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Original Article

Dentistry Section

Comparative Analysis of GCF Resistin Levels in Obese Subjects with and without Periodontal Disease

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ABSTRACT

Introduction: Resistin is an adipocyte derived hormone that has been shown to play a substantial role in the development of insulin resistance. Resistin acts as a pro-inflammatory molecule and stimulates the synthesis and secretion of pro-inflammatory cytokines. Recent studies have reported the association of Gingival Crevicular Fluid (GCF) resistin levels with periodontal condition.

Aim: The aim of this study was to assess and compare the GCF resistin levels in obese subjects with periodontal health and disease and to correlate the disease severity with GCF resistin levels.

Materials and Methods: Ninety subjects of both the sexes with age between 20–45 years were selected for the study and were categorized into four groups: 25 obese or overweight subjects with generalized chronic periodontitis (Group-I), 25 obese or overweight subjects with healthy periodontium (Group-II), 25 non-obese subjects with generalized chronic periodontitis (Group-III) and 15 non obese subjects with healthy periodontium (Group-IV). The demographic variables like age, Body Mass Index (BMI), Waist Circumference (WC) were recorded and the clinical periodontal parameters such as Plaque Index (PI), Gingival Index (GI), and Clinical Attachment Level (CAL) were also assessed in all the groups. GCF was collected and assessed for resistin levels.

Results: The mean GCF resistin levels in Groups I, II, III & IV were 15.14, 9.06, 12.74 and 5.41 ng/dl respectively and the difference in mean GCF resistin level was statistically significant with the p-value<0.001. The mean GCF resistin levels in Group-I was higher compared to Group II and III and the differences in mean GCF resistin levels were statistically significant. GCF resistin levels were positively correlated with BMI, WC and CAL in Group I and CAL correlated with GCF resistin in Group III and this correlation was statistically significant.

Conclusion: From our study we report that obese subjects with periodontitis have more GCF resistin levels compared to non-obese subjects with healthy periodontium.

Keywords: Adiposity, ELISA, Periodontal disease

INTRODUCTION

Obesity creates the risk of many chronic health problems, is associated with high mortality and exists in multiple clustered, behavioural risk factors. Similarly periodontal disease is one of the most common chronic diseases. Obesity plays a role in modulating the initiation and progression of periodontal disease. Recent meta-analyses have demonstrated a positive association of overweight/obesity with periodontitis [1-3]. The adipose tissue is considered as an endocrine organ, since this tissue secretes various bioactive molecules called as adipocytokines and these adipokines are responsible for dysregulation of immune responses [4]. Adipokines include novel and highly active molecules like leptin, resistin, adiponectin, visfatin, chemerin, apelin and cytokines like Tumor Necrosis Factor-α (TNF-α), InterLeukin-1 and 6 (IL- 1 & 6) [5]. These adipocytokines play an important role in initiation of periodontal disease.

Resistin is an adipocyte derived hormone, that has been shown to play a substantial role in the development of insulin resistance [6]. Resistin is a family member of tissue specific signalling molecules called Resistin Like Molecules (RELMs). There are four members in the mouse RELMs family: Resistin, RELMα, RELMβ and RELMγ. Only two counterparts were found in humans: Resistin and RELMβ. Human resistin is also called as FIZZ3 i.e., found in the inflammatory zone and adipocyte secreted factor, which is a 12.5 kDa cysteine rich peptide containing 108 amino acids in humans and 114 amino acids in mice, it was found that human resistin is mainly synthesized by non-adipocytes inflammatory resident cells such as peripheral blood mononuclear cells, bone marrow cells, monocytes and most predominantly by macrophages [7]. The proportion of adipose tissue macrophages is found to be increased with obesity and this may explain the enhanced production of this hormone in obese individuals. Resistin acts as a pro-inflammatory molecule and stimulates the synthesis and secretion of pro-inflammatory cytokines: TNF-α, IL-6, IL-12 and Monocyte Chemoattractant Protein (MCP)-1. Furugen et al., reported that increased serum resistin levels were significantly associated with periodontal condition in Japanese population [8]. Due to lack of studies related to obesity with periodontal disease and GCF resistin levels in South Indian population, our study was aimed to estimate and compare the GCF resistin levels in obese subjects with periodontal health and disease and to correlate the disease severity with GCF resistin levels.

MATERIALS AND METHODS

The present case control study included 90 subjects of both the sexes with age between 20-45 years. The study participants were selected from the outpatient pool of Thaimoogambigai Dental College, Chennai. The study was conducted from May 2015 to December 2015. The ethical clearance was obtained from DR.MGR University Ethical Committee, Chennai, India. The protocol was explained and written informed consent was obtained from all the participants.

Individuals within 20–45 years of age and with clinical signs for their respective groups and also subjects having minimum number of 20 natural teeth were included in the study.

Pregnancy, previous or current smokers, menopause, cardiovascular disorders, thyroid disorders, diabetes mellitus patients, use of antioxidant supplements, patients on long term steroid medications, patient who had taken anti-inflammatory or antibiotics within previous three months and underwent periodontal treatment in the past six months were excluded from the study.

The subjects were screened for periodontal status and were categorized into four groups as, 25 obese or overweight subjects with generalized chronic periodontitis (Group-I), 25 obese or overweight subjects with healthy periodontium (Group-II), 25 non-obese subjects with generalized chronic periodontitis (Group-III) and 15 non obese subjects with healthy periodontium (Group-IV).

Overweight and obesity were defined by subjects having BMI in a range of >25 kg/m^2, and WC>90cm (men) and >80cm (women) and categorized into healthy periodontium, and chronic periodontitis, based on gingival index and clinical attachment level [9-11]. Subjects with healthy periodontium had gingival index score of 0. Generalized chronic periodontitis is defined by having clinical attachment loss of 3-5mm at more than 30% of sites [11]. The clinical periodontal parameters like Plaque Index (PI), Gingival Index (GI) and Clinical Attachment Level (CAL) were measured. Plaque index was recorded at four sites (mesiobuccal, midbuccal, distobuccal and mid palatal sites) around each tooth [12]. Four gingival areas of the tooth (facial, mesial, distal and lingual) were assessed for GI [13]. CAL was recorded at six sites per tooth and measured in millimetres. CAL was measured as the distance in millimetres from the cementoenamel junction to the bottom of the periodontal pocket. BMI was calculated by dividing weight of an individual in kilograms by height of an individual in meters squared [10].

GCF Collection

GCF was collected from the site with maximum CAL for the Groups I and III, whereas in the periodontally healthy Groups II and IV, GCF was collected from multiple sites to ensure adequate amount of collection. The GCF was collected in the forenoon at the same time of the day, to allow for circadian variation seen in GCF volume. A calibrated volumetric pipette of 5µL capacity was placed extra-sulcularly for collection of GCF. The sample of 2µL capacity was collected for 20 min. The collected sample was then transferred to a sterilized plastic vial with the help of air spray. The sample was transported to lab and the vial was stored at –71ºC. GCF samples were obtained to measure resistin levels using ELISA kit.

BIOCHEMICAL ANALYSIS

GCF samples were analyzed for resistin using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) (Raybio Human Resistin ELISA Kit). In this assay, an antibody specific for human resistin was coated on a 96-well plate. The standards and samples should be pipetted into the wells in the microtitre plate. The resistin found in the sample will bind to antibody in the wells. The biotinylated anti-human resistin antibody was added to the wells. The unbound biotinylated antibody was removed after washing. Then Horseradish Peroxidise (HRP) conjugated streptavidin was pipetted onto the wells and the TetraMethylBenzidine (TMB) substrate was added after washing the wells. Colour was developed, in proportion to the amount of resistin bound. Addition of stop solution changed the colour from blue to yellow. The intensity of the color was measured at 450nm. Analyses were performed according to the manufacturer's protocol. The GCF resistin values were obtained from ELISA reader. Results were calculated using the standard curves created in each assay. The total amount of resistin in GCF was expressed as ng/ml.

STATISTICAL ANALYSIS

The statistical analysis was performed using SPSS software program. The mean values of demographic and clinical parameters were compared by one way ANOVA. GI and CAL were compared between Group I and Group III by independent samples t-test. Pearson correlation analysis was done to correlate WC, BMI, GI, CAL, PI with GCF resistin levels.

RESULTS

A total of 90 subjects (39 males and 51 females) were included in this study. Our results showed that mean age of Group I (38.56) was higher on comparison with other groups. Similarly, the mean BMI scores of Groups I and II were higher than Group III and IV, which was statistically significant (p<0.001). The mean WC was higher in Group I and II than Group III and IV and the difference was statistically significant (p<0.001). The mean plaque index score was more in Group I and III, which was statistical significant (p<0.001). The mean GCF resistin levels in Groups I, II, III & IV were 15.14, 9.06, 12.74 and 5.41 ng/dl respectively and the difference in mean GCF resistin level was statistically significant with the p-value<0.001 [Table/Fig-1]. On comparing the mean values of clinical parameters like GI and CAL between Groups I and III, found to be non-significant [Table/Fig-2]. GCF resistin levels were positively correlated with BMI, WC, PI, GI and CAL and this correlation was statistically significant [Table/Fig-3].

Variable	Group	N	Mean	Std. Dev	f-value	p-value
Age	Group-I	25	38.56	5.378	7.475	<0.001
	Group-II	25	31.40	6.825		
	Group-III	25	35.96	5.806		
	Group-IV	15	31.40	6.706		
	Total	90	34.66	6.786		
BMI	Group-I	25	31.84	2.055	173.495	<0.001
	Group-II	25	31.08	2.613		
	Group-III	25	22.40	.645		
	Group-IV	15	22.47	1.356		
	Total	90	27.44	4.883		
WC (cm)	Group-I	25	99.56	3.731	181.059	<0.001
	Group-II	25	102.68	7.186		
	Group-III	25	77.36	2.447		
	Group-IV	15	79.07	3.654		
	Total	90	90.84	12.511		
Plaque Index	Group-I	25	2.560	0.3215	197.433	<0.001
	Group-II	25	1.060	0.5470		
	Group-III	25	2.776	0.2107		
	Group-IV	15	0.413	0.2642		
	Total	90	1.846	1.0184		
GCF Resistin (ng/ml)	Group-I	25	15.1408	1.65510	224.077	<0.001
	Group-II	25	9.0680	0.77545		
	Group-III	25	12.7472	1.32158		
	Group-IV	15	5.4107	1.00264		
	Total	90	11.1673	3.67278		

[Table/Fig-1]: One way ANOVA to compare mean values of clinical parameters between groups.

Variable	Group	N	Mean	Std. Dev	t-value	p-value
Gingival Index	Group-I	25	2.748	0.2874	1.591	0.120
	Group-III	25	2.852	0.1558		
CAL (mm)	Group-I	25	5.688	0.8151	1.451	0.154
	Group-III	25	5.968	0.5162		

[Table/Fig-2]: Independent samples t-test to compare mean values between Group-I and Group-III.

		WC (cm)	Plaque Index	Gingival Index	CAL (mm)	GCF Resistin (ng/ml)
BMI	Correlation	0.902	-0.009	-0.107	-0.084	0.341
	p-value	<0.001	0.931	0.317	0.429	0.001
	N	90	90	90	90	90
WC (cm)	Correlation		-0.117	-0.241	-0.231	0.206
	p-value		0.274	0.022	0.029	0.051
	N		90	90	90	90
Plaque Index	Correlation			0.919	0.914	0.829
	p-value			<0.001	<0.001	<0.001
	N			90	90	90
Gingival Index	Correlation				0.981	0.837
	p-value				<0.001	<0.001
	N				90	90
CAL (mm)	Correlation					0.859
	p-value					<0.001
	N					90

[Table/Fig-3]: Pearson correlations test for comparison of clinical parameters with GCF resistin levels.

DISCUSSION

Obesity is an abnormal accumulation of body fat and is associated with increased risk of illness, disability and death and is considered a risk for cardiovascular diseases, some cancers and type 2 diabetes [14,15]. Periodontitis appears to affect more frequently and be more severe in obese individuals than in normal weight individuals [2]. Adipose tissue secretes bioactive molecules known as adipocytokines and they under normal physiological conditions may play an important role in energy homeostasis, mobilization of fat and triacyl glceryl storage leading to central obesity [16]. Resistin has also been studied in the GCF as an inflammatory mediator during the induction and resolution of experimental gingivitis in humans [17]. Resistin may hold value as an inflammatory mediator, because it has been associated with obesity and periodontal disease.

In the light of information, the present study was aimed to estimate and compare the GCF resistin levels in obese and non-obese subjects with or without periodontal disease.

In the present study design, the influence of age and sex on resistin concentrations was minimized by including both males and females in the study and selecting patients within the specified age group (20-45 years). On comparing the demographic variables like BMI, WC, Group I and II had higher mean value compared to other groups similar to studies by Swathi et al., [18]. In our study, the extra-sulcular (un-stimulated) method of GCF collection using micro-capillary pipettes was used to ensure atraumatism, to obtain an undiluted sample of native GCF, the volume of which could be accurately assessed and to avoid non-specific attachment of the analyte to filter-paper fibres [19]. Obese individuals were characterized based on WC and BMI jointly, since WC has shown superiority to BMI in predicting obesity related disorders [20]. Overall, the results demonstrate that the mean GCF resistin levels were increased in subjects who were obese and had periodontitis (Group-I) (15.14ng/dl) compared to other groups which was statistically significant ($p<0.001$) similar to previous studies [21,22]. These findings indicate that obesity and periodontitis can, independently or jointly, alter the GCF resistin levels, mostly in favour of pro-inflammation.

In this study, GCF resistin levels were up-regulated in both groups with periodontitis (Group I and III) (15.14 and 12.75 ng/dl) suggesting that periodontal inflammation may modulate the GCF levels of this proinflammatory marker independent of obesity. These results were supported by previous studies in which subjects with periodontitis showed significantly higher serum and GCF resistin concentrations [8,22]. One possible explanation of these findings is that peripheral mononuclear cells and macrophages, rather than adipocytes, release resistin in humans because this cytokine is associated with the activation of inflammatory process and expression of IL-6 by these cells [4].

GCF resistin levels were increased in obese subjects with healthy periodontium compared to non obese subjects with healthy periodontium, accept the fact that obesity upregulates the adipokine resistin secretion. The adiposity parameters like BMI, WC, and periodontal parameters such as PI, GI, CAL were positively correlated with GCF resistin levels, which was similar to study by Swathi et al., [18].

LIMITATIONS

Being an observational study with small group sample size proved the causal association of GCF resistin levels with obese subjects with periodontitis. Future longitudinal studies involving more participants from similar population with intervention trials can overcome these limitations.

CONCLUSION

Our study results show that obesity and periodontal inflammation were associated with high resistin levels. This study therefore highlights that resistin can be considered as an inflammatory mediator, since it has been associated with obesity and periodontal disease.

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