

Salivary Irisin and periodontal clinical parameters in patients of chronic periodontitis and healthy individuals: A novel salivary myokine for periodontal disease

Safi Ullah Khan¹, Sarah Ghafoor², Saba Khaliq³, Ali Raza Syed⁴

Abstract

Objective: To evaluate changes in the levels of salivary irisin in chronic periodontitis, and to correlate the two.

Methods: The analytical cross-sectional study was conducted at Fatima Memorial Hospital & College of Dentistry, Lahore, Pakistan, from September 2017 to March 2018, and comprised patients of either gender visiting the periodontic out-patient department. The subjects were divided into group I, which had periodontally healthy controls, and group II, which had an equal number of chronic periodontitis patients. Chronic periodontitis was assessed on basis of pocket probing depth, clinical attachment level, plaque percentage and bleeding on probing. Also, 4ml of un-stimulated saliva was collected for the quantification of irisin protein using enzyme-linked immunosorbent assay. Data was analysed using SPSS 25.

Results: Of the 40 subjects, there were 20 (50%) in group I with 10 (50%) males and 10 (50%) females having an overall mean age of 37.60 ± 2.58 years. The remaining 20 (50%) subjects were in group II with 16 (80%) males and 4 (20%) females having an overall mean age of 43.25 ± 6.10 years. Mean salivary irisin level in group II was 6.80 ± 3.97 ng/ml compared to 3.99 ± 2.48 ng/ml in group I ($p=0.009$). Periodontal clinical parameters in both the groups were positively but non-significantly correlated with salivary irisin levels ($p>0.05$) except for decreased plaque percentage in group I ($p<0.05$).

Conclusion: Salivary irisin levels increased in chronic periodontitis and decreased with decreasing plaque percentage in healthy individuals, indicating that this myokine can act as a biomarker for chronic periodontal disease.

Keywords: Irisin, Chronic periodontitis, Probing pocket depth, Clinical attachment loss, Plaque, Bleeding on probing.

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Introduction

Periodontitis is a pathological condition in which an inflammatory cascade is observed in the supporting structures of the tooth forming the periodontium. These tissues include gingiva and periodontal ligament as the two soft connective tissue elements and alveolar bone and cementum being the two hard connective tissue elements of the periodontium. Periodontitis can be subdivided into three types; chronic periodontitis (CP), aggressive periodontitis (AP), and necrotising periodontal disease (NPD).¹ CP can have risk factors which are either local or systemic in nature and these factors have their own clinical manifestations towards disease progression.^{2,3} CP has its peculiar microbiology which is related to its disease pathogenesis. It includes various species of anaerobic as well as microaerophilic bacteria.⁴ CP disease progression is a result of endotoxins produced by these bacteria which have disastrous effects on the periodontium. The clinical sequela observed is periodontal pocket formation and consequent clinical attachment loss, leading to tooth mobility and subsequent tooth-loss.¹ Intra-oral views of a

patient with CP are shown in Figure.

Salivary proteins have rendered themselves as reliable diagnostic tools for various ailments of the human body, such as the detection of human immunodeficiency virus (HIV) activity, for reporting an episode of acute myocardial infarction (AMI), and detection of steroid hormones.⁵ Saliva plays a vital action in controlling inflammatory conditions in a periodontal disease through a variety of proteins present in it which have been regarded as robust markers for CP initiation or predictions, such as hepatocyte growth factor (HGF), transforming growth factor-beta (TGF- β), interleukin-1 β (IL-1 β), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), matrix metalloproteinase-8 (MMP-8), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1).^{6,7}

Myokines refer to a family of proteins which are secreted by the skeletal muscle fibres as a result of physical activity. More recently, it has been shown that myokines are not produced by skeletal muscle fibres alone, rather there are various other body tissues that are capable of producing these molecules, including salivary glands.⁸

Irisin is one such novel myokine which was first discovered in 2012.⁹ It is called the 'exercise hormone' as it is released

^{1,2}Department of Oral Biology, University of Health Sciences, Lahore, Pakistan;

³Department of Physiology and Cell Biology, University of Health Sciences,

Lahore, Pakistan; ⁴Department of Periodontology, FMH College of Dentistry, Lahore, Pakistan.

Correspondence: Sarah Ghafoor. e-mail: sarahghafoor@uhs.edu.pk

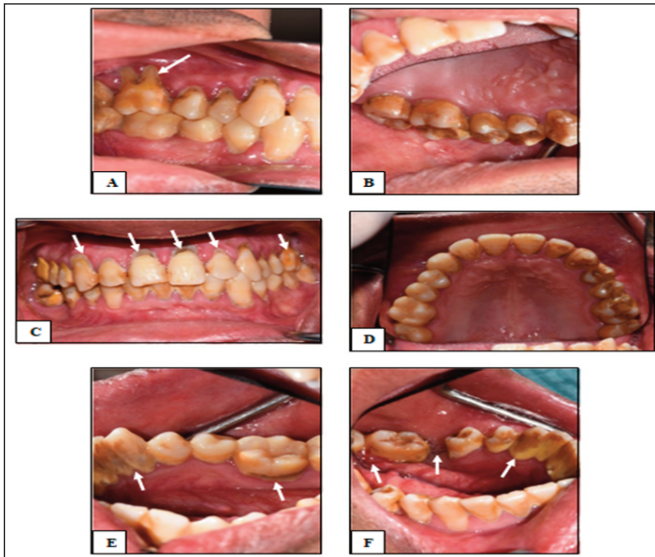


Figure: Intra-oral views of a patient with chronic periodontitis (CP); A: Buccal view of left maxillary dentition showing evident gingival inflammation and recession leading to clinical attachment loss (CAL). Arrow shows grade II furcation involvement in maxillary left 1st molar with clinically visible mesiobuccal and distobuccal roots of the respective tooth; B: Palatal view of left maxillary dentition as depicted in A, showing excessive plaque deposits and staining; C: Anteroposterior view of complete maxillary and mandibular dentition showing labial and buccal aspects of these teeth. Arrows show generalised gingival recession and inflammation, associated CAL, abundant plaque and calculus deposits along with staining; D: Occlusal view of the maxillary dentition showing severe staining on the left quadrant side; E: Lingual view of right mandibular dentition depicting gingival recession along with plaque and calculus deposits as well. In particular, arrows show calculus deposit on lingual surface of right mandibular canine and gingival recession on lingual surface of right mandibular first molar; F: Lingual view of left mandibular dentition with arrows showing bleeding on probing (BoP) on the distolingual and middle lingual sites of the left mandibular 2nd molar, missing left mandibular 1st molar due to CP and plaque and calculus deposits on lingual aspect of left mandibular canine.

by skeletal muscles of the human body when exercise is done.¹⁰ It is found effective when used in the form of a 'hormone therapy' in the treatment of obesity and Type 2 diabetes mellitus (T2DM).^{9,11} The release of irisin by cardiac muscles is higher than the skeletal muscles as a consequence of exercise.¹² Irisin has been found to be present in all three paired major salivary glands as well, and is produced the most by parotid gland and the least by sublingual gland, with a moderate level released by submandibular gland.^{11,12} Irisin is also expressed and regulated differently in human periodontal ligament cells, dental pulp stem cells and osteoblasts.¹³ Other than this, irisin has been found to be involved in cementoblast differentiation as well.¹⁴

Periodontal clinical parameters, such as probing pocket depth (PPD), clinical attachment level (CAL), plaque percentage and bleeding on probing (BoP), are effective tools to monitor periodontal health as well as periodontal disease states. These parameters respond to disturbances and changes of the gingival and sub-gingival microbiota.

However, some individuals are more prone to develop CP and are shown to be less responsive to definitive bacterial control procedures for prevention and treatment of CP. These patients are also more likely to develop CP-related systemic diseases, such as diabetes mellitus (DM), cardiovascular diseases (CVDs), rheumatoid arthritis (RA), cognitive impairment, obesity, metabolic syndrome (MS) and cancer. In such patients, it is not clear whether the current periodontal clinical parameters alone are ample enough to determine disease initiation and treatment prognosis. In this regard, biomarkers may be of high value to support the information recorded by routine clinical parameters. Saliva-based biomarkers may help to improve diagnostic validity in timely identification of CP and are likely to present with decisive contributions towards a superior evaluation of both the grade and stage of CP as well.^{15,16} Although irisin was first classified as a myokine as it was produced by muscle fibres, its recent identification to be produced by the salivary glands and its availability in saliva paves the way for further investigation into its role as a possible biomarker in state of periodontal health or disease. To our knowledge, no study has been conducted to evaluate changes in levels of salivary irisin in CP patients and healthy individuals. The current study was planned to fill the gap by evaluating changes in the levels of salivary irisin in CP, and by exploring the correlation between the two.

Materials and Methods

This analytical cross-sectional study was conducted at Fatima Memorial Hospital & College of Medicine and Dentistry (FMHCMD), Lahore, Pakistan, from September 2017 to March 2018, after approval from the ethics review board of FMHCMD and the University of Health Sciences (UHS), Lahore, the sample size was calculated by using the following formula;¹⁷

$$n = \frac{\left(Z_{1-\beta} + Z_{1-\frac{\alpha}{2}} \right)^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

where Z = Standard score, μ = Mean of the sample, σ = Standard deviation of the sample, α = Probability of Type-I error and β = Probability of Type-II error. The desired power of study was 90%, desired level of significance 5%, $Z_{1-\beta}$ = Z score for power of study 1.28, $Z_{1-\alpha/2}$ = Z score for level of significance 1.96, μ_1 = estimated mean of irisin in control group 316.21, μ_2 = estimated mean of irisin in diseased group 250.68, $\sigma_1(2)$ = standard deviation of irisin in control group = 78.04, and $\sigma_2(2)$ = standard deviation of irisin in diseased group 13.22.¹²

The sample was collected using convenience sampling technique, from among the patients of either gender aged 35-54 years and having a minimum of 20 teeth presenting to the outpatient department (OPD) of the FMHCMD Department of Periodontology. Individuals with acute or chronic medical ailments, such as DM, hypertension (HTN) and hyperlipidaemia, as well as pregnant or lactating women, tobacco and alcohol consumers, patients with on-going oral conditions, such as gingivitis-only, leukoplakia, erythroplakia, lichen planus, oral carcinoma and xerostomia, patients taking any oral medication for the preceding three months, those with a positive history of radiotherapy or chemotherapy, and those having undergone any periodontal treatment during the preceding year were excluded.

After taking informed consent from the participants, they were divided into group I, which had periodontally healthy controls, and group II, which had an equal number of patients with generalised CP with pocket probing depth $\geq 5\text{mm}$ and clinical attachment level (CAL) $\geq 2\text{mm}$.¹⁸

Clinical parameters of PPD, CAL, plaque percentage and bleeding on probing (BoP) were examined and recorded using William's probe (Hu-Friedy, Chicago, IL, United States). The procedure was conducted by the same examiner to avoid any bias. PPD was determined as the distance starting from the gingival margin and extending up to the base of the gingival sulcus or the periodontal pocket. William's probe with a controlled force of $\sim 0.25\text{ N}$ was applied at the apical end of the sulcus/periodontal pocket in a direction parallel to the long axis of the tooth at 6 particular sites. These sites were distofacial, facial, mesiofacial, distolingual, lingual and mesiolingual for each tooth present in the oral cavity.¹⁹ For CAL determination, the gingival margin levels in respect to cemento-enamel junction (CEJ) of each tooth at the same 6 sites as done for PPD were first calculated. When the gingival margin was observed at CEJ, it was recorded as 0mm. When margin of the gingiva was covering the CEJ, it was recorded with a '-' sign with value in mm, and when it was seen apical to the CEJ due to gingival recession, it was recorded with '+' sign with value in mm. Once this was done, CAL was calculated for each of the 6 specific tooth sites per tooth using the formula:²⁰

$$\text{Clinical attachment level/loss} = (\text{Periodontal probing depth}) + (\text{Gingival Margin to CEJ level})$$

Plaque percentage and BoP were determined for the same 6 tooth sites for each tooth and were recorded according to a dichotomous (yes/no) evaluation pattern. The existence of dental plaque was estimated if, after allowing an easy passage of periodontal probe along the margin of

the gingiva, there was observed a film on the respective probe. The appearance of bleeding was evaluated 30 sec after probing was done.²¹ Data of all of clinical parameters were recorded for each study participant onto their respective odontogram manually, and was shifted to an online periodontal chart (<http://www.periodontalchart-online.com/uk/index.asp>) through which the values for mean PPD, CAL, plaque percentage and BoP values for all the participants were calculated.²⁰

Following periodontal clinical assessment, salivary samples were obtained from all the participants. Collection of unstimulated saliva was done in the morning between 8am and 10am. The participants were required not to eat and drink anything, and not to undergo any oral hygiene maintenance procedure for at least 1 hour before saliva collection. The participants rinsed their mouth with distilled drinking water for at least one minute and then swallowed or expectorated it. After the oral rinse, the participants were asked to passively drool into a 15ml sterile falcon tube which was placed on ice intermittently while more saliva was obtained from the participant within the oral cavity. The quantity of saliva obtained from each participant was 4ml. The saliva samples were kept at -80°C till further utilisation.²² Salivary irisin concentration of each participant was calculated in duplicates using the Human Salivary Irisin (Fibronectin Type III Domain Containing 5 [FNDC5]) enzyme-linked immunosorbent assay (ELISA_ kit (Elabscience Biotechnology Company Limited; Catalog No: E-EL-H2254). The optical density (OD) of each of the 96-well micro ELISA plate was determined spectrophotometrically at 450nm wavelength by microplate reader (Biochrom EZ Read 400) by using Galapagos software.

Data was analysed using statistical package for social sciences (SPSS) Version 25. Correlations between salivary irisin concentration and periodontal clinical parameters were done using Pearson correlation analysis. For all analysis, a value of $p < 0.05$ was considered as statistically significant.

Results

Out of the 40 participants, there were 20(50%) in group I with 10(50%) males and 10(50%) females having an overall mean age of 37.60 ± 2.58 years. The remaining 20(50%) subjects were in group II with 16(80%) males and 4(20%) females having an overall mean age of 43.25 ± 6.10 years (Table 1). Mean salivary irisin level in group II was 6.80 ± 3.97 ng/ml compared to 3.99 ± 2.48 ng/ml in group I ($p = 0.009$) suggesting that salivary Irisin levels are higher in CP patients as compared to the healthy controls (Table 2). Variations in periodontal clinical parameters, PPD, CAL, plaque percentage and BoP, were assessed for healthy

Table-1: Demographic characteristics.

Variable	Group-I (n=20) Healthy controls n (%)	Group-II (n=20) Patients with CP n (%)
Gender		
Male	10 (50)	16 (80)
Female	10 (50)	04 (20)
Mean Age (years)	37.60±2.58	43.25±6.10

Table-2: Comparison of salivary irisin concentrations in the groups.

Variable	Healthy controls (mean±SD)	Patients of CP (mean±SD)	p-value
Concentration of salivary Irisin (ng/ml)	3.99±2.48	6.80±3.97	0.009

CP: Chronic periodontitis, SD: Standard deviation.

Table-3: Periodontal clinical parameters in healthy controls and patients of chronic periodontitis (CP).

Variable	PPD (mm) (Mean±SD)	CAL (mm) (Mean±SD)	Plaque (%) (Mean±SD)	BoP (%) (Mean±SD)
Group-I (n=20) Healthy controls	1.87±0.30	0.08±0.11	31±7.54	11.05±7.56
Group-II (n=20) Patients with CP	2.79±0.62	1.05±0.47	72.75±10.27	40.50±19.78
p-value (group-I versus group-II)	0.000	0.000	0.000	0.000

PPD: Pocket probing depth, CAL: Clinical attachment level, BoP: Bleeding on probing, SD: Standard deviation.

Table-4: Correlation between salivary irisin and periodontal clinical parameters of healthy controls and patients of chronic periodontitis (CP).

Groups	Description	PPD	CAL	Plaque	BoP
Healthy Controls	Correlation (r) value with respect to Salivary Irisin	0.433	0.317	0.533	0.417
	p-value	0.056	0.173	0.015	0.067
Patients of CP	Correlation (r) value with respect to Salivary Irisin	0.112	0.032	0.273	0.032
	p-value	0.639	0.895	0.244	0.893

PPD: Pocket probing depth, CAL: Clinical attachment level, BoP: Bleeding on probing.

controls as well as for patients with CP. The mean±SD values of these periodontal clinical parameters when compared for the two groups gave significant statistical differences ($p \leq 0.05$) suggesting that all these aforementioned periodontal clinical parameters were higher in CP patients as compared to healthy controls (Table 3). Salivary Irisin concentrations and the periodontal clinical parameters (PPD, CAL, Plaque percentage and BoP) of healthy controls as well as of patients of CP gave a positive yet insignificant correlation among them except for 'plaque percentage' of healthy controls which was both positively as well as significantly correlated with Irisin concentration suggesting that increased plaque deposits in healthy controls is directly proportional to increased salivary Irisin levels in this group (Table 4).

Discussion

The current study is the first to report changes in salivary irisin levels in CP patients compared to healthy controls. A study on salivary irisin in patients of Prader-Willi syndrome showed raised irisin levels.²³ Two previous studies, one on patients of acute myocardial infarction, and the other on obese individuals after taking a Turkish bath or performing exercise, also demonstrated a variation in concentration of salivary irisin over specific period of time with a decrease in salivary irisin concentration followed by a gradual increase in its concentration.^{11,12}

Irisin modulates the inflammatory response in pathologies which are associated with constant chronic inflammation such as Type 2 diabetes mellitus (T2DM), obesity, dysfunction of the immune system, asthma and neurological or coronary heart disease, and its detectable levels are of clinical importance for the physicians to monitor the severity of the particular diseased process.²⁴ Previous research has demonstrated that Irisin may reduce the symptoms of inflammatory bowel diseases by inhibiting the production of pro-inflammatory cytokines. In vitro studies on a type of macrophage, RAW (Ralph, rAschke, Watson) 264.7 macrophage revealed that irisin causes the up-regulation of immunocompetent cells. Irisin increases the production and function of macrophages, enhances their phagocytic potential and limits the release of reactive oxygen species (ROS), keeping the cell viability intact.²⁵ Irisin causes a reduction in the increased formation of deleterious hydrogen peroxide (H_2O_2) by macrophages. This anti-oxidative function of irisin is a result of increased production of particular anti-oxidative enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase 9 (Cat-9).²⁶ The increased levels of irisin (50nM, 100nM) decreased the cell count of apoptotic cells, reduced the expression of toll-like receptors 4 (TLR4) present at the cell surface, and also lessened the quantity of myeloid differentiation primary response 88 (MyD88) adapter protein in lipopolysaccharide (LPS) stimulation-dependent macrophages. Besides, reduction of mitogen-activated protein kinase (MAPK) cascade signalling pathways and a simultaneous decrease in the quantity of nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB), c-Jun N-terminal kinases (JNK) and extracellular signal-regulated kinase (ERK) phosphorylation were also found. This caused a reduction in the secretion of important pro-inflammatory cytokines, such as interleukin 1-beta (IL-1β), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), keratinocyte chemoattractant (KC), macrophage chemotactic protein (MCP)-1, and high mobility group box 1 (HMGB1) protein. These studies led to a plethora of information regarding the anti-inflammatory and protective functions of irisin in

the progression of pathologies related to chronic inflammation.²⁷

The pathogenesis of CP is also regulated by certain key pro-inflammatory markers. The sequence of destruction of periodontal tissues in CP is initiated from the deposition of dental plaque. Bacteria present in dental plaque influence the local tissue as well as immune cells through intrinsic and secreted microbe-associated molecular patterns (MAMPs), like LPS and specific antigens, like fimbrial proteins. The periodontium which is healthy remains intact by an efficient innate response towards commensal (non-pathogenic) microflora of dental plaque localised only at the gingival/plaque margin. Neutrophils perform a key inflammatory function here controlled by low levels of cytokines, like IL-1 β and interleukin-8 (IL-8). An ecological change in dental plaque rendering it to be composed of a more pathogenic microflora causes an increased immune reaction via influence of cytokine responses exhibited by a broad variety of periodontal and immune cells. The initiation of periodontitis is controlled by a robust activation of intrinsic periodontal cells, a primary and afterwards a secondary cytokine response causing an activation of innate effector responses and in particular incorporation and activation of neutrophils, in response to increased IL-1 β and IL-8 and osteoclasts in response to receptor activator of NF- κ B ligand (RANKL). Increased local functioning of neutrophils in the periodontium is mirrored by enhanced concentrations of MMP-8 neutrophil collagenase, MMP-9 neutrophil gelatinase and β -glucuronidase. Activated macrophages and T- and B-lymphocytes may also lead to the cytokine localisation via production of TNF- α , IL-6, interleukin-12 (IL-12), interleukin-17 (IL-17) and interleukin-18 (IL-18), and the harmony of these pro-inflammatory cytokines along with immunosuppressive mediators, like interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β), may be key elements responsible for disease establishment. Continuation of this pro-inflammatory response leads to tissue destruction involving diminution of soft connective tissues of periodontium such as gingiva's lamina propria and the periodontal ligament, and of the alveolar bone as well, which ultimately manifests itself as the poor state of tooth functioning. This is the characteristic of CP disease.⁷

Early disease detection has always been the prime focus for medical and dental practitioners to reduce morbidity and mortality rates. CP is the second most prevalent dental condition in Pakistan after dental caries and has got serious detrimental effects for the oral structures, including teeth and their biological supporting apparatus.²⁸ CP is detrimental to the overall wellbeing of the individual. In this regard, the role of a salivary diagnostic marker for CP

would be of high importance. Though the exact mechanism of how irisin controls biological markers in CP is still unknown, increased levels of salivary irisin observed in CP patients in the current study paves way for establishing a relationship between this protein and CP. It can be speculated that increased level of irisin may be suggestive of its role as the 'salivary biomarker' for CP disease.

Conclusion

Irisin concentrations were higher in CP patients compared to healthy controls. PPD, CAL, plaque percentage and BoP in both the groups had a positive but non-significant correlation with salivary irisin levels except for plaque percentage in healthy controls.

Disclaimer: The text is based on an M. Phil thesis.

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