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Estimation of levels of Apelin in Gingival Crevicular Fluid of Periodontitis patients: An Immunological Analysis

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Abstract

Introduction: Periodontitis has been suggested to contribute to adipose tissue inflammation and dysregulation of adipokines may increase both risk and development of chronic and autoimmune diseases. Apelin, a type adipokine may serve as a beneficial biomarker for assessment of metabolic risk factors. Presence and severity of periodontitis and obesity might influence apelin. Apelin levels in gingival crevicular fluid (GCF) of patients with and without periodontal disease and obesity may help to clarify possible relation between obesity and periodontal disease. The aim of the study was to assess levels of apelin in periodontitis and to identify association between apelin and periodontal disease.

Materials and Methods: A total of 75 subjects (Group 1 - 25 healthy subjects, Group 2 - 25 periodontitis subjects and Group 3 - 25 periodontitis with obesity subjects) were included in this study. Clinical periodontal parameters Plaque Index (PI), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) were recorded for all the 3 groups. GCF was collected using microcapillary pipettes, transferred to airtight plastic vials and stored at −80°C until assayed. Samples were assayed for apelin levels using a commercially available ELISA kit.

Results: A statistically significant increase in GCF levels of apelin were observed in periodontitis with obesity group when compared with periodontitis and healthy groups and statistically significant correlation was also noticed between GCF levels of apelin, BMI and clinical periodontal parameters.

Conclusion: Within the limitations of present study, it can be concluded that the levels of apelin in GCF are higher in

periodontitis with obesity subjects in correlation with higher BMI and clinical periodontal parameters suggesting that there might be a link between obesity, periodontitis and apelin. Hence, apelin can be considered as a potential diagnostic biomarker for periodontal disease.

Keywords: Apelin, Periodontitis, Obesity, Gingival Crevicular Fluid, ELISA.

Introduction

Periodontitis is a chronic inflammatory disease of the periodontium, which is caused by pathogenic bacteria in combination with other risk factors. It is a pathological sign of the host response against periodontal pathogens and results in the destruction of tooth supporting tissues, progressive attachment loss and bone loss [1].

The periodontal pathogens and their components can evoke the inflammatory host response and the production of inflammatory mediators in periodontal tissues [2]. Periodontal disease is associated with several systemic diseases and conditions and it affects both glucose and lipid metabolism that may be important factors in the development of both type 2 diabetes and cardiovascular disease [3].

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 [4]. The mechanisms underlying these associations are not well understood so far, but adipokines may be a pathomechanistic link. Specifically, the relationship between obesity and deep periodontal pockets was independent of glucose tolerance status, suggesting a direct association between obesity and periodontitis [5]. The imbalance between proinflammatory and anti-inflammatory adipokines results in a low-grade inflammatory state, as observed in obesity and diabetes mellitus [6].

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 [7]. Adipose tissue depots are the most vulnerable target to mediate significant immune cell infiltration and inflammation contributing to systemic inflammation and insulin resistance (IR) in obese humans [8].

Adipokines are bioactive molecules that are secreted by the adipose tissue. Some of the adipokines are key regulators of the inflammatory response and are crucial for the progression of periodontitis. Several adipokines could serve as the monitoring molecules that reflect overall and oral disease conditions that include periodontitis[9].

It has become increasingly clear that nutrition and body weight are modifying factors [10]. Increased body weight assessed as body mass index (BMI), waist circumference or waist-to- hip ratio is associated with higher incidence of periodontitis [11]. These systemic conditions modify the course, as well as the nature, of the host response to the bacterial challenge. 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) [12].

Apelin serves as a beneficial biomarker for assessment of metabolic risk factors. Apelin may be released by many cell types in the oral cavity in response to bacterial activity. Many cell types including osteoblasts which are key players in bone metabolism are known to secrete apelin and osteoblasts in particular proliferate in response to this molecule [13].

Periodontitis is associated with insulin resistance, and insulin-induced apelin expression has been demonstrated in adipocytes [14], but there are limited studies in the literature that assessed the levels of apelin in periodontal disease. So, aim of the study is to assess the levels of

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apelin in gingival crevicular fluid (GCF) of periodontitis patients and to identify its association with periodontal disease.

Materials And Methods

Seventy five patients were recruited from outpatient Department of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bangalore and ethical clearance for the study was obtained from the institutional ethical committee.

Study population consisted of 3 groups i.e., Group 1 – Healthy subjects (n=25), Group 2 – Periodontitis subjects (n=25) and Group 3 – Periodontitis with obesity subjects (n=25). Subjects with BMI ≥ 25 for periodontitis with obesity group, subjects with ≥20 completely erupted teeth, presence of BOP, periodontal pockets of PPD ≥5mm and ≥2 mm clinical attachment loss were included in this study. Subjects with diabetes mellitus and hypertension, immunological disorders, arthritis/osteoporosis, history of periodontal intervention within the last 6 months and antibiotic treatment within the last 3 months were excluded from the study. Informed consent was obtained from the patients who agreed to participate in this study.

BMI was calculated by measuring the height (in centimetres) and weight (in kilograms) of the patient. If the BMI of the patient was >25, then the patient was said to be obese and were included in Group 3. Patients were included in groups 2 and 3 if they were diagnosed with periodontitis. All the clinical periodontal parameters Plaque Index (PI), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) were recorded for all the 3 groups by a single examiner. UNC-15 probe was used to record PPD and CAL at six sites per tooth at the time of examination. PI was interpreted by the scoring criteria given by Silness J and Loe H in 1964.

GCF was collected from the sulcus around the tooth which exhibit the deepest pocket (most representative) by placing a capillary tube parallel to the tooth surface so as to collect 5 microliters of GCF from the sulcus after proper isolation using cotton rolls. Collected GCF samples were immediately transferred to airtight plastic vials and mixed with 0.5cc phosphate buffer solution to attain neutral pH and stored at −80°C until assayed. Samples were assayed for apelin levels using a commercially available ELISA kit.

Assessment of Apelin levels using Enzyme Linked Immunosuppressant Assay (ELISA):

Samples were assayed for apelin levels using a commercially available ELISA kit (KinesisDx human Apelin, ELISA), with a minimum detection level of 0.756 pg/ml and assay range of 7.5pg/ml to 120pg/ml. Samples were analysed at the Central Research Laboratory, Department of Microbiology, Ramaiah Medical College and Hospital, Bangalore, India. All reagents and samples were allowed to warm to room temperature (18-25°C) before use. All the samples and standards were run in duplication. The standards were prepared according to the manufacturer's instructions. 50µL of Standards and 40µL samples were added to the wells. Then 10µL of Biotin conjugate and 50µL of HRP (Horseradish Peroxidase) conjugate were added to the wells. The plate was covered and incubated for 1 hour at 37°C in the incubator. After 1 hour the wells were aspirated and washed using the 1X wash buffer for 4 times. Substrate A and Substrate B of 50µL each were then added to the wells, gently mixed and incubated for 10 minutes at 37°C in dark. Later, 50µL of stop solution was added to the wells which showed the colour change from blue to yellow. The intensity of the colour was measured within 15 minutes at 450nm in microplate reader.

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Statistical Analysis

Statistical Package for Social Sciences (SPSS) for Windows, Version 22.0. Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. One-way ANOVA test followed by Tukey's post hoc analysis was used to compare the mean values of BMI & clinical parameters between different groups. Kruskal Wallis test followed by Mann Whitney U test and post hoc Analysis was used to compare the mean GCF Apelin levels between 3 study groups. Spearman's Correlation test was used to estimate the relationship between periodontal parameters and GCF Apelin levels in different study groups. The level of significance (P-Value) was set at P<0.05.

Results

Comparison of PI was done for 3 groups using One-way ANOVA test where the mean values were 0.89±0.38, 1.30±0.43 and 1.49±0.30 for healthy, periodontitis and periodontitis with obesity group respectively which was statistically significant. Intergroup comparison of PI among 3 groups was done using Tukey's post hoc test. Statistically significant results were observed on comparing Group 1 versus Group 2, and Group 1 versus Group 3 (p<0.001) .

PPD in 3 groups showed mean values of 1.72±0.27, 5.96±0.68 and 6.32±0.90 for healthy, periodontitis and periodontitis with obesity group respectively which was statistically significant. Intergroup comparison of PPD among 3 groups was done using Tukey's post hoc test. Statistically significant results were observed on comparing Group 1 versus Group 2, and Group 1 versus Group 3 (p<0.001).

Comparison of CAL was done for 3 groups showed mean values were 0.56±0.87, 4.88±0.78 and 5.16±0.90 for healthy, periodontitis and periodontitis with obesity group respectively which was statistically significant. Intergroup comparison of CAL among 3 groups was done using Tukey's post hoc test. Statistically significant results were observed on comparing Group 1 versus Group 2, and Group 1 versus Group 3 (p<0.001).

GCF Apelin Levels

Intragroup comparison of GCF Apelin levels was done for 3 groups using Kruskal Wallis test where the mean values were 76.54±8.38, 89.66±7.81 and 103.66±20.81 for healthy, periodontitis and periodontitis with obesity group respectively which was statistically significant (p<0.001). Intergroup comparison of GCF Apelin levels among 3 groups was done using Mann Whitney Post hoc analysis. Statistically significant results were observed on comparing Group 1 versus Group 2, and Group 1 versus Group 3 and Group 2 versus Group 3 (p<0.001) (Table 1, Graph 1).

A statistically significant difference was observed between GCF Apelin levels and BMI in Group 1 healthy subjects (p-0.04*). Between the GCF Apelin levels, PPD and CAL there was a statistically significant difference (p<0.001) in Group 2 periodontitis subjects. Between the GCF Apelin levels, PPD, CAL and BMI there was a statistically significant difference (p<0.05) in Group 3 periodontitis with obesity subjects (Table 2, Graph 2, Graph 3,Graph 4).

Discussion

Apelin is a novel amino peptide expressed in the adipocytes of humans and is encoded by the APLN gene. It is an endogenous ligand for G-protein coupled APJ receptor that is expressed at the surface of cell types. It is widely expressed in heart, lung, kidney, liver, adipose tissue, gastrointestinal tract, brain, adrenal glands, endothelium and human plasma. The functions of apelin include fluid homeostasis, cardiovascular function and insulin secretion. The synthesis of apelin in adipocytes is triggered by insulin and its plasma levels are reported to

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increase in association with insulin resistance, hyperinsulinemia, and diabetes mellitus. Apelin was likely to be involved in the pathophysiology of T2DM and CVD, and this could be explained by different mechanisms such as the level of apelin in obese T2DM patients that correlated closely with BMI and the elevated levels that may be a result of insulin resistance compensatory reaction. Apelin correlates with states of insulin resistance and obesity and decreases insulin secretion [15].

Apelin may also inhibit the release of insulin, aggravating the disorders of glucose metabolism which was also proved by a study [16] by coordinating with other factors associated with increased circulating free fatty acids, apelin may cause the occurrence of insulin resistance [17]. It has been reported that apelin correlated with oxidative stress and inflammation markers [18] understanding the contribution of such an adipokine in obesity-associated disorders appears to be of major importance.

Obesity creates the risk of many chronic health problems associated with high mortality and exists in multiple clustered, behavioural risk factors. Obesity leads to the downregulation of anti-inflammatory factors and upregulation of apelin that activates endothelial cells and promote dysfunctional phenotype [19]. Similarly, periodontal disease is one of the most common chronic diseases that is associated with several systemic diseases and conditions and it affects both glucose and lipid metabolism that may be important factors in the development of both type 2 diabetes and cardiovascular disease. Obesity might represent a systemic condition capable of influencing the initiation and progression of periodontal disease as noted first using a ligature-induced periodontitis model in rat [20]. Obesity is another common risk factor to be considered in this regard and it has a significant association with periodontitis via mechanisms associated with BMI, body fat, and maximum oxygen consumption [21].

Increased body weight, assessed as body mass index (BMI), waist circumference or waist - to- hip ratio is associated with higher incidence of periodontitis [22]. These systemic conditions modify the course, as well as the nature, of the host response to the bacterial challenge. Lipopolysaccharides and endotoxins secreted by bacteria with the progress of periodontal disease leads to an increase in the inflammatory state, this in turn causes reduction in growth factor levels and increase in secretion of interleukins and inflammatory biomarkers which are secreted primarily into the plasma, traces of which are found in gingival crevicular fluid and saliva. These biological fluids can therefore be utilised to detect adipokine levels. Evaluation of biomarkers in tissue fluids have considerable shortcomings. Serum analysis is complex and invasive as drawing of blood is involved, saliva has higher chances of contamination and has poor levels of these biomarkers [23].

Periodontitis has been suggested to contribute to adipose tissue inflammation by promoting insulin resistance and associated with insulin resistance, and insulin-induced apelin expression has been demonstrated in adipocytes [24, 25]. The presence and severity of periodontitis and obesity might influence apelin. So, the present study analysed the apelin levels in GCF of healthy, periodontitis and periodontitis with obesity subjects. The results of the study indicate an increase in the GCF apelin levels in subjects with periodontitis and obesity.

In this study, BMI was significantly higher in periodontitis with obesity subjects when compared to the other two groups which was in accordance with the previous study conducted [26]. It was also observed higher BMI in the severe obstructive sleep apnea syndrome group than the

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control group that would suggest a link between periodontal disease and obesity [27].

Plaque Index scores in this study were slightly higher in periodontitis and periodontitis with obesity groups when compared to the healthy group which was statistically significant and this result was in accordance with the previous study conducted [26]. PPD and CAL were statistically significant in periodontitis and periodontitis with obesity group when compared to healthy group. Slightly more levels were observed in periodontitis with obesity group which would suggest that obesity compromises the periodontium leading to destruction of the periodontal supporting tissues causing deeper periodontal pockets and attachment loss.

In this study, the GCF apelin levels were higher in periodontitis with obesity group when compared to the healthy and periodontitis groups which was in accordance with the previous study [27] where they observed the increased levels of salivary apelin in obstructive sleep apnea syndrome in comparison with the controls. In a previous study done it was observed that there were higher concentrations of serum apelin in obese diabetic groups compared to non-obese controls which were linked with an increase in multiplicity of metabolic risk factors, suggesting that apelin serves as a beneficial biomarker for assessment of metabolic risk factors which is in accordance with this study [26]. The limitations of the present study would be smaller sample size and observational nature of the study.

Conclusion

Apelin is a type of adipokine, which might be a key regulator of the inflammatory response and is crucial for the progression of periodontitis. A positive correlation was noticed between GCF levels of apelin, BMI and clinical periodontal parameters that included plaque index, probing pocket depth and clinical attachment loss. Hence, apelin can be considered as a potential diagnostic biomarker for periodontal disease.

To the best of our knowledge, this is the first study to be conducted to detect the apelin levels in GCF of subjects with periodontitis and obesity. Within the limitations of the present study, it can be concluded that the levels of apelin in GCF are higher in periodontitis with obesity subjects suggesting that there might be a link between obesity, periodontitis and apelin levels. Further longitudinal studies with larger sample size could be carried out to assess the link between apelin and periodontal disease and strengthen the findings of this study.

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Legend Table and Figures

Table 1: Comparison of mean GCF Apelin Levels b/w groups

Comparison of mean GCF Apelin Levels b/w groups using Kruskal Wallis test followed by Mann Whitney Post hoc Analysis							
Parameters	Groups	N	Mean	SD	P-Value[a]	Sig. Diff	P-Value[b]
GCF Apelin	Group 1	25	76.54	8.38	<0.001*	G1 vs G2	0.003*
	Group 2	25	89.66	7.81		G1 vs G3	<0.001*
	Group 3	25	103.66	20.81		G2 vs G3	0.002*

Graph 1: Comparing Mean GCF Apelin Levels between 3 groups

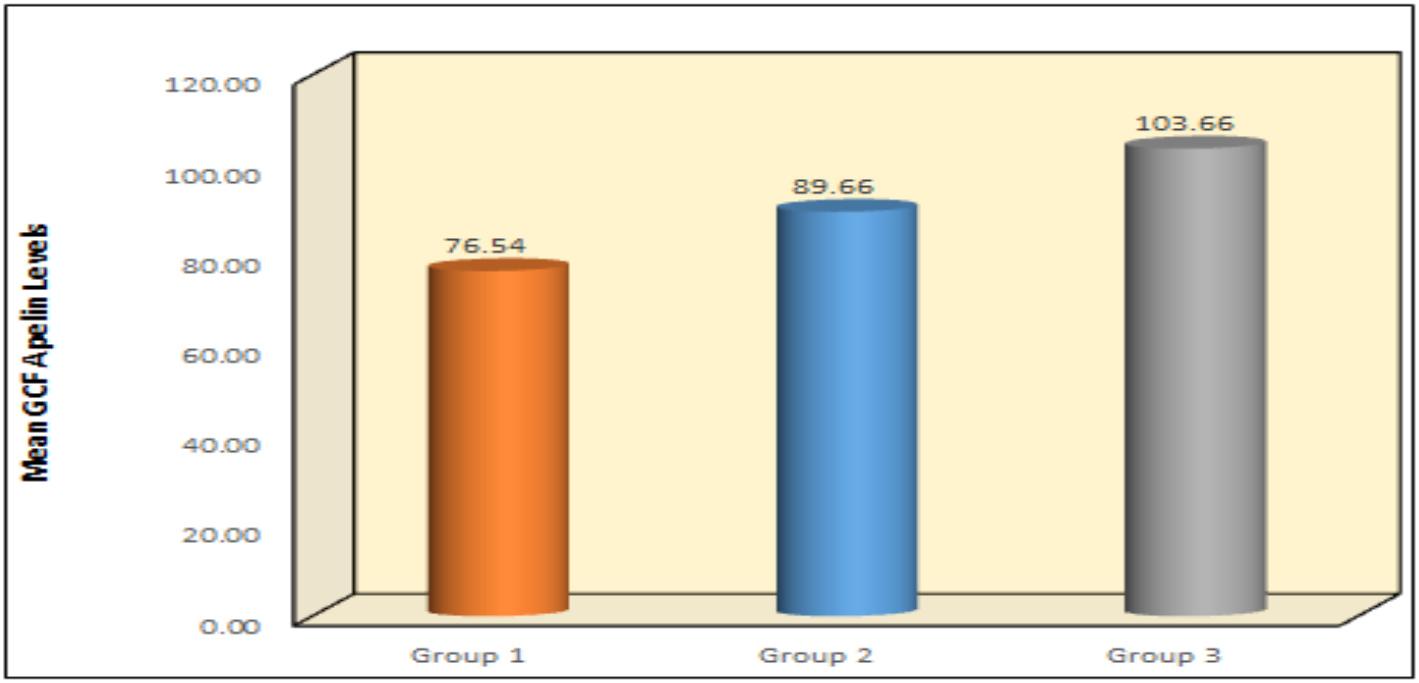

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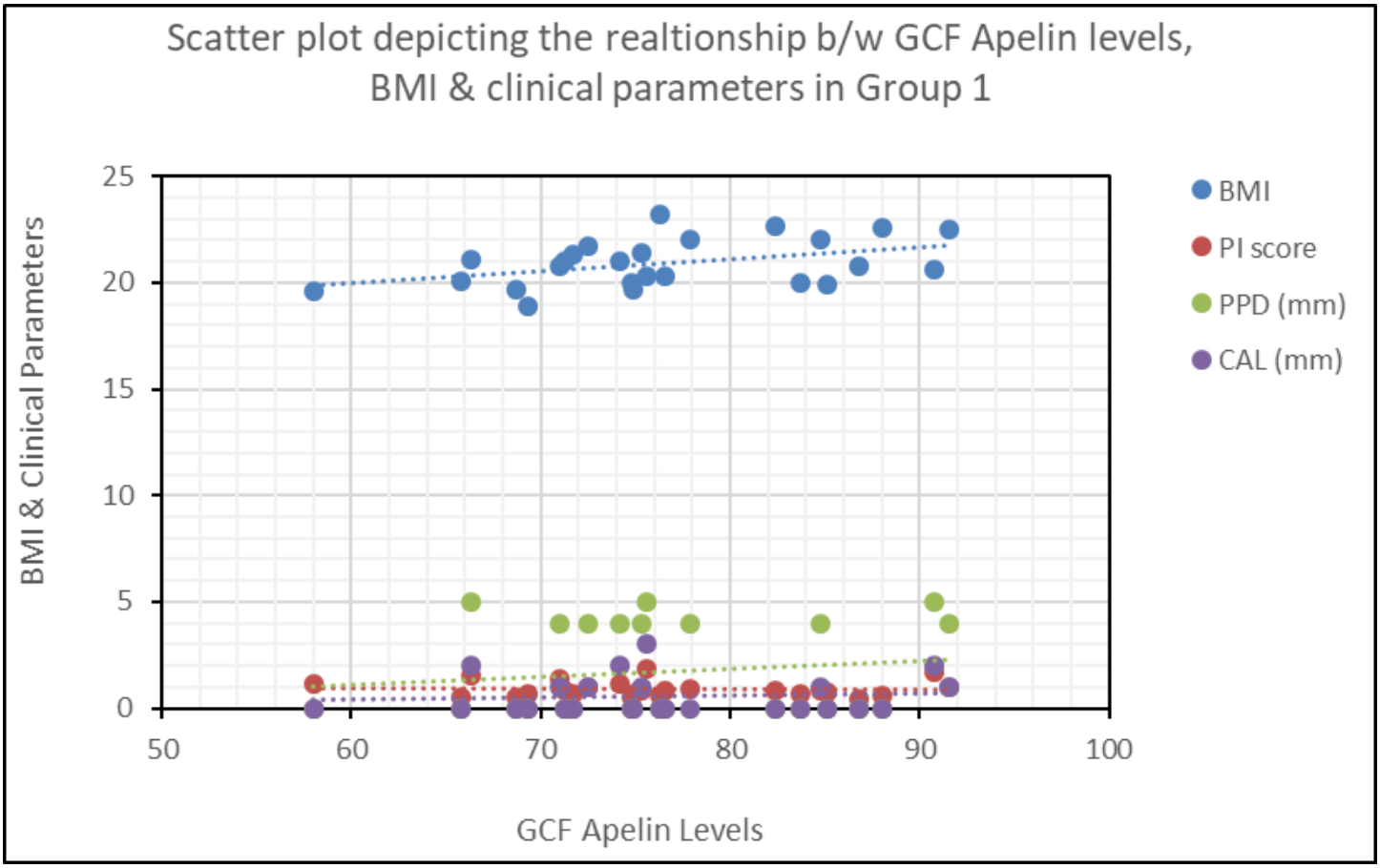
Table 2: Spearman's correlation test to assess the relationship b/w GCF Apelin levels, BMI & Clinical Parameters in each study group

Spearman's correlation test to assess the relationship b/w GCF Apelin levels, BMI & Clinical Parameters in each study group							
Groups	Variable	Values	PI score	PPD (mm)	CAL (mm)	BMI	Age (yrs)
Group 1	GCF Apelin	Rho	-0.02	0.12	0.06	0.41	0.15
		P-Value	0.92	0.58	0.76	0.04*	0.47
Group 2	GCF Apelin	Rho	0.16	0.61	0.65	0.30	0.28
		P-Value	0.45	0.001*	<0.001*	0.14	0.18
Group 3	GCF Apelin	Rho	0.37	0.59	0.44	0.49	0.03
		P-Value	0.07	0.002*	0.03*	0.01*	0.91

Graph 2: Scatter plot depicting relationship between GCF Apelin levels, BMI and clinical parameters in Group 1



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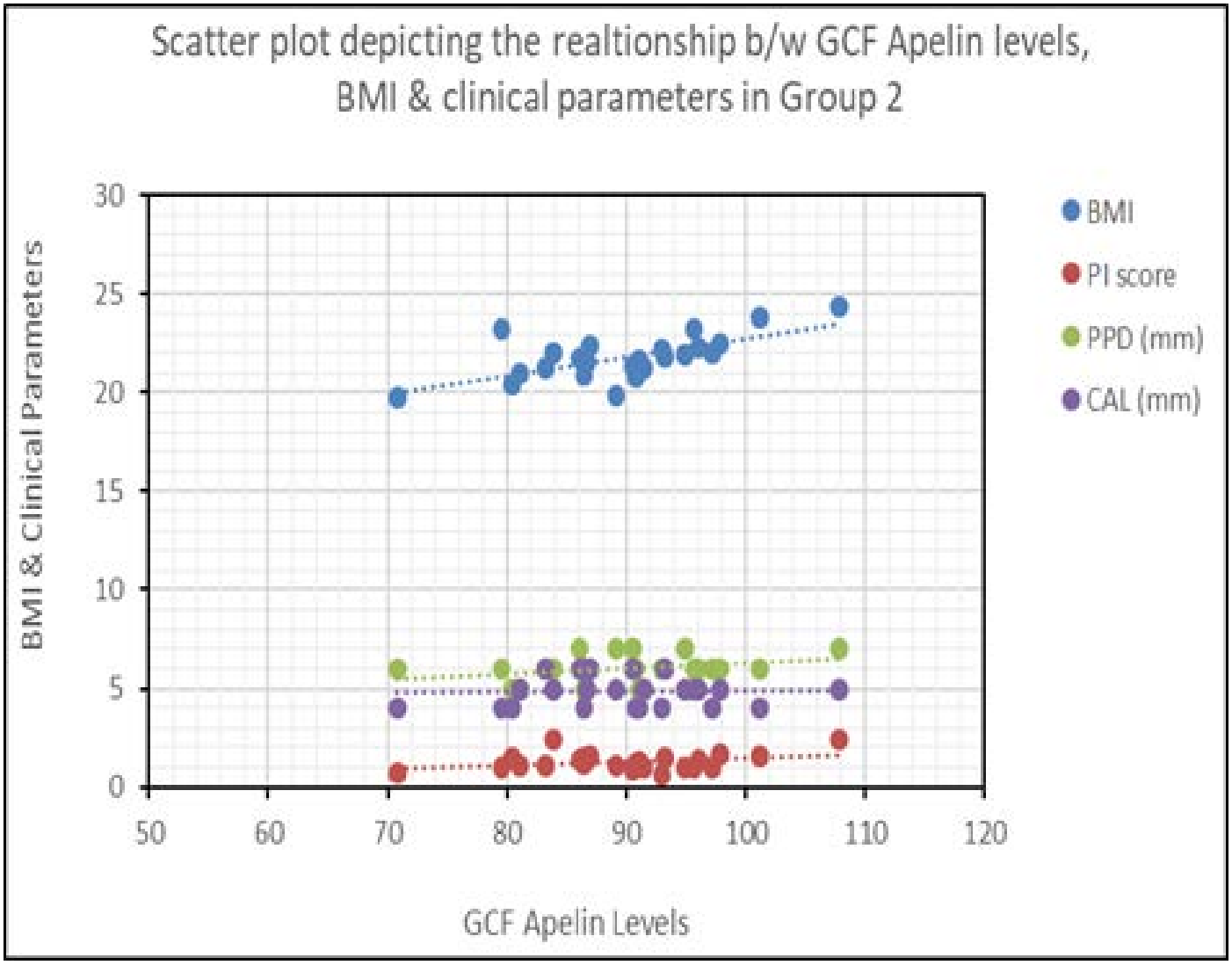
Graph 3: Scatter plot depicting relationship between GCF Apelin levels, BMI and clinical parameters in Group 2



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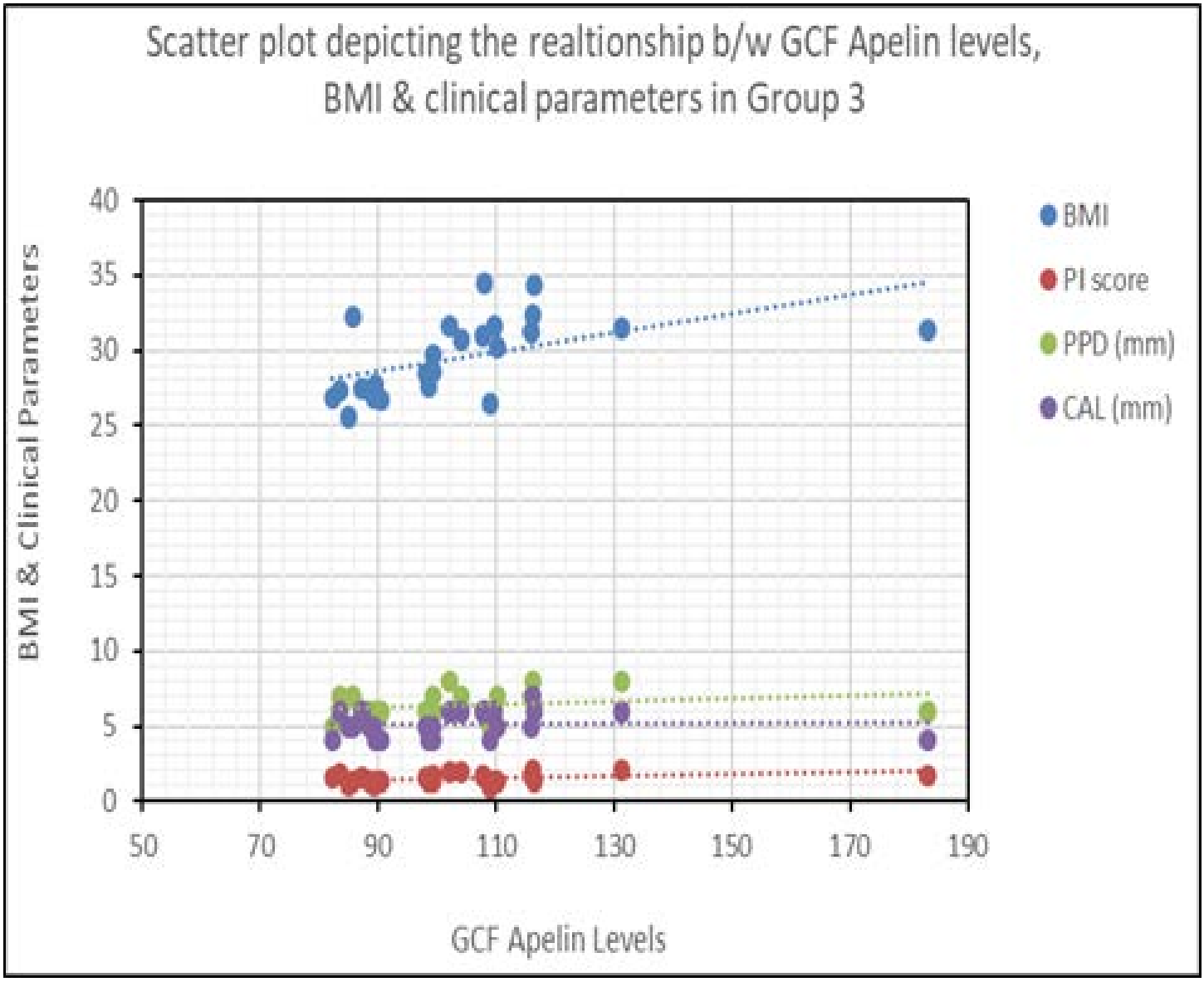
Graph 4: Scatter plot depicting relationship between GCF Apelin levels, BMI and clinical parameters in Group 3



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