enrolled 88 participants in total were split into two groups for this investigation. 44 participants as the control group has a periodontal tissue that was in good condition, with BOP less than 10%, PPD less than 3 mm, and none CAL[19]. Periodontitis patients (n = 44) showed identifiable interproximal CAL in more 2 of no neighboring teeth or greater 3 mm CAL on either the buccal (facial) or lingual/palatal facets, linked with PPD > 3 mm in two teeth [20]. Additionally, the participants were generalized, stage 1–4 (grade A–C) unstable (PPD ≥ 4 mm with BOP or PPD >5 mm with or without BOP) periodontitis and be free of risk factors[20]. Each participant then had a clinical evaluation after salivary samples were taken.

2.1. Clinical Examination

Saliva samples were collected prior to doing any periodontal examinations. The clinical indicators to be assessed in sequential series included periodontal indicators [PI, BOP, PPD, and CAL]. PI then BOP recorded classifying either existing one score or not zero score[21,22]. A probe (Michigan O probe) was used to do the periodontal assessment at six locations per tooth, with the exception of plaque scores, which were only obtained on four faces for every tooth. The periodontal checkup did not include wisdom teeth. The same examiner examined the clinical characteristics for every tooth that was in place.

2.2. Saliva collection and analysis

The subjects of the examination were told to abstain from consuming anything but the water during a minimum of one hour before the samples were collected. To remove any food particulates, germs, and desquamated epithelium, the participants thoroughly washed their mouths with drinking water for 15 seconds. They then waiting for the water to clear for two minutes before the sample was taken. Study participants' saliva was taken from nine to twelve clocked using a standardized, drool and passively salivation technique to obtain entire saliva samples. Following unstimulated saliva collection, the samples were centrifuged for 20 minutes at 3000 rpm in order to separate the salivary supernatants from the debris from the cells. Prior to ELISA analysis, have been stored at -20 °C.

2.3. Statistical

The data was statistically analyzed via SPSS for Window, versions 28. Constant records were represented by mean and standard deviation, whereas categorical variables represented by numeral and proportion. The data distribution's normality was determined using the Shapiro-Wilk test. T-test performed that establishing the meaningfully variances among the study individuals to evaluate the false supposition. Furthermore, the Correlation coefficient identified noteworthy associations among various indicators and variably features, p-values < 0.05 were considered to be statistically noteworthy.

3. Result

Population information and periodontal characteristics of the 88 participants are demonstrated in Table 1. Participants had periodontitis were 40.2 ± 3.8 years old on average, while control subjects were 39.8 ± 1.4 years old. The periodontits group's male to female ratio was 25/19, whereas the healthy group's was 20/24. Age and sex variations among both groups under investigation were not statistically noteworthy.

Additionally, relative to the control group, the present findings revealed a significant rise in the mean values of PI, BOP, PPD, and CAL among participants had periodontitis (0.73 ± 0.21, 0.82 ± 0.22, 8.43 ± 0.26, and 7.38 ± 0.05, respectively).

Moreover, Table 2 illustrations that the mean salivary quantities of NLRC4 and TNF- α, were significantly greater in the periodontitis group (2.52 ±1.09 ng/ml, and 178.64 ± 1.54 pg/ml resp) in comparison to the healthy group (0.31 ± 0.84 ng/ml, and 57.26 ± 1.03 pg/ml resp).

This investigation found significantly relationships of the salivary concentrations of NLRC4 and TNF-α and clinical characteristics (PI, BOP, PPD, and CAL), as indicated in Table 3 via correlation coefficient (r).

Remarkably, Table 4 displays a substantially significant connection between NLRC4 and TNF- α values in saliva (r = 0.612, p < 0.05).

Table 1 Demographic data and clinical parameters of the study groups

Parameters	Healthy group (control)	Periodontitis group	p-value
Age	39.8 ± 1.4	40.2 ± 3.8	0.073 NS
Sex Male Female	20 (40.0%) 24 (60.0%)	25 (66.3%) 19 (33.7%)	 0.890 NS
PI	0.11 ± 0.20	0.73 ± 0.21	0.01
BOP	0.04 ± 0.07	0.82 ± 0.22	0.03
PPD	0.000 ± 0.000	8.43 ± 0.26	0.04
CAL	0.000 ± 0.000	7.38 ± 0.05	0.02

p ≤ 0.05, significant.

Table 2 Salivary level of NLRC4and TNF-α of the study groups

Parameters	Healthy group	Periodontitis group	p-value
NLRC4 (ng/ml)	0.31 ± 0.84	2.52 ±1.09	0.000
TNF-α (pg/ml)	57.26 ± 1.03	178.64 ± 1.54	0.000

p ≤ 0.001, significant; ±: standard deviation.

Table 3 Correlation between salivary biomarkers and clinical parameters

Salivary NLRC4			**Salivary TNF- α**	
Parameters	r	p-value	r	p-value
PI	0.395	0.03	0.685	0.01
BOP	0.624	0.02	0.732	0.02
PPD	0.521	0.00	0.872	0.04
CAL	0.643	0.01	0.884	0.01

r: correlation coefficient; statistically significant at $p \leq 0.05$:

Table 4 Correlation between NLRC4 and TNF- α

	Salivary NLRC4	
Salivary TNF- α	r	p-value
	0.612	0.01

statistically significant at $p \leq 0.01$

4. Discussion

The inflammatory cytokines stimulator, NLRC4 inflammasome, is concerned the host cell necrosis and inflamed apoptosis [23,24]. NLRC4 inflammasome concentrations were substantially greater in the periodontal disease group versus an intact periodontium group. This implies a relationship between the increase in salivary NLRC4 and the degree of periodontium injury during inflammation. NLRC4 concentration rises as a result of the induction of several type of periodontal cells thru stimulatory of inflammation to create inflammatory chemicals such IL-18 and TNF- α. These are believed to play an essential function in connective tissue degradation. Inflammasomes have been implicated in the pathophysiology of periodontal disease by boosting the release of inflammatory chemicals, it was linked to periodontium deterioration [25].

The prior study used the IHC technique to examine NLRC4 expression in various forms of periodontal disease. The results of this investigation showed that, in comparison to healthy controls, NLRC4 was more expressed in the epithelium of diseased sites. However, the gum tissue of the group with periodontal disorders had higher levels of NLRC4 activating., the limited sample size prevented it from reaching significance [26].

Moreover, a relationship with statistical significance was found between the characteristics of periodontitis. (PI, BOP, PPD, and CAL) and NLRC4 concentration. These results showed a potentially role of NLRC4 in periodontitis pathway. The activation of the NLRC4 and their inflammatory response in periodontal tissue and bone increased destruction of periodontitis. There was no previous study to compare with it.

It's been proposed that caspase-1 is triggered by NLRC4 inflammasome formed responding the infections via several pathogens. It was shown that when surface Toll-like receptors like TLR5, which detect extracellular flagellin, are absent, NLRC4-dependent activation of caspase-1 takes place [27]. While NRLC4 ligation starts the synthesis of cytokines and their processing by caspase-1, TLR5 ligation only stimulates the production of pro-IL-1β and not its release [27]. Prior in vitro studies assessed NLRC4's potential function in various cells activated by P. gingivalis[28.] According to the animal investigation, mice with NLRC4 activity had higher levels of an inflammatory process caused destruction of bones [27].

TNF- α is an inflammatory molecule, it regulates induction of the white blood cells that cause inflammation, alteration in permeation of the microvascular, and promotion bone breakdown [29]. The patient in this study had a greater salivary TNF- α concentration versus the health indivdules. This supports research via Varghese et al. [29] and Ehsan et al. [30] showed a considerable increase in TNF- α levels in people had periodontitis related to healthy people.

As well, a substantial relationship was seen among periodontitis characteristics and TNF- α values. The findings suggest that TNF- α may have detrimental effects at tissues of periodontium. The result was consistent to previous investigations

[31,32] these revealed this cytokine was present in greater amounts at periodontitis than those with intact periodontion. Additionally, TNF- α and IL-6 were found to be possible biomarkers of diagnosis for periodontitis.

Conversely, Ng et al.[33] and Rathinasamy et al.[34] did not find any statistically significant variation in salivary TNF- α levels between patients with periodontitis and those in good health. Furthermore, the findings of the Teles et al.[35] study differed from those of this investigation; they did not discover any connotation among TNF- α values and measures of periodontal. They explained their results by claiming that potential inhibitors found in entire saliva inhibited cytokines. A noteworthy discovery in this research was the statistically significant positive correlation between salivary TNF- α and (PI, BOP, PPD, and CAL). The cytokine's role in the development of periodontal disease was also proven by earlier Iraqi investigations, which showed a statistically significant positive association with TNF- α heights and periodontium damage indices [35,36].

Likewise, Kurtis et al. [37] showing a high correlation between salivary TNF- α levels and periodontal clinical indicators in samples of people with aggressive and chronic periodontitis symptoms. In contrast to the findings of study, Varghese et al.[29] found no substantial relationship between the various indicators of periodontitis and TNF- α. This finding might be explained by the marker's widespread dilution in entire saliva, which prevents it from reflecting the minute changes in periodontal characteristics [29].

Remarkably, the heights of inflammatory molecules TNF- α, NRLC4 and the various indicators of periodontitis showed a strong positive correlation in the current study, confirming the idea that these biomarkers may be important function in starting the mechanisms that cause persistent periodontal inflammation. Furthermore, a significant relationship of NLRC4 and TNF-α was observed at periodontitis. Thus, NRLC4, TNF- α salivary biomarkers that contribute to the pathophysiology of periodontitis were identified. These biomarkers can be utilized to diagnose periodontitis and discriminate it from periodontal health.

The present study was limited in that it concentrated on nonsmokers who were systemically healthy and did not take periodontitis severity into account. Future study should measure the connection of the severity of periodontitis and NRLC4, TNF- α values. It is required to conduct additional research involving different kinds of samples, like gingival tissues and GCF.

5. Conclusion

Salivary NLRC4and TNF- α concentrations were higher in patients had periodontitis than the participants had intact periodontium, and significantly connected with indices of periodontal disease. Moreover, the study's findings further supported the interaction between NLRC4 and TNF- α salivary levels, which may be a significant factor in initiating the mechanisms that cause chronic inflammation in clinical periodontitis. Furthermore, a significant relationship of NLRC4 and TNF-α was identified at periodontitis cases. The salivary biomarkers NLRC4and TNF- α were observed which plays an important role in the development of periodontitis and can be utilized for diagnosing periodontitis and distinguish it from periodontal health. Further, the NLRC4 inflammasome can be activated by sterile signal molecule or perio-pathogen, which result in release of TNF- α and may contribute to the breakdown of the periodontal apparatus.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interst to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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