Original Article

DOI: 10.7860/JCDR/2015/12327.5849

GCF Resistin As A Novel Marker in Patients with Chronic Periodontitis and Rheumatoid Arthritis

Dentistry Section

MANOJ MITTAL[1], BASIT HASSAN[2], KHUSHBOO DESAI[3], SHILPA DUSEJA[4], SANTOSH KUMAR[5], SHARASCHANDRA G. REDDY[6]

ABSTRACT

Background: The associational studies between periodontitis and rheumatoid arthritis are less documented, although they are found to have similar inflammatory pathogenesis. Resistin, a novel adipokine is suggested to be a common link between periodontitis and rheumatoid arthritis. The aim of the present study was to reinforce the inter-relationship between periodontitis and rheumatoid arthritis by using resistin as a potent inflammatory marker.

Materials and Methods: Hundred patients (aged >30 y) of either sex were selected for this study and were divided equally into four groups of 25 patients each. Group A consisted of healthy individuals, Group B consisted of patients with chronic periodontitis, Group C of patients with rheumatoid arthritis and Group D had patients suffering from both arthritis and periodontitis. Periodontal parameters assessed were plaque index (PI), modified gingival index (GI) and probing depth (PD). Panoramic radiographs were taken to confirm the diagnosis of periodontitis. Rheumatoid arthritis was confirmed by the rheumatologists and seropositivity for rheumatoid factor (RF) was checked. Resistin levels were analysed in GCF collected from all the four groups and statistical analysis was done by using Pearson correlation coefficient.

Results: The GCF of all the patients showed presence of resistin. The level of resistin was highest in Group D patients and least in Group A patients. On analysing the samples together positive co-relation was found between GCF resistin and PD, PI, GI and RF.

Conclusion: Resistin levels are increased in both chronic periodontitis and rheumatoid arthritis. Therefore, the increased level of GCF resistin can be regarded as potential inflammatory marker for periodontitis and rheumatoid arthritis.

Keywords: Adipokines, Inflammatory diseases, Periodontal disease

INTRODUCTION

It is truly said that "Oral cavity is the reflection of the whole body". According to Hunter [1] microorganisms and their toxins enter the body through mouth. From ages periodontitis is considered to be an important risk factor for rheumatoid arthritis, cardiovascular disease, cerebrovascular disease and respiratory disease [2].

Periodontal disease is an inflammatory disease of oral cavity caused by various microorganisms [3]. It causes destruction of bone and supporting structures around the tooth whereas rheumatoid arthritis is a disease of the joints that causes persistent inflammation of the synovial membrane leading to swelling and stiffness of the concerned joints. Both the diseases follow similar pathogenic mechanism with pro-inflammatory cytokines like interleukin 1, tumour necrosis factor α, prostaglandin E2 causing destruction in both conditions [2,4-6].

Recently the role of adipokines i.e. leptin, adiponectin and resistin have been identified in inflammation-related diseases. Resistin is an adipocyte derived cysteine rich secretory protein expressed in low levels in human adipose cells, lung tissue, resting endothelial cells and in placenta [7]. Expression of resistin is increased by the higher levels of pro-inflammatory cytokines and the effects of resistin are mediated through NF-κB signalling pathway. Abundance of resistin is detected in peripheral blood mononuclear cells and macrophages suggesting its link with the inflammatory processes [8,9]. So, with the increase in severity of inflammatory diseases like periodontitis and arthritis the levels of resistin have found to be increased. Gokhale NH et al., [10], Devanoorkar A et al., [11], Patel SP et al., [12], Dikbas O et al., [13] have done studies to correlate this association and found positive results.

Role of adipokines in inflammatory diseases, including rheumatoid arthritis and periodontitis have greatly increased and an obvious implication of the role of these proteins in the pathogenesis and progression of these diseases is still missing. Resistin can now be considered a new biomarker that can bridge the biological link between arthritis and periodontitis [14]. So, the present study was carried out to reinforce the co-relation between periodontitis and rheumatoid arthritis (RA) [4-6] by using GCF resistin which has not been used much in earlier researches.

MATERIALS AND METHODS

The present study was conducted in the Department of Periodontology. Ethical committee approval was taken with the number KSD-290 and informed consent from the participants was obtained.

An eligible sample was selected based on following criteria:

Inclusion Criteria: Adults of age > 30 y of either sex.

Exclusion Criteria: Patients having any metabolic diseases like obesity, cushing's syndrome and diabetes, patients with known history of cardiovascular and cerebrovascular diseases, tobacco users and alcoholics, pregnant and lactating females, patients on antibiotics from last six months, patients taking medications for RA.

STUDY DESIGN

One thousand fifty patients were screened from the total patient pool coming to the out-patient department over a period of six months. A single examiner checked the periodontal parameters like plaque index (PI) [15], modified gingival index (GI) [16] and full mouth probing depth (PD). Panoramic radiographs were taken. Patients having rheumatoid arthritis were confirmed by their medical records and seropositivity for rheumatoid factor (RF) was checked. Hundred

patients were randomly selected and divided into four groups of 25 patients each. Group A consisted of healthy individuals with PI and GI ≤ 1, no teeth with probing depth ≥ 5 and patients who did not meet the criteria given under American College of Rheumatology Classification for RA [17]. Group B consisted of systemically healthy patients with chronic periodontitis. Periodontitis was defined as PI and GI > 1, atleast > 4 teeth in each quadrant having probing depth ≥ 5mm showing radiographic evidence of bone loss. Group C consisted of patients with rheumatoid arthritis who met the criteria given under American College of Rheumatology Classification for RA but did not have chronic periodontitis as defined above. Group D had patients suffering from both arthritis and periodontitis.

Collection of GCF

Collection of GCF was done with the help of micro pipettes {Top-Tech Biomedicals, Maharashtra, India}. Sampling for Group A and C was done from multiple sites to obtain adequate quantity of GCF and for Group B and D was done from single site with the deepest probing depth. Micropipettes were placed at the entrance of the sulcus after proper isolation and removal of supragingival plaque. Samples contaminated with blood or saliva were discarded. 5 microliters of GCF was collected in maximum of 10 minutes. Collected samples were stored at -20^{0}C until evaluation [18].

Quantitative assessment of resistin was done by commercially available ELISA Kit {Resistin (human) ELISA Kit, AdipoGen, Incheon, South Korea} for all the four groups following the manufacturer's instructions. GCF samples were diluted upto 100 µl by using diluent in the kit. A microtiter plate was taken and its wells were precoated with monoclonal antibody specific for resistin followed by pipetting of standards and samples into the wells for binding to the coated antibody. Incubation at 37°C for one hour followed by removal of unbound compounds by extensive washing was done. Then a detection antibody was added which was again incubated at 37°C for one hour. After removal of excess detection antibody, detector was added that was incubated for one hour at 37°C. A final washing was carried out and a substrate was added at room temperature. Stop solution was then added after 20 min to control the reaction. The intensity of the colour reaction was measured at 450 nm within 30 min which gives colour that is directly proportional to the concentration of resistin in the samples.

STATISTICAL ANALYSIS

Parametric tests were then carried out to compare PI, GI, PD and resistin levels among all four groups followed by one way ANOVA and Tukey HSD procedure. Pearson correlation coefficient was used to analyse the correlation of resistin with other clinical parameters taken in the study. The statistical significance was set at $p<0.05$ and data were analysed using SPSS software®.

RESULTS

The periodontal parameters and resistin levels of all the four groups are shown in [Table/Fig-1]. Resistin levels were found the least in Group A (12.12±4.85) and were found the maximum in Group D (41.13±9.82). On intergroup comparison [Table/Fig-2], it was found that the resistin levels showed maximum significance when Groups A and D were compared (0.0001). On analysing the correlation among resistin level and PI, GI, PD for all the groups by Pearson correlation coefficient, negative correlation was found in Group A (0.5127) where as positive correlation was found in all the other three groups [Table/Fig-3].

Variables	Group A	Group B	Group C	Group D
PI	1.71±0.52	2.51±0.48	1.62±0.81	1.95±0.41
GI	0.67±0.21	2.41±0.42	0.51±0.15	2.64±0.32
PD(mm)	2.91±0.62	6.95±0.96	2.84±0.61	7.15±0.72
RF	N*	N*	P†	P†
Resistin (ng/µl)	12.12±4.85	22.45±6.82	32.45±9.87	41.13±9.82

[Table/Fig-1]: Values of PI, GI, PD and Resistin with presence or absence of Rheumatoid Factor Values are presented as mean±standard deviation
PI: Plaque Index, GI: Modified Gingival Index, PD: Probing Depth, RF: Rheumatoid Factor *absence, †presence

	PI	GI	PD(mm)	Resistin (ng/µl)
Group A versus Group B	0.0081*	0.0000*	0.0018*	0.0238*
Group A versus Group C	0.082	0.925	0.878	0.0198*
Group A versus Group D	0.0843	0.0014*	0.0001*	0.0001*
Group B versus Group C	0.0324*	0.0021*	0.0210*	0.498
Group B versus Group D	0.521	0.983	0.982	0.034*
Group C versus Group D	0.456	0.0002*	0.0016*	0.0378

[Table/Fig-2]: Consolidated pairwise comparison among the four groups for PI, GI, PD and Resistin
PI: Plaque Index, GI: Modified Gingival Index, PD: Probing Depth, RF: Rheumatoid Factor *p-value <0.05 (statistically significant)

Parameter		Group A	Group B	Group C	Group D
PI	Correlation coefficient	- 0.5127	0.3510	0.3143	0.6007
	p-value	0.1231*	0.1983	0.2132	0.0389*
GI	Correlation coefficient	- 0.2896	0.3789	0.3912	0.2675
	p-value	0.1785	0.1632	0.1389	0.3890
PD	Correlation coefficient	- 0.1391	0.0231	0.2154	0.5897
	p-value	0.5823	0.7981	0.4323	0.0467*

[Table/Fig-3]: Pearson correlation analysis among Resistin and PI, GI, PD in all four groups
PI: Plaque Index, GI: Modified Gingival Index, PD: Probing Depth
*p-value <0.05 (statistically significant)

DISCUSSION

Oral cavity and systemic diseases are two entities that can affect each other. Golub et al., [19] proposed a "two-hit" model for chronic periodontitis and systemic diseases like arthritis and osteoporosis. They concluded that the periodontopathic bacteria provided one "hit", whereas systemic inflammations elevating levels of pro-inflammatory biomarkers like CRP, IL-6, and MMP-9 in serum or plasma act as a second "hit".

Similarly both RA and periodontitis are tissue destructive diseases where bone remodelling is constantly adjusted by the host against the noxious agents. Maintenance of the balance between the pro and anti-inflammatory cytokines determines the level of destruction of extracellular matrices in both the diseases. Inflammatory mediators like interleukin 1 (IL-1), PGE2, IL-6 and TNF-α effect the normal coupling mechanism causing more bone destruction [20]. They have common immune response as their protective antibody response is mediated by genes regulating monocyte/T cell response. Immunogenetic mapping studies have linked both the diseases to HLA-DR region of chromosome 5 in the area of TNF-α genes [21]. Various studies have also suggested that specific species of bacteria like *Porphyromonas gingivalis* causing periodontitis may also be involved in the pathogenesis of RA [22,23]. Also, the levels of anti-cyclic citrullinated peptide (CCP) antibody which are increased in RA also target *Porphyromonas gingivalis*, a major pathogen of periodontitis [24].

Resistin is an inflammatogenic cytokine expressed by macrophages and PMNs in inflammatory conditions. It acts as a pro-inflammatory molecule stimulating the synthesis of TNF-α and IL-12 [9]. Its level is also increased by neutrophilic stimulation done by LPS secreted by *Porphyromonas gingivalis* [24]. Its role has also been found to be present in osteoclast differentiation by increasing the levels of ICAM-1 and VCAM-1, thus affecting the bone metabolism [25]. So, it can be considered as a marker for studying the severity of inflammatory diseases like periodontitis and RA.

In the present study clinical parameters for periodontitis and resistin levels were checked and compared in all groups of patients. On analysing it was found that resistin levels were higher in Groups

B, C and D [Table/Fig-1] due to the presence of the underlying inflammatory diseases. This result was in accordance to studies done by Furugen R et al., [26], Saito T et al., [27] Migita K et al., [28], Gokhale NH et al., [10], Devnoorkar A et al., [11] and Patel SP et al., [12]. On intergroup comparison between Groups A and B, Groups A and C and Groups A and D, the resistin levels were higher in all the diseased conditions but the rise in resistin level was of maximum significance when Groups A and D were compared [Table/Fig-2]. On comparing Groups B and C no statistically significant difference in resistin was found. While comparing Groups B and D and C and D, it was noted that the resistin levels were although higher in all the three groups, but the levels in group D were significantly higher than the other two groups. This greater increase in resistin level of Group D can be attributed to the combined effect of both the inflammatory conditions. When resistin levels were correlated with the clinical parameters of the study it was found that positive correlation was found between resistin and PI, GI, PD thus indicating its role in inflammatory conditions [Table/Fig-3]. This result is in accordance with the study done by Choi et al., [29].

The discovery of adipokines has profoundly changed the understanding of the functions of adipose tissue. The adipokines effect the interaction between metabolic disorders, white adipose tissue, inflammatory and immunological diseases [14]. Accumulating evidence on the modulation of GCF levels of many adipokines encourages their future exploitation as soluble biomarkers of disease activity and therapeutic response [30].

CONCLUSION

Use of biomarkers is done in the field of medicine to assess the various underlying biological or pathological processes in the body. With the recent advent in the technology, early detection and timely therapeutic intervention have become easily attainable. Resistin, is one such new biomarker which can be used to diagnose as well as monitor the inflammatory diseases.The clinical significance of using such biomarkers is the prediction of individuals with high risk. It can also be possibly used as a newer agent for therapeutic intervention which can lower the risk of various inflammatory diseases. Since the sample size was small which is the limitation of the present study, different longitudinal and interventional studies with higher sample size are needed to better address the use of adipokines as clinical diagnostic markers.

REFERENCES

[1] Hunter W. Oral sepsis as a cause of disease. *Br Med J*. 1900;2(2065):215-16.
[2] Mercado FB, Marshall RI, Bartold PM. Inter-relationships between rheumatoid arthritis and periodontal disease. *J Clin Periodontol*. 2003;30(9):761-72.
[3] Shah C, Modi B, Budhiraja S, Desai K. A short term comparative clinical evaluation of diode laser and hand instruments for gingival curettage. *Adv Hum Biol*. 2013;3(1):37-42.
[4] Abou-Raya A, Abou-Raya S, Abu-Elkheir H. Periodontal disease and rheumatoid arthritis: Is there a link? *Scand J Rheumatol*. 2005;34(5):408-10.
[5] Bingham III CO, Moni M. Periodontal disease and Rheumatoid arthritis. *Curr Opin Rheumatol*. 2013;25(3):345-53.
[6] Koziel J, Mydel P, Potempa J. The link between periodontal disease and rheumatoid arthritis: an updated review. *Curr Rheumatol Rep.* 2014;16(3):408. doi: 10.1007/s11926-014-0408-09.
[7] Shanshan Pang, Yingying Le. Role of resistin in inflammation and inflammation-related diseases. *Cell Mol Immunol*. 2006;3(1):29-34.
[8] Iacopino AM. Periodontitis and diabetes interrelationships: Role of inflammation. *Ann Periodontol*. 2001;6(1):125-37.
[9] Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun*. 2003;300(2):472-76.
[10] Gokhale NH, Acharya AB, Patil VS, Trivedi DJ, Setty S, Thakur SL. Resistin levels in gingival crevicular fluid of patients with chronic periodontitis and type II diabetes mellitus. *J Periodontol*. 2014;85(4):610-17.
[11] Devanoorkar A, Dwarakanath CD, Gundanavar G, Kathariya R, Patil SR. Evaluation of serum resistin levels in periodontal health and disease and effects of non-surgical periodontal therapy on its levels. *Dis Markers*. 2012;32(5):289-94.
[12] Patel SP, Raju PA. Resistin in serum and gingival crevicular fluid as a marker of periodontal inflammation and its correlation with single nucleotide polymorphism in human resistin gene at -420. *Contemp Clin Dent*. 2013;4(2):192-97.
[13] Dikbas O, Tosun M, Bes C, Tonuk SB, Aksehirli OY, Soy M. Serum levels of visfatin, resistin and adiponectin in patients with psoriatic arthritis and associations with disease severity. *Int J Rheum Dis*. 2014;8. doi: 10.1111/1756-185X.12444. [Epub ahead of print].
[14] Del Prete A, Salvi V, Sozzani S. Adipokine as potential biomarkers in rheumatoid arthritis. *Mediators Inflamm. 2014;2014:425068*. 2014;2014:425068. 11 pages. Doi:10.1155/2014/425068.
[15] Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. *J Periodontol*. 1970;41(1):41–43.
[16] Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. *Clin Prev Dent.* 1986;8(1):3–6.
[17] Neogi T, Aletaha D, Silman AJ, Naden RL, Felson DT, Aggarwal R, et al. American College of Rheumatology Ad Hoc Committee on Clinical guidelines. Guidelines for the management of rheumatoid arthritis. *Arthritis Rheum*. 1996;39(5):713-22.
[18] Sueda T, Bang J, Cimasoni G. Collection of gingival fluid for quantitative analysis. *J Dent Res*. 1969;48(1):159-62.
[19] Golub LM, Payne JB, Reinhardt RA, Nieman G. Can systemic diseases co-induce (not just exacerbate) periodontitis? A hypothetical "two-hit" model. *J Dent Res*. 2006;85(2):102-05.
[20] Marotte H, Farge P, Gaudin P, Alexandre C, Mougin B, Miossec P. The association between periodontal disease and joint destruction in rheumatoid arthritis extends the link between the HLA-DR shared epitope and severity of bone destruction. *Ann Rheum Dis*. 2006;65(7):905-09.
[21] Bendtzen K, Morling N, Fomsgaard A, Svenson M, Jokobsen B, Odum N, et al. Association between HLA-DR2 and production of tumour necrosis factor α and interleukin-1 by mononuclear cells activated by lipopolysaccharide. *Scand J Immunol*. 1988;28(5):599-606.
[22] Okuda K, Ebihara Y. Relationships between chronic oral infectious diseases and systemic diseases. *Bull Tokyo Dent Coll*. 1998;39(3):165-74.
[23] Meurman JH, Janket S, Qvarnstrom M, Nuutinen P. Dental infections and serum inflammatory markers in patients with and without severe heart disease. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2003;96(6):695-700.
[24] Mikuls TR, Payne JB, Reinhard RA, Thiele GM, Maziarz E, Cannella AC, et al. Antibody responses to Prophyromonasgingivalis (P.gingivalis) in subjects with rheumatoid arthritis and periodontitis. *IntImmunopharmacol*. 2009;9(1):38-42.
[25] Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, et al. Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. *BiochemBiophys Res Commun*. 2004;314(2):415-19.
[26] Furugen R, Hayashida H, Yamaguchi N, Yoshihara A, Ogawa H, Miyazaki H, et al. The relationship between periodontal condition and serum levels of resistin and adiponectin in elderly Japanese. *J Periodontal Res*. 2008;43(5):556-62.
[27] Saito T, Yamaguchi N, Shimazaki Y, Hayashida H, Yonemoto K, Doi Y, et al. Serum levels of resistin and adiponectin in women with periodontitis: The Hisayama study. *J Dent Res*. 2008;87(4):319-22.
[28] Migita K, Maeda Y, Miyashita T, Kimura H, Nakamura M, Ishibashi H, et al. The serum levels of resistin in rheumatoid arthritis patients. *Clin Exp Rheumatol*. 2006;24(6):698-701.
[29] Choi BY, Kim JH, Kim KH, Kim KR, Joo SH, Yoon MJ, et al. Elevated Serum Resistin Levels In Rheumatoid Arthritis With Periodontitis. [abstract]. *Arthritis Rheum.* 2013;65(Suppl 10):2414.
[30] Devanoorkar A, Kathariya R, Guttiganur N, Gopalakrishnan D, Bagchi P. Resisitn: a potential biomarker for periodontitis influenced diabetes mellitus and diabetes induced periodontitis. *Dis Markers.* 2014;2014:930206.

PARTICULARS OF CONTRIBUTORS:
1. Professor and HOD, Department of Periodontology, RKDF Dental College, Bhopal, Madhya Pradesh, India.
2. Senior Lecturer, Department of Conservative Dentistry and Endodontics, RKDF Dental College, Bhopal, Madhya Pradesh, India.
3. Senior Lecturer, Department of Periodontics, Karnavati School of Dentistry, Gandhinagar, India.
4. Reader, Department of Periodontics, Karnavati School of Dentistry, Gandhinagar, Gujarat, India.
5. Reader, Department of Periodontics, Karnavati School of Dentistry, Gandhinagar, Gujarat, India.
6. Reader, Department of Conservative Dentistry and Endodontics, Vydehi Institute of Dental Sciences, Bengaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Shilpa Duseja,
Karnavati School of Dentistry, 907/A, Uvarsad, Gandhinagar, Gujarat, India.
E-mail : budhirajashilpa@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Nov 27, 2014**
Date of Peer Review: **Feb 08, 2015**
Date of Acceptance: **Mar 14, 2015**
Date of Publishing: **Apr 01, 2015**