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ORIGINAL ARTICLE

Leucine-rich alpha-2 glycoprotein (LRG): A novel acute phase protein expressed in Stage 3 Grade C periodontitis before and after periodontal therapy

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Abstract

Background: Leucine-rich alpha-2 glycoprotein (LRG) is a novel acute phase protein involved in inflammation-associated diseases and that considered to be induced by multiple proinflammatory cytokines. This study aimed to investigate gingival crevicular fluid (GCF) and serum levels of LRG, interleukin (IL)-6 and tumor necrosis factor (TNF)-α in patients with Stage 3 periodontitis before and after non-surgical periodontal treatment.

Methods: Twenty-five Stage 3 periodontitis and twenty-five periodontally healthy individuals were enrolled in the study. Clinical periodontal measurements were recorded; periodontitis patients received non-surgical periodontal treatment, and GCF and serum samples were obtained at baseline and at 6 weeks after treatment. LRG, IL-6 and TNF-α were determined by ELISA.

Results: GCF and serum LRG, IL-6 and TNF-α were significantly higher in periodontitis group than healthy controls ($P < .001$). A significant decrease in GCF and serum LRG, IL-6 and TNF-α was detected after periodontal treatment compared with baseline values of periodontitis patients ($P < .001$).

Conclusion: Our findings revealed that LRG expression was increased in Stage 3 periodontitis both locally and systemically, and non-surgical periodontal therapy was effective in reducing LRG levels in GCF and serum of these patients.

KEYWORDS
gingival crevicular fluid, interleukin-6, leucine-rich alpha-2 glycoprotein, periodontitis, serum, tumor necrosis factor-alpha

1 | INTRODUCTION

Periodontitis is a globally prevalent microbial infection-induced inflammatory disease that gradually and irreversibly causes the destruction of tooth supporting hard and soft tissues and ultimately tooth loss. The onset, severity and progression of the disease are clearly known to be mainly associated with the host immune-inflammatory response against periodontal pathogens or their products.[1,2] Activated host immune response during the bacterial stimuli leads to the local and systemic release of various inflammatory mediators including cytokines and acute phase proteins, resulting in the tissue breakdown.[3–5] Acute phase proteins are important innate immune components synthesized by cytokine induction in the acute phase response which is the earliest and the primary host reaction.[6,7] The concentrations of these proteins in body fluids are related to severity of

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the disease, extent of tissue destruction, disease activity or healing status.[8–10] Thus, acute phase proteins can provide diagnostic and prognostic information in relation to inflammatory status of periodontitis.

Leucine-rich alpha-2 glycoprotein (LRG), a novel acute phase protein, is an approximately 50 kDa glycoprotein containing repetitive sequences with a leucine-rich motif.[11] The biological functions of LRG are remain unclear, however recent researches indicated that it can modulate angiogenesis, cell proliferation and differrentiation.[11] LRG is mainly secreted by the liver cells and neutrophils.[12] Clinical evidence revealed excessive LRG expression associated with a variety of inflammatory diseases such as inflammatory bowel disease, ulcerative colitis, asthma, myocardial infarction, heart failure, and rheumatoid arthritis.[13–21] Moreover, studies have demonstrated that LRG increase was related to clinical disease activity.[11,13,18–21] Accordingly, LRG has been suggested to be a clinically useful marker for inflammatory conditions.

It is well-known that proinflammatory cytokines including interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α are the key molecules in the modulation of inflammatory immune responses in periodontitis.[3,22,23] Of these, interleukin (IL)-6 is the major regulator of the acute phase protein synthesis by stimulating the hepatocytes in the liver.[24] Existing knowledge shows that LRG is produced not only in the liver but also in the lesion sites.[11,13] In addition, its expression shown to be stimulated not only by IL-6 but also by TNF-α which can activate nuclear factor kappa B ligand.[11] Studies also reported that IL-6 acts synergistically with TNF-α in LRG expression and the secretion of the glycoprotein is greatly enhanced in the presence of multiple pro-inflammatory cytokines.[11,25] Both IL-6 and TNF-α are well-established and upregulated cytokines in periodontal diseases,[3,9,10,26–28] and on the one hand, these cytokines could stimulate the LRG release in periodontal inflammation.

Currently, there is no data on the relationship between LRG and periodontal disease. Periodontitis is clearly known to be associated acute phase response that represents the systemic counterpart to the local inflammatory response.[29] Besides, LRG could serve as a specific biomarker of inflammation sites because of its local production. Accordingly, it would be important to investigate this glycoprotein in both gingival crevicular fluid and serum. The hypothesis of the present study is that gingival crevicular fluid (GCF) and serum LRG levels might be increased in Stage 3 periodontitis and decreased following periodontal treatment considering that it can be induced by IL-6 or TNF-α. Thus, the aim of this study was three-fold: First, to comparatively investigate GCF and serum LRG, IL-6, and TNF-α levels of Stage 3 periodontitis and healthy individuals. Second, to evaluate the changes of these molecules levels in GCF and serum of the periodontitis patients before and after non-surgical periodontal treatment. Third, to explore the possible correlations of biochemical parameters with clinical parameters and also with each other.

2 | MATERIALS AND METHODS

2.1 | Study population and clinical evaluation

A total of 50 systemically healthy and non-smoker participants with age range 26 to 49 years (25 females and 25 males; mean age: 37.12 ± 5.30 years) were recruited for the present study from the Department of Periodontology, Faculty of Dentistry, Zonguldak Bulent Ecevit University, Zonguldak, Turkey between September 2019 and January 2020. The study protocol was approved by the Ethics Committee of Giresun University, Giresun, Turkey (Protocol number:KAEK-92) and registered at ClinicalTrials.gov (NCT04383561). Before the enrollment, the aim and the procedures of the study were explained and the written informed consent was provided from all participants in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Medical and dental histories were taken from all volunteers, and the patients were excluded if they had any systemic disorder, a history of smoking, current pregnancy or lactation, received any periodontal treatment in the previous 6 months or used antibiotic, anti-inflammatory drugs or any other drugs within the past 6 months.

All individuals having 20 teeth or more except third molars underwent full mouth periodontal clinical and radiographic examination. Clinical periodontal evaluation consisted of the measurements of probing depth (PD), clinical attachment loss (CAL), gingival index (GI),[30] plaque index (PI)[31] and bleeding on probing (BOP)[32] scores. All the measurements were recorded at six sites around each tooth using a manual periodontal probe* by a single calibrated examiner experienced in clinical trials (UB) who was masked regarding the study conception and hypothesis. Tooth loss because of periodontitis was also assessed by self-reporting of the participants. For calibration of the investigator, 10 individuals were selected randomly prior to study commencement and examined on two separate occasions, 48 hours apart. The investigator's measurements were accepted sufficiently reproducible if those taken at baseline and at 48 hours differed by no more than 10% at the millimeter level.

* Williams probe, Hu-Friedy, Chicago, IL.

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Considering the periodontal status, the participants were classified as periodontally healthy controls (n = 25) and Stage 3 periodontitis ($n = 25$), and that were matched for age and sex. Diagnoses were carried out based on the criteria stated in the consensus report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.[33,34] Healthy controls included volunteers with an intact periodontium who had BOP < 10% and PD ≤ 3 mm without clinical attachment loss or radiographic sign of alveolar bone destruction. They also had no history of periodontitis. The inclusion criteria for generalized Stage 3 periodontitis patients were interdental CAL ≥ 5 mm, PD ≥ 6 mm and radiographic bone loss extending to the mid-third of the root or beyond. They showed no more than four teeth loss because of periodontitis. According to the extent and distribution of the disease, periodontitis patients had CAL ≥ 5 mm affecting 30% or more of the teeth. In addition, the grade of periodontitis has been determined as indirect evidence of the rate of progression by radiographic bone loss/age. Radiographic bone loss was determined using the tooth with the most severe bone loss as a percentage of root length. Because the ratio of the greatest radiographic bone loss (%)/age was > 1.0, all the patients with periodontitis were Grade C.

2.2 | GCF and serum sampling

All of the samples were collected one day after the clinical periodontal measurements to avoid the blood contamination in the morning following an overnight fast. To obtain serum samples, 5 mL of venous blood were taken from the antecubital vein by a standard venipuncture method. Serum was isolated by centrifugation at 1,500 × g for 15 minutes at 4°C and then immediately frozen and stored at −80°C.

Following the serum sampling, GCF was collected from the buccal aspects of two interproximal sites of single-rooted teeth in each individual using filter paper strips.[*] In healthy control group, GCF samples were obtained from the sites with no attachment loss and no BOP or clinical signs of inflammation. In periodontitis group, the sites with PD of 6-7 mm showing radiographic bone loss and BOP were selected. Before GCF sampling, the sites were isolated with cotton rolls and if present, the supragingival plaque was gently removed with a sterile curette and air-dried. Then, the paper strips were placed carefully into the gingival crevice until feeling of mild resistance and left there for 30 seconds. Strips contaminated with blood or saliva were discarded. The absorbed fluid volume of per strip was measured with a calibrated electronic device,[†] whereupon the strips were transferred into an individual sterile polypropylene tube immediately and frozen and stored at −80°C until the analysis.

2.3 | Non-surgical periodontal treatment

Periodontitis patients received non-surgical periodontal treatment by the same researcher (UB) after the baseline serum and GCF sampling. Non-surgical periodontal treatment included motivation, oral hygiene education and scaling and root planing (SRP) procedures. SRP was performed using ultrasonic devices and manual scalers and specific curettes on a quadrant-by-quadrant basis once a week and completed in four weeks. Every visit took up 45 to 60 minutes. Root surfaces were instrumented under local anesthesia if necessary. No antibiotics or any medications were prescribed during the treatment. Oral hygiene instructions including the modified-Bass brushing technique and interdental cleaning procedures (dental floss or interproximal brushes based on patient's requirement) were elucidated to individuals. Motivation and the hygiene education were reinstructed at each visit during the treatment period. At 6 weeks after the completion of the treatment, clinical periodontal measurements were repeated and GCF and serum samples were taken in patients with periodontitis. GCF sampling was performed from the same sites at baseline and at 6 weeks after treatment.

2.4 | Analysis of LRG, IL-6, and TNF-α levels

On the day of analysis, 300 μL phosphate buffered saline (pH 7.4) was added to each Eppendorf centrifuge tube containing the strips. All tubes were vortexed for 1 minute and then centrifuged at 10,000 × g for 5 minutes +4°C. Afterwards, the supernatants were obtained. The frozen serum samples were thawed before the assays. The levels of LRG, IL-6 and TNF-α in GCF and serum were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits.[‡,§,¶] The assays were carried out on 40 μL samples of GCF and 40 μL of serum for each molecule according to the instructions provided by the manufacturer. Standards and samples were added to appropriate microtiter plate wells with a biotinconjugated

[*] Periopaper, Proflow, Amityville, NY.

[†] Periotron 8010, Oraflow, Amityville, NY.
[‡] Human LRG ELISA kit, Sunred Biotechnology, Shanghai, China.
[§] Human IL-6 ELISA kit, Sunred Biotechnology, Shanghai, China.
[¶] Human TNF-α ELISA kit, Sunred Biotechnology, Shanghai, China.

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antibody preparation specific for LRG, IL-6 and TNF-α, and incubated at room temperature for 60 minutes. Then, the plates were washed and chromogen solution was added and incubated for 10 minutes. The enzyme-substrate reaction was terminated by the addition of stop solution and incubated for the color change. The absorbances were read at 450 nm by ELISA plate reader, and the concentrations were calculated by comparing the absorbance readings of the samples with the standard curves included in each assay kit.

The detection ranges for LRG, IL-6 and TNF-α were 6 to 1800 ng/mL, 3 to 600 ng/L and 3 to 900 ng/L, respectively. Total amounts of LRG, IL-6 and TNF-α were calculated because of the dilutions and the results were expressed as total proteins in the 30 seconds GCF samples.

2.5 | Statistical analysis

Statistical power calculation to estimate minimum sample was performed for GCF total amount of TNF-α as there is no data on LRG in periodontal diseases. According to this analysis, 25 participants were required to achieve 90% power at 0.70 effect size and 0.05 statistical significance level.[28] A post-hoc power calculation was also performed for GCF LRG, and the observed power was 98%.

The distribution of all variables was examined by Shapiro-Wilk normality test. Because the data were not normally distributed, comparisons of the biochemical and clinical parameters between groups were determined using the Kruskal-Wallis nonparametric test, followed by post-hoc group comparisons with the Bonferroni-adjusted Mann–Whitney U test. For the Bonferroni correction, $\alpha = 0.05/2 = 0.025$ was considered to be statistically significant. The Wilcoxon signed-rank test (paired observations) was performed to compare the baseline values with those after treatment. Sex ratio between groups was evaluated with x^2 test. The Spearman's rank correlation analysis was performed in all groups to detect the possible correlations of GCF and serum levels of LRG, IL-6 and TNF-α with the clinical parameters and also with each other. All data analyses were conducted using statistical software* and significance level was taken as $P < .05$ statistically.

3 | RESULTS

3.1 | Demographics and clinical findings

Demographic variables of the study groups are outlined in Table 1. There were no significant differences in age and sex distribution between the study groups ($P > .05$). The number of teeth was lower in periodontitis patients than those of healthy controls ($P < .01$).

TABLE 1 Demographic characteristics of the study groups

	Healthy ($n = 25$)	Periodontitis ($n = 25$)
Demographic variables		
Age (years)	37 (26–44)	38 (30–49)
Sex (F/M)	13/12	12/13
Number of teeth	28 (25–28)	26 (24–28)*

*Significantly different from healthy controls ($P < 0.05$).

Full-mouth and sampling-sites clinical periodontal findings of the study groups are outlined in Table 2. All clinical parameters namely PD, CAL, GI, PI, and BOP of both full-mouth and the sampling-sites in periodontitis patients were significantly higher than the healthy control group ($P < .001$). Significant decreases were found in all these clinical peridontal parameters for whole mouth as well as the sampling sites at 6 weeks after non-surgical periodontal treatment in patients with periodontitis ($P < .001$). Full-mouth clinical periodontal parameters were higher after treatment in periodontitis patients compared to the those of healthy controls ($P < 0.001$). There were significant differences in the sampling-sites clinical parameters except PI and BOP ($P > 0.05$) between post-treatment values of periodontitis group and the healthy controls ($P < 0.001$).

3.2 | Biochemical findings

GCF LRG, IL-6 and TNF-α total amounts are outlined in Figure 1. Stage 3 periodontitis patients showed significantly elevated GCF LRG total amounts compared to periodontally healthy individuals ($P < .001$, Figure 1A). GCF total amounts of IL-6 and TNF-α were also found to be higher in periodontitis group than in healthy controls ($P < .001$ for both, Figures 1B and 1C). The levels of both LRG and IL-6 and TNF-α were significantly decreased in GCF of periodontitis patients following the periodontal therapy ($P < 0.001$ for all, Figure 1A–C). GCF levels of LRG, IL-6 and TNF-α were higher in periodontitis patients than those of healthy controls after treatment ($P < .01$ for all, Figure 1A-C).

Serum levels of LRG, IL-6 and TNF-α are presented in Figure 2. Similarly to GCF results, there were significant increases in serum LRG, IL-6 and TNF-α levels in patients with periodontitis compared to the healthy group ($P < .001$ for all, Figure 2A–C). The results also indicated significant reductions in serum levels of LRG, IL-6 and TNF-α after non-surgical periodontal treatment compared to the baseline values of periodontiitis patients ($P < 0.001$ for

*SPSS v19.0, Chicago, IL.

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TABLE 2 Clinical periodontal measurements of the study groups

	Healthy (n = 25)	Periodontitis (n = 25)	
		Baseline	After treatment
Periodontal parameters			
Full-mouth			
PD (mm)	1.79 (1.07–2.16)	4.70 (4.11–5.40)*	2.72 (2.21–2.96)†,*
CAL (mm)	0.00 (0.00–0.00)	5.54 (4.36–7.52)*	3.77 (2.96–4.60)†,*
GI	0.12 (0.00–0.30)	2.53 (1.88–2.89)*	0.40 (0.16–0.87)†,*
PI	0.30 (0.13–0.63)	1.92 (1.30–2.69)*	0.51 (0.27–0.88)†,*
BOP (%)	0.00 (0.00–1.78)	86.66 (76.79–96.80)*	8.69 (6.41–9.87)†,*
Sampling site			
PD (mm)	2.00 (1.00–2.00)	6.00 (6.00–7.00)*	3.00 (2.00–3.00)†,*
CAL (mm)	0.00 (0.00–0.00)	7.00 (6.00–8.00)*	4.00 (3.00–5.00)†,*
GI	0.00 (0.00–0.00)	2.00 (2.00–3.00)*	0.00 (0.00–0.00)†,*
PI	0.00 (0.00–1.00)	2.00 (2.00–3.00)*	0.00 (0.00–1.00)†
BOP (%)	0.00 (0.00–0.00)	100.00 (100.00–100.00)*	0.00 (0.00–0.00)†
GCF volume (μL)	0.14 (0.09–0.18)	0.80 (0.51–1.00)*	0.28 (0.20–0.35)†,*

BOP, bleeding on probing; CAL, clinical attachment loss; GCF, gingival crevicular fluid; GI, gingival index; PD, probing depth; PI, plaque index.
Data are expressed as median (minimum-maximum). Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney U test (unpaired observations). Wilcoxon signed-rank test (paired observations). Bonferroni correction $\alpha = 0.05/2 = 0.025$.
*Significantly different from healthy controls ($P<.05$).
†Significantly different from baseline ($P<.05$).

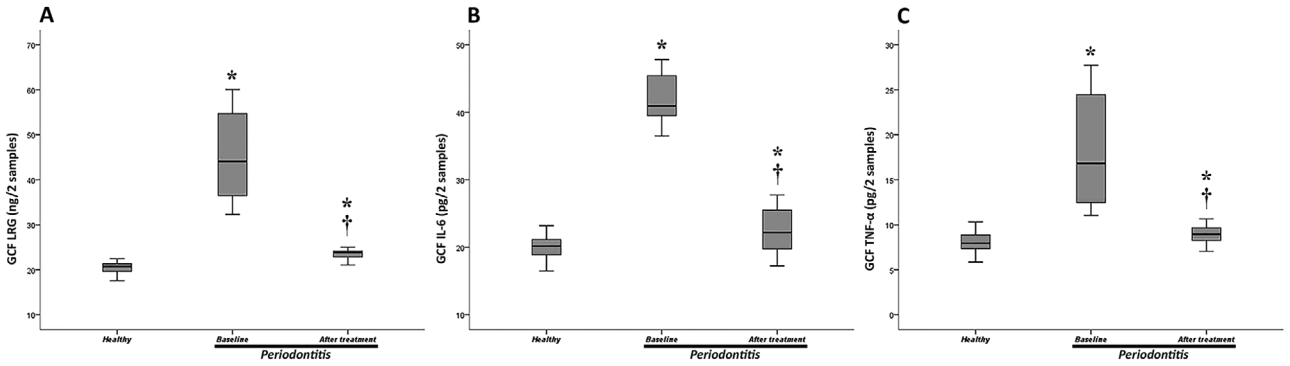

FIGURE 1 GCF total amounts of LRG, IL-6 and TNF-α in periodontal health and in periodontitis before and after non-surgical periodontal treatment. (**A**) GCF LRG levels (ng/2 samples); (**B**) GCF IL-6 levels (pg/2 samples); (**C**) GCF TNF-α levels (pg/2 samples). *Significantly different from healthy controls (Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney U test). †Significantly different from baseline (Wilcoxon signed-rank test). Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values

all, Figure 2A–C). Compared to the healthy individuals, serum LRG levels were significantly higher in periodontitis patients after therapy ($P < 0.001$), whereas serum IL-6 and TNF-α levels were similar ($P > 0.05$) (Figure 2A–C).

3.3 | Correlations

GCF LRG, IL-6, and TNF-α total amounts were positively correlated with sampling-site PD, CAL and GI ($P < .001$ for all, Table 2). GCF LRG showed strong correlation with GCF levels of both IL-6 and TNF-α ($P < .001$ for both, Table 3). There was also positive relationship between IL-6 and TNF-α in GCF ($P < .001$). Significant positive correlations were found between the clinical parameters of full-mouth PD, CAL and GI and serum levels of LRG, IL-6 and TNF-α ($P < .001$ for all, Table 4). Serum LRG levels were strongly and positively correlated with serum IL-6 and TNF-α levels ($P < .001$ for both, Table 4). A significant positive correlation was also observed between

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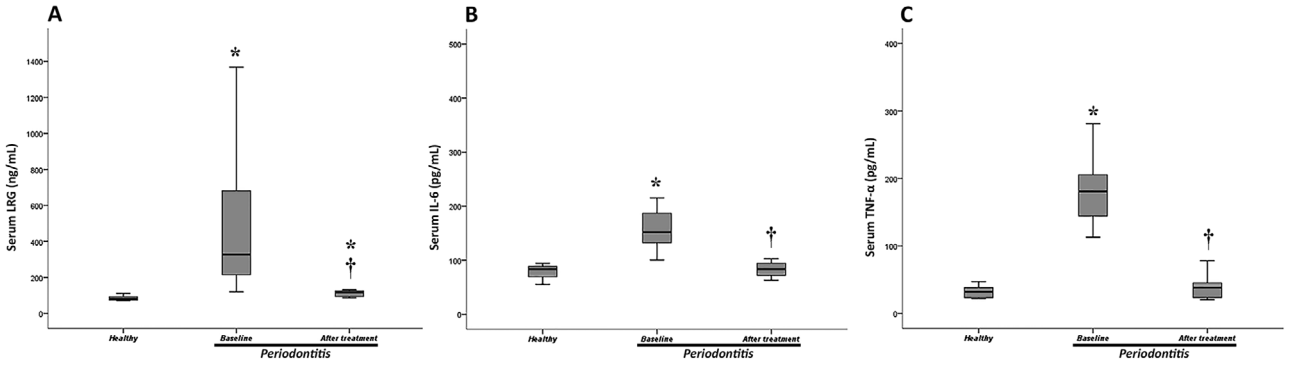

FIGURE 2 Serum levels of LRG, IL-6 and TNF-α in periodontal health and in periodontitis before and after non-surgical periodontal treatment. (**A**) Serum LRG levels (ng/mL); (**B**) Serum IL-6 levels (pg/mL); (**C**) Serum TNF-α levels (pg/mL). *Significantly different from healthy controls (Kruskal–Wallis/Bonferroni-adjusted Mann–Whitney U test). †Significantly different from baseline (Wilcoxon signed-rank test). Data are presented as box and whisker plots. The median value is indicated by the line within the box plot. The box extends from the 25th to the 75th percentiles. Whiskers extend to show the highest and lowest values

serum levels of IL-6 and TNF-α ($P < .001$, Table 4). In addition, GCF and serum LRG, IL-6 and TNF-α were positively related to each other ($P < .001$ for all, Table 4).

4 | DISCUSSION

The current study investigated GCF and serum levels of LRG, IL-6 and TNF-α in patients with Stage 3 periodontitis and that also examined the changes across the levels of these molecules after non-surgical periodontal treatment. LRG is an acute phase protein isolated from human serum and secreted by mainly hepatocytes and neutrophils.[12,35,36] Although its function is unclear, increasing evidence shows that LRG secretion was elevated in various diseases characterized by inflammation.[13–15,17,19–21,25] Clinical studies revealed that serum LRG levels were elevated in patients with rheumatoid arthritis and decreased after treatment. Furthermore, the authors reported the relationship between serum LRG levels and the disease activity. Hence, LRG is proposed to be a novel inflammatory marker clinically useful in rheumatoid arthritis.[11,18–20] When taken into consideration these observations and the similarities between the pathogenesis of rheumatoid arthritis and the periodontitis, we postulated that LRG could have an important role related to the inflammatory process of periodontitis. To the best of our knowledge, this is the first study presenting data on the GCF and serum LRG levels in periodontal disease. According to our findings, both GCF and serum LRG were significantly higher in periodontitis patients compared to the healthy individuals. These increased levels of LRG in periodontitis patients were also found to be decreased after non-surgical periodontal treatment. Moreover, there were strong correlations between GCF and serum LRG and the clinical parameters, PD, CAL and GI. The present data support the view of that LRG can be a valuable acute phase protein in inflammatory conditions.

It is clear that periodontitis is characterized by chronic progressive inflammation and the disease progression or response to periodontal treatment is a result of activated host immune inflammatory response.[37] However, the current diagnostic approaches that are based solely on the measurements of clinical periodontal parameters such as PD, CAL, GI, BOP, PI, and radiographic findings do not reflect the current state of disease activity or the future risk of disease progression. Additionally, many patients are identified at late stages of the disease because the periodontitis progression is silent. Thus, identification of a reliable biomarker of periodontal tissue breakdown to establish early diagnosis and for monitoring the disease progression and personalizing the treatment could be a great benefit in clinical practice.

For the present research, both GCF and serum were obtained from the participants. GCF closely reflects the inflammatory burden of periodontal tissues and it provides information at site-specific level. Importantly, continuous local inflammation of the periodontium with upregulation of proinflammatory responses and immune mediators can contribute the systemic inflammation. Serum gives information about this systemic inflammatory response caused by periodontal destruction.[38] It is also important to notice that LRG, an acute phase protein, is shown to produce in inflamed tissues in addition to the liver.[11,13] That is also why both GCF and serum analyzed in this study. Data were presented as total amount of a constituent in the GCF rather than its concentration, which is the more appreciate and reliable indicator to identify the GCF constituents related to periodontal disease.[39,40]

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TABLE 3 Spearman rank correlations of GCF LRG, IL-6 and TNF-α with sampling site clinical parameters and with each other

	PD (mm)	CAL (mm)	GI	GCF LRG (ng/2 samples)	GCF IL-6 (pg/2 samples)	GCF TNF-α (pg/2 samples)
GCF LRG (ng/2 samples)	0.829*	0.871*	0.812*	1.000	0.922*	0.960*
GCF IL-6 (pg/2 samples)	0.750*	0.778*	0.789*	0.922*	1.000	0.939*
GCF TNF-α (pg/2 samples)	0.755*	0.796*	0.778*	0.960*	0.939*	1.000

CAL, clinical attachment loss; GCF, gingival crevicular fluid; GI, gingival index; IL-6, interleukin-6; LRG, leucine-rich alpha-2 glycoprotein; PD, probing depth; TNF-α, tumor necrosis factor-α.
*Statistically significant ($P<.05$).

TABLE 4 Spearman rank correlations of serum LRG, IL-6 and TNF-α with full-mouth clinical parameters, GCF levels and with each other

	PD (mm)	CAL (mm)	GI	GCF LRG (ng/2 samples)	GCF IL-6 (pg/2 samples)	GCF TNF-α (pg/2 samples)	Serum LRG (ng/mL)	Serum IL-6 (pg/mL)	Serum TNF-α (pg/mL)
Serum LRG (ng/mL)	0.833*	0.855*	0.826*	0.953*	0.961*	0.933*	1.000	0.930*	0.836*
Serum IL-6 (pg/mL)	0.704*	0.733*	0.717*	0.888*	0.977*	0.925*	0.930*	1.000	0.839*
Serum TNF-α (pg/mL)	0.722*	0.716*	0.724*	0.830*	0.847*	0.875*	0.836*	0.839*	1.000

CAL, clinical attachment loss; GCF, gingival crevicular fluid; GI, gingival index; IL-6, interleukin-6; LRG, leucine-rich alpha-2 glycoprotein; PD, probing depth; TNF-α, tumor necrosis factor-α.
*Statistically significant ($P<.05$).

Consistent with our hypothesis, both GCF and serum levels of LRG were dramatically increased in patients with periodontitis, and significant reductions in LRG levels were detected both locally and systemically in parallel to the clinical periodontal healing provided by non-surgical periodontal treatment. When the GCF findings of LRG taken into consideration, this novel glycoprotein might be produced locally by inflammatory cells in periodontal tissues. In addition, such a locally produced potential biomarker may aid the identification and monitoring of diseased tooth sites. Our findings also demonstrated that periodontal tissue breakdown caused the systemic release of LRG by the liver. Furthermore, GCF and serum LRG were significantly correlated with each other and also with PD, CAL and GI clinical parameters. We therefore suggested that LRG might have a potential diagnostic and prognostic value both at the site level and at the patient level. This longitudinal trial presents the first data on the link between LRG and periodontitis, and there is no other study conducted on the LRG in periodontal diseases to compare our findings. On the other hand, the current findings conform with the results of systemic inflammatory diseases suggesting that LRG is a useful biomarker of disease activity.[11,13,18–20]

One of the hypotheses in our study was that IL-6 and TNF-α could have a role in LRG expression. IL-6 is well-documented as the main stimulator of systemic acute phase response.[7,24] However, there is an important point to taken into account that LRG has been shown to be stimulated not only by IL-6 but also by TNF-α.[11,25] Therefore, we also focused on the IL-6 and TNF-α in GCF and serum. As expected, we observed the increased levels of these proinflammatory cytokines in Stage 3 periodontitis patients compared to healthy individuals. In addition, a marked decrease was found following the treatment in patients with periodontitis. A dramatic increase of especially serum IL-6 and TNF-α in periodontitis patients may point out the increased systemic inflammatory response and the future risk of systemic vascular damage. However, non-surgical periodontal therapy was effective in improving clinical periodontal status of periodontitis patients as well as in reducing both local and systemic levels of these proinflammatory cytokines. Despite methodological differences, the current results were conform with the earlier studies.[9,26–28] Notably, both TNF-α and IL-6 are well-defined cytokines in periodontal inflammation and it has been evidenced for years by many researchers.[3,9,10,23,26–28] So, the present findings of IL-6 and TNF-α are not surprising. Plus, given that the levels of both TNF-α and IL-6 correlated well with the clinical indicators of periodontitis severity, all these results confirm that periodontitis patients indeed experienced an inflammatory phase and the success of periodontal treatment and the resolution of inflammation. Although the clinical parameters and the

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GCF LRG, IL-6 and TNF-α levels of periodontitis patients did not decrease to the levels seen in healthy individuals after treatment, our results clearly demonstrated that non-surgical periodontal therapy provided significant changes in both GCF and serum LRG, IL-6 and TNF-α levels in parallel to clinical periodontal healing. One of the important findings of the current study implies that levels of LRG were positively correlated with TNF-α and IL-6 in both GCF and serum. All these observations lead us to speculate that both IL-6 and TNF-α are involved in the LRG expression in the pathogenesis of periodontitis. In a supportive way of the previous studies,[11,13,19,20,41] the current findings suggest that, unlike the other acute phase reactants, LRG is capable of showing the disease activity via the stimulation by TNF-α if IL-6 is inactivated. From a clinical point of view and based on our results, LRG may be a promising biomarker for Stage 3 periodontitis.

The lack of the other stages of periodontitis can be considered as a limitation of this study and further investigations examining LRG levels in different stages of periodontitis with long-term follow-up period would clarify its role in periodontal disease. Additionally, although the power of the study was large enough, the small sample size may be another limitation of the study.

5 | CONCLUSION

LRG appears to be a new acute phase protein expressed in patients with Stage 3 periodontitis and non-surgical periodontal therapy was successful to reduce the levels of this molecule in GCF and serum. Thus, the expression of LRG might be a consequence of the inflammatory burden of both local and systemic inflammation in Stage 3 periodontitis. Further prospective and large-scale researches are warranted to confirm our findings and to better understand the role of this novel protein in periodontal diseases.

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AUTHOR CONTRIBUTIONS

Zeynep Pinar Keles Yucel contributed to study conception, design, data analysis and interpretation, drafted and critically revised the manuscript. Umut Balli contributed to data acquisition, data analysis and interpretation. The authors gave final approval and agreed to be accountable for all aspects of the work.

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REFERENCES

1. Kornman KS. Mapping the pathogenesis of periodontitis: a new look. *J Periodontol*. 2008;79:1560-1568.
2. Kinane DF. Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. *Periodontol 2000*. 2000;24(1):215-225.
3. Preshaw PM, Taylor JJ. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? *J Clin Periodontol*. 2011;38(Suppl. 11):60-84.
4. Graves D. Cytokines that promote periodontal tissue destruction. *J Periodontol*. 2008;79(Suppl. 8):1585-1591.
5. Slade GD, Offenbacher S, Beck JD, et al. Acutephase inflammatory response to periodontal disease in the US population. *J Dent Res*. 2000;79:49-57.
6. Koj A. Initiation of acute phase response and synthesis of cytokines. *Biochim Biophys Acta*. 1996;1317:84-94.
7. Moshage H. Cytokines and the hepatic acute phase response. *J Pathol*. 1997;181:257-266.
8. Chandy S, Joseph K, Sankaranarayanan A, et al. Evaluation of C-reactive protein and fibrinogen in patients with chronic and aggressive periodontitis: a clinico-biochemical study. *J Clin Diagn Res*. 2017;11(3):ZC41-ZC45.
9. Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H. The effect of periodontal treatment on serum Leptin, Interleukin-6, and C-Reactive protein. *J Periodontol*. 2010;81:1118-1123.
10. Keles ZP, Keles GC, Avci B, Cetinkaya BO, Emingil G. Analysis of YKL-40 acute-phase protein and interleukin-6 levels in periodontal disease. *J Periodontol*. 2014;85(9):1240-1246.
11. Naka T, Fujimoto M. LRG is a novel inflammatory marker clinically useful for the evaluation of disease activity in rheumatoid arthritis and inflammatory bowel disease. *Immunol Med*. 2018;41:2,62-67.
12. O'Donnell LC, Druhan LJ, Avalos BR. Molecular characterization and expression analysis of leucinerich alpha2-glycoprotein, a novel marker of granulocytic differentiation. *J Leukoc Biol*. 2002;72:478-485.
13. Serada S, Fujimoto M, Terabe F, et al. Serum leucine-rich alpha-2 glycoprotein is a disease activity biomarker in ulcerative colitis. *Inflamm Bowel Dis*. 2012;18:2169-2179.
14. Honda H, Fujimoto M, Miyamoto S, et al. Sputum leucine-rich alpha-2 glycoprotein as a marker of airway inflammation in asthma. *PLoS One*. 2016;11(9):e0162672.
15. Xuan C, Li H, Li LL, et al. Screening and identification of pregnancy zone protein and leucine-rich alpha-2-glycoprotein as potential serum biomarkers for early-onset myocardial infarction using protein profile analysis. *Proteomics Clin Appl*. 2019;13(3):e1800079.
16. Watson CJ, Ledwidge MT, Phelan D, et al. Proteomic analysis of coronary sinüs serum reveals leucine-rich α2-glycoprotein as a novel biomarker of ventricular dysfunction and heart failure. *Circ Heart Fail*. 2011;4:188-197.

19433670, 2021, 1, Downloaded from https://aap.onlinelibrary.wiley.com/doi/10.1002/JPER.20-0358 by Bezmi-I Alem Vakif University, Wiley Online Library on [11/08/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

17. Shinzaki S, Matsuoka K, Iijima H, et al. Leucine-rich alpha-2 glycoprotein is a serum biomarker of mucosal healing in ulcerative colitis. *ECCOJC*. 2017;11:84-91.
18. Serada S, Fujimoto M, Ogata A, et al. iTRAQbased proteomic identification of leucine-rich alpha-2 glycoprotein as a novel inflammatory biomarker in autoimmune diseases. *Ann Rheum Dis*. 2010;69:770-774.
19. Fujimoto M, Serada S, Suzuki K, et al. Leucine-rich a2-glycoprotein as a potential biomarker for joint inflammation during anti-interleukin-6 biologic therapy in rheumatoid arthritis. *Arthritis Rheumatol*. 2015;67:2056-2060.
20. Ha YJ, Kang EJ, Lee SW, et al. Usefulness of serum leucine-rich alpha-2 glycoprotein as a disease activity biomarker in patients with rheumatoid arthritis. *J Korean Med Sci*. 2014;29(9):1199-1204.
21. Shimizu M, Inoue N, Mizuta M, Nakagishi Y, Yachie A. serum leucine-rich α2-glycoprotein as a biomarker for monitoring disease activity in patients with systemic juvenile idiopathic arthritis. *J Immunol Res*. 2019;2019:3140204. https://doi.org/10.1155/2019/3140204.
22. Buduneli N, Kinane DF. Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis. *J Clin Periodontol*. 2011;38(Suppl. 11):85-105.
23. Garlet GP. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. *J Dent Res*. 2010;89:1349-1363.
24. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J*. 1990;265:621-636.
25. Shirai R, Hirano F, Ohkura N, Ikeda K, Inoue S. Up-regulation of the expression of leucine-rich alpha(2)-glycoprotein in hepatocytes by the mediators of acute-phase response. *Biochem Biophys Res Commun*. 2009;382(4):776-779.
26. Gokul K, Faizuddin M, Pradeep AR. Estimation of the level of tumor necrosis factor- a in gingival crevicular fluid and serum in periodontal health & disease: a biochemical study. *Indian J Dent Res*. 2012;23:348-352.
27. Kurtis B, Tuter G, Serdar M, et al. Gingival crevicular fluid levels of monocyte chemoattractant protein-1 and tumor necrosis factor-alpha in patients with chronic and aggressive periodontitis. *J Periodontol*. 2005;76:1849-1855.
28. Türer ÇC, Durmuş D, Balli U, Güven B. Effect of non-surgical periodontal treatment on gingival crevicular fluid and serum endocan, vascular endothelial growth factor-a, and tumor necrosis factor-alpha levels. *J Periodontol*. 2017;88(5):493-501.
29. Gomes-Filho IS, Coelho JMF, da Cruz SS, et al. Chronic periodontitis and C-reactive protein levels. *J Periodontol*. 2011;82(7):969-978.
30. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533-551.
31. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121-135.
32. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *Int Dent J*. 1975;25:229-235.
33. Chapple ILC, Mealey BL, Van Dyke TE, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: consensus report of workgroup 1 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. *J Periodontol*. 2018;89(Suppl 1):S74-S84.
34. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: consensus report of workgroup 2 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. *J Periodontol*. 2018;89(Suppl 1):S173-S182.
35. Haupt H, Baudner S. Isolation and characterization of an unknown, leucine-rich 3.1-S-alpha2-glycoprotein from human serum (author's transl). *Hoppe Seylers Z Physiol Chem*. 1977;358:639-646.
36. Shirai R, Hirano F, Ohkura N, Ikeda K, Inoue S. Up-regulation of the expression of leucine-rich alpha(2)-glycoprotein in hepatocytes by the mediators of acute-phase response. *Biochem Biophys Res Commun*. 2009;382:776-779.
37. Offenbacher S, Barros SP, Beck JD. Rethinking periodontal inflammation. *J Periodontol*. 2008;79:1577-1584.
38. D'Aiuto F, Parkar M, Andreou G, et al. Periodontitis and systemic inflammation: control of the local infection is associated with a reduction in serum inflammatory markers. *J Dent Res*. 2004;83:156-160.
39. Lin SJ, Chen YL, Kuo MY, Li CL, Lu HK. Measurement of gp130 cytokines oncostatin M and IL-6 in gingival crevicular fluid of patients with chronic periodontitis. *Cytokine*. 2005;30:160-167.
40. Lamster IB, Oshrain RL, Fiorello LA, Celenti RS, Gordon JM. A comparison of 4 methods of data presentation for lysosomal enzyme activity in gingival crevicular fluid. *J Clin Periodontol*. 1988;15:347-352.
41. Chong PF, Sakai Y, Torisu H, et al. Leucine-rich alpha-2 glycoprotein in the cerebrospinal fluid is a potential inflammatory biomarker for meningitis. *J Neurol Sci*. 2018;392:51-55.

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