Original article

Evaluating Salivary Apelin Level among Chronic Periodontitis Patients, with and without Type 2 Diabetes Mellitus utilizing Enzyme-Linked Immunosorbent Assay: A Comparative Study

Tanvi Hirani [1], Md. Ahsanul Haq [2], Neetha J Shetty [3], Viral Patel [4], Rahnuma Ahmad [5], Susmita Sinha [6], Siddhartha Dutta [7], Mainul Haque [8], Santosh Kumar [9]

Abstract

Background: Periodontitis is a chronic inflammatory disorder mainly due to a decrease in the immune system caused by periodontal pathogens. The relationship of apelin among chronic generalized periodontitis (CGP) and type 2 diabetes mellitus (T2DM) has been reported. This study intends to assess the level of apelin in saliva from patients of healthy individuals, individuals with CGP, and CGP + T2DM, and evaluate the role of apelin adipokine in inflammation and insulin resistance. New treatment modalities can be invented using apelin in host modulation therapies. ***Methods:*** The study comprised 300 patients that were enrolled according to their clinical findings and were divided into three groups chronic periodontitis (n=100), chronic periodontitis with T2DM (n=100), and healthy controls (n=100). Probing pocket depth (PPD), clinical attachment level (CAL), and salivary Apelin (AP) were obtained. With the help of the enzyme-linked immunosorbent assay (ELISA) kit, the salivary apelin level was measured. A statistical software package was used for the surveying of collected data. ***Results:*** Group three[CGP+DM] shows the highest salivary apelin level compared to CGP patients without DM. It was 15.43±2.05ng/dl in CGP and 24.08 ± 3.60ng/dl in CGP with DM. All three groups found a statistically significant difference in mean Apelin Level, PPD, and CAL. ***Conclusion:*** This study indicated a possible role of adipokines in inflammation and glucose level regulation in patients with T2DM and CGP. In the coming era, apelin levels in saliva can be used for screening objectives in large populations to assure the risk of destructive periodontal disease.

Keywords: Assessment, Salivary Apelin, Concentration, Chronic Periodontitis, Type 2 Diabetes Mellitus (T2DM), ELISA, Compare, Contrast.

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Introduction

Periodontitis is a chronic inflammatory disorder mainly due to a decrease in the immune defense system caused by periodontal pathogens. Interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNF-α), considered pro-inflammatory cytokines, have been elevated in periodontal diseases[1]. An increase in pro-inflammatory cytokines has a significant role in the inception of periodontal tissue destruction and ultimately impacts the pathogenesis

1. Research Scholor, Department of Periodontology, Gujarat University, Ahmedabad, Gujarat, and Department of Periodontology, Karnavati School of dentistry, Uvarsad, Gandhinagar, India
2. Infectious Diseases Division, icddr,b, Mohakhali, Dhaka, Bangladesh.
3. Department of Periodontology and Implantology, Manipal College of Dental Sciences, MAHE, Mangalore, Karnataka 575001, India.
4. Department of Periodontology and Implantology, College of Dental Science and Research Center, Gujarat University, Ahmedabad, Gujarat, India.
5. Department of Physiology, Medical College for Women and Hospital, Dhaka, Bangladesh.
6. Department of Physiology, Khulna City Medical College and Hospital, Khulna, Bangladesh.
7. Department of Pharmacology, All India Institute of Medical Sciences, Rajkot, Gujarat, India.
8. Unit of Pharmacology. Faculty of Medicine and Defence Health, Universiti Pertahanan Nasional Malaysia (National Defence University of Malaysia), Kem Perdana Sungai Besi, 57000 Kuala Lumpur, Malaysia.
9. Department of Periodontology and Implantology, Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat, India.

Correspondence:

Santosh Kumar. Department of Periodontology and Implantology, Karnavati School of Dentistry, Karnavati University, Gandhinagar, Gujarat-382422, India. **Email**: drsantoshkumar2004@gmail.com. Cell Phone: +91 96018 00379.

Mainul Haque.Unit of Pharmacology. Faculty of Medicine and Defence Health, Universiti Pertahanan Nasional Malaysia (National Defence University of Malaysia), Kem Perdana Sungai Besi, 57000 Kuala Lumpur, Malaysia. **Email**: runurono@gmail.com. mainul@upnm.edu.my Cell Phone: +60109265543.

of systemic diseases [2].

Diabetes mellitus (DM) is a metabolic disorder characterized by a high blood sugar level (hyperglycemia) over a prolonged period. DM is mainly of two types type 1 and 2 (T2DM). T2DM is a commonly observed form of DM, previously known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes [3]. In T2DM, cells fail to respond to insulin appropriately, leading to insulin resistance, and/or a lack of insulin may develop [4]. A remarkable increase in the extensiveness of obesity among children has led to more cases of T2DM in younger and older adults [5]. Periodontal disease often denotes an early sign and is considered the sixth complication of DM [6]. Different type of studies has shown a bidirectional interrelationship between T2DM and periodontitis. The non-enzymatic reaction of glucose in the hyperglycaemic state of DM increases the production of advanced glycation end products (AGE), which alter the cellular function and modulate tissue structure (Figure 1) [7].

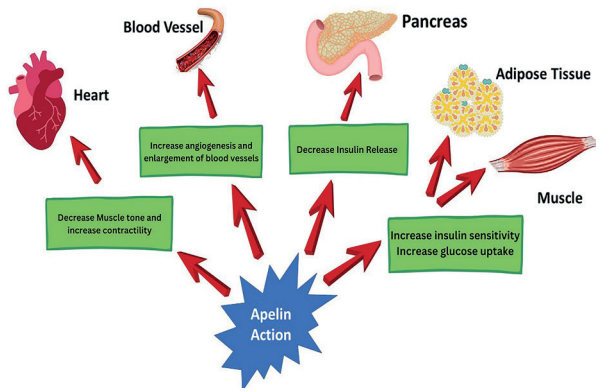

Figure 1: Illustrating the bidirectional relationship between Type 2 Diabetes Mellitus and Periodontitis. ***Image Credit****: Rahnuma Ahmad.*

Periodontal disease is a chronic inflammatory disease considered a potential risk in cardiovascular and respiratory diseases. Diabetes and these systemic disorders have been capable of affecting the periodontium or treatment of periodontal disease [1, 8]. Adipose tissue is an effective endocrine organ that secretes approximately 50 biologically progressive substances, generally termed adipokine or adipocytokines. Leptin was observed as the first adipokine in 1994 [9]. After that, hundreds of adipokines were invented [10], and their role in periodontal diseases has been calculated [2]. Periodontitis has been suggested to contribute to adipose tissue inflammation by promoting insulin resistance. Periodontitis is associated with insulin resistance, and insulin-induced apelin expression has been demonstrated in adipocytes. The presence and severity of periodontitis might influence salivary apelin, and IL-6, originating from buccal adipocytes, may be involved in this response.

Apelin is a molecule released from endothelial cells. It shows its action through various autocrine and paracrine mechanisms by its attachment to the apelin receptor (APJ), a G-protein coupled receptor expressed on cardiomyocytes, muscle cells, and fibroblasts [11-13].

Additionally, the apelin gene comprises 77 amino acid preprotein, which affects different systems, mainly regulating cardiovascular homeostasis [14]. It regulates insulin secretion, glucose, and lipid metabolism in other vital tissues [15]. Moreover, it stimulates glucose transport like insulin [16]. It is observed to stimulate glucose uptake in the muscle of obese and insulin-resistant experimental animals, indicating an overall increase in insulin sensitivity (Figure 2) [17, 18].

Evidence reveals that periodontitis can promote inflammation of adipose tissue and can develop insulin resistance (IR) [19]. Periodontitis is associated with IR and insulin-induced apelin expression in the adipocytes [20]. The buccal adipocytes release salivary apelin and other adipokines, and the quantity and response might depend on the severity of the periodontitis [21-23]. Afterward, adipokines act as selective biomarkers in serum and saliva to detect diabetes and periodontitis [24].

Objectives of the Research

This study analyzed salivary apelin levels in patients with chronic generalized periodontitis (CGP) and chronic generalized periodontitis + Diabetes Mellitus (CGP+DM) compared to healthy controls to find the association between apelin levels and diabetes.

Materials and Methods

This comparative study was carried out at the department of periodontology at Karnavati School of Dentistry, Gujarat, India. Modified Cochrane formula analysis was utilized to calculate the sample size. The estimated total sample size was 300. Henceforth, 100 research participants in each group were enrolled healthy patients as the control group, CGP, and CGP+DM. The study was conducted between May 2021 to December 2021.

Inclusion criteria included the patients aged 30-45 years with diabetes and periodontitis. Exclusion

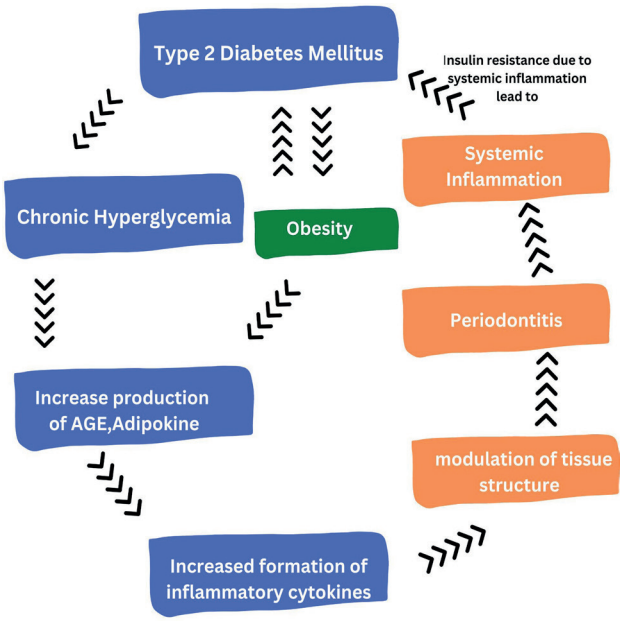

Figure 2: Illustrating the action of Apelin on different organs, including adipose tissue, muscle, pancreas, heart, and blood vessels. ***Image Credit****: Rahnuma Ahmad.*

criteria had (1) <30 years of age; (2) Any previous periodontal treatment (3) aggressive periodontitis; (4) <25 teeth; (5) Any medication and/or antibiotics therapy three months before starting the study; (6) Other than diabetes any known systemic disease (7) pregnancy or lactation, (8) Individuals with the habit of smoking. Moreover, our study samples did not suffer from other known systemic diseases.

The study was carried out per Helsinki's declaration (Version 2008). The Research Ethics Committee approved this study, Karnavati School of Dentistry [Reference Number: KU/17/KSD246; Dated: February 18, 2021]. Research participants have explained the study protocol, and written informed consent was obtained from all research participants.

Sample Selection

As per classification by the European Federation of Periodontology [25], chronic periodontitis was evaluated in all the patients. A single examiner conducted a thorough examination with the help of a UNC 15 (University of North Carolina) periodontal probe and checked all four sites of the tooth. CP was present when the patient showed CAL ≥3 mm and PPD ≥4 mm in ≥3 interproximal sites.

Diabetes Examination

Random plasma glucose was estimated in all the individuals enrolled in the study. According to the American diabetic association's criteria [26], random plasma glucose ≥200 mg/dl (11.1 mmol/l) with classic symptoms of hyperglycemia or hyperglycaemic crisis was considered diabetic and included in the study group of chronic periodontitis with T2DM. As this study follow the American diabetic association's criteria, we also conducted the hemoglobin A1c (HbA1c) test to confirm diabetes status.

Saliva Sample Collection and Biomarker Analysis

The salivary secretion was collected based on an unstimulated saliva collection procedure. It was collected from all the individuals between 9 AM and 12 PM to decrease diurnal variations. Mouth rinsing with water was done 10 minutes before collecting the sample. Patients were asked to drain the saliva passively from the lower lip into a pre-labeled sterile container for about almost 20 minutes till the ~5 ml of saliva was collected [3]. All the cellular debris and turbidity of saliva samples were removed by centrifuging (10,000 x g) for 5 minutes. After that, 0.5 ml of saliva was instantly shifted to 1.5 ml diluents (5x concentrated phosphate buffered saline solution) and kept at - 80° centigrade till the commencement of the ELISA test.

Analysis of Apelin Adipokine

The level of apelin adipokine in saliva was determined using the ELISA method. The Human Apelin Elisa kit (Apelin EIA Kit™, Sigma Aldrich, USA) assessed the apelin level. All the analyses were carried out according to the manufacturer's instructions by the laboratory, which is blinded to clinical data. The minimum apelin detectable concentration differs from kit to kit. This study utilized the kit's minimum detectable concentrations of apelin, which were 0.031 µg/ml.

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Statistical Analysis

Statistical analysis was performed using STATA-15 (StataCorp, College Station, Texas, USA), and graphics were prepared using GraphPad Prism 8.3.2. Outcomes and independent variables were reported as means with standard deviation (SD) for continuous observation and numbers with percent for categorical variables. There were some significant observations noted in independent features between the 3 groups. Older participants, along with more males,

arterial hypertension, hypercholesterolemia, higher education, and obese participants,wererecorded in CGP and CGP+DM groups compared to healthy patients. To reduce the unequal selection of independent factors, multiple regression models were used to evaluate the effects of the CGP and CGP+DM group compared to the healthy patients' group on apelin, Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) by including all independent variables in the model.

Results

A total of 300 participants were enrolled in the study. Of these, 200 patients were of chronic generalized periodontitis.The study comprised three groups. Group 1 consisted of 100 healthy controls; Group 2 consisted of 100 patients with CGP, and group 3 included 100 patients with CGP+DM. Out of 300 samples, 181 were male, and 119 were female. No statistically significant ($p>0.05$) difference was found in gender distribution in all three groups. The prevalence of hypercholesterolemia in the participants with CGP (27%) and CGP+DM (26%) was higher as compared to the healthy ones (14%). A similar trend was also observed with arterial hypertension, where the participants with CGP (22%) and CGP+DM (20%) had a higher prevalence as compared to the healthy controls (8%). On the assessment of the Body Mass Index of the participants, almost all of the participants (100%) in the CGP+DM were obese as compared to 50% obese in the CGP group [Table 1].

Table 1: Baseline Characteristics of The Study Participants Among Three Groups.

	Healthy participants (n=100)	CGP (n=100)	CGP+DM (n=100)
Age	35.5±3.73	41.1±4.11	45.3±3.69
Sex			
Male	60(60.0%)	63(63.0%)	58(58.0%)
Female	40(40.0%)	37(37.0%)	42(42.0%)
Arterial hypertension	8(8.0%)	22(22.0%)	20(20.0%)
Hypercholesterolemia	14(14.0%)	27(27.0%)	26(26.0%)
Depression	2(2.0%)	1(1.0%)	8(8.0%)
Education			
Low education	34(34.0%)	0	0
High education	66(66.0%)	100(100%)	100(100%)
BMI, category			
Normal	30(30.0%)	15(15.0%)	0
Underweight			
Overweight	48(48.0%)	35(35.0%)	0
Obese	22(22.0%)	50(50.0%)	100(100.0%)

Notes: CGP: Chronic Generalized periodontitis, T2DM: Type 2 Diabetes Mellitus, BMI: Body Mass Index. Data were presented as mean±SD or number with percent in the parenthesis.

A comparative evaluation of the level of Apelin, CAL & PPD among chronic generalized periodontitis patients with and without T2DM. Group three (CGP+DM) shows the highest salivary apelin level compared to CGP patients without DM. It was 15.43±2.05ng/dl in the CGP group and 24.08±3.60ng/dl in CGP with DM (Figure 3A). The mean PPD was 3.9±0.55 in CGP patients and 3.6±0.49 in CGP with DM patients (Figure 3B). CAL was found more among CGP patients with DM (4.5±0.50) (Figure 3C). Figure 3 reveals a comparative evaluation of the level of Apelin, periodontal pocket depth (PPD), and clinical attachment level (CAL) among chronic generalized periodontitis patients with and without T2DM.

Multiple regression model showed that apelin was higher by 8.17 units (95% CI=7.29, 9.05; $p<0.001$) and 17.0 units (95% CI=15.8, 18.2; $p<0.001$) in CGP and CGP+DM patients respectively compared to healthy controls. The participants with arterial hypertension showed a significantly lower level of apelin (β=-1.49; 95% CI=-2.87, -0.10, $p=0.035$). Both the PPD and CAL increased by 1.73 units (95% CI=1.55, 1.91; $p<0.001$) and 2.49 units (95% CI=2.23, 2.75; $p<0.001$), respectively, in the CGP patients' group compared to healthy patients. Similarly, a significant increase was noted in PPD and CAL by 1.39 units (95% CI=1.15, 1.63; $p<0.001$) and 3.00 units (95% CI=2.64, 3.34; $p<0.001$), respectively, in CGP+DM group compared to healthy patients (Table 2). The obese patients had a higher CAL level thantypical BMI patients (β=0.28; 95% CI=0.001, 0.57; $p=0.049$).

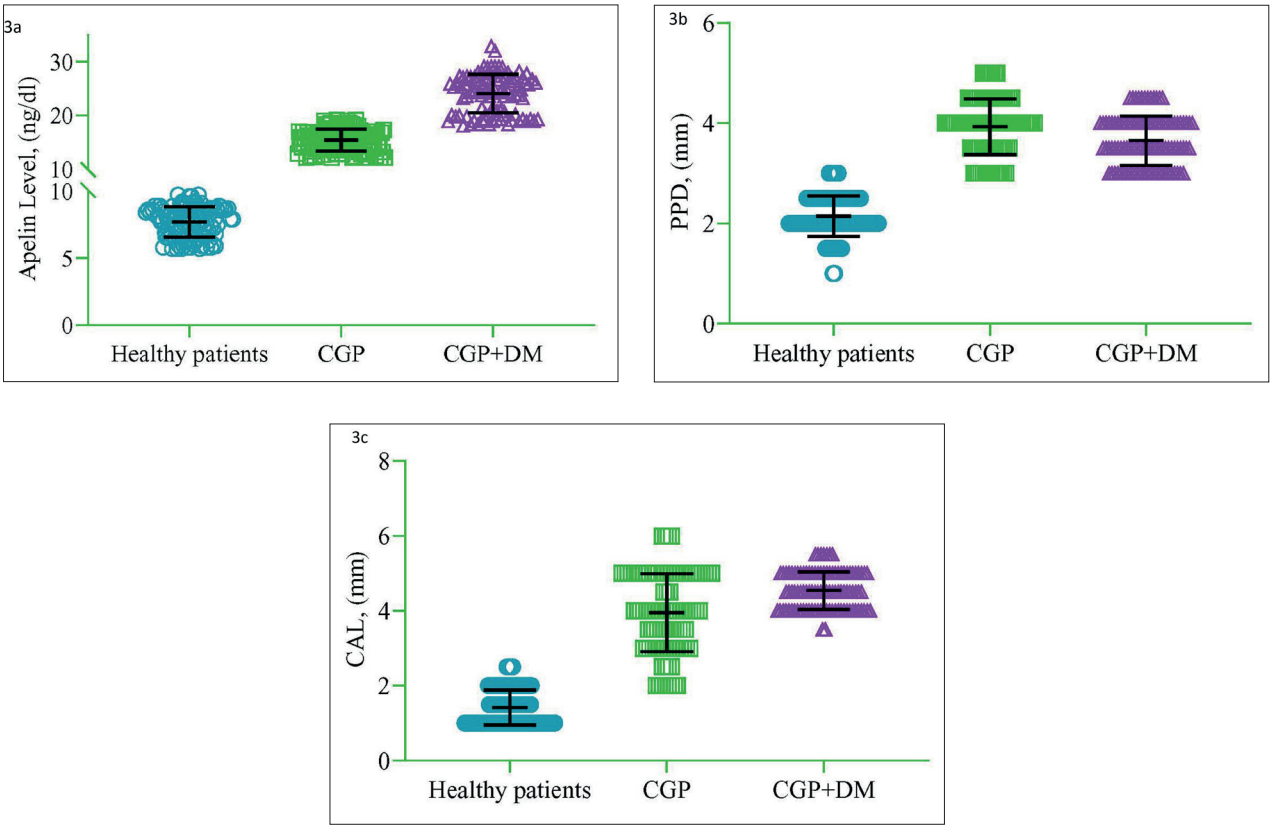

Figure 3 (a, b, and C): Comparative evaluation of Apelin, CAL & PPD among chronic generalized periodontitis patients with and without type II diabetes Mellitus. Notes: periodontal pocket depth (PPD), clinical attachment level (CAL), and chronic generalized periodontitis (CGP).

Discussion

This study aimed to evaluate the role of apelin adipokine in inflammation and insulin resistance. In chronic inflammatory conditions, these adipokines are crucial to immune regulation. The investigation of saliva is believed to be an excellent substitute for serum analysis [27, 28]. Saliva can be easily collected and surveyed; therefore, it can be used on a large scale for diagnostic purposes. Saliva comprises various biochemical molecules, including adipokine, easily detected in the oral cavity by active transport from the serum.

The present study compared the salivary apelinlevels in patients with periodontitis with or without diabetes and healthy controls. We observed that the group with periodontitis and diabetes had the highest salivary apelin level compared to other groups. A study by Vinitha et al. reported similar findings and observed that salivary apelin levels were significantly higher (p=0.014) in T2DM [29]. Sarhat et al., in their study, reported similar findings with an increase in salivary apelin level along with other molecules like IL-17 and vaspin in diabetic patients with chronic periodontitis (CP) [30].

Li et al., in their study, compared the basal apelin levels in normoglycemic individuals with patients with impaired glucose tolerance (IGT) and diabetics. They reported that apelin levels were significantly higher in patients with IGT and diabetes compared to the healthy controls ($p<0.05$ and $p<0.01$). They repeated the same test 2 hours after loading the patients with glucose and observed that the apelin levels were significantly higher than the basal levels in all the groups ($p<0.05$). Also, the apelin levels were found to be substantially correlated with HOMA-IR index (HOMA-IR), body mass index (BMI), free fatty acids, high-density lipoprotein, low-density lipoprotein, fasting blood glucose, and insulin level in simple regression analysis whereas multiple regression analysis showed that independent related factors like HOMA-IR, BMI, and total cholesterol were influencing plasma apelin levels [31]. The above findings from the study suggest that insulin, fasting/ feeding state, blood glucse, and cholesterol might

Table 2: Comparison of Apelin, CAL & PPD among chronic generalized periodontitis patients with and without type II diabetes Mellitus compared to healthy patients.

	Apelin		PPD		CAL	
	β-Coff (95% CI)	p-value	β-Coff(95% CI)	p-value	β-Coff(95% CI)	p-value
Healthy patients	Ref.		Ref.		Ref.	
CGP	8.17(7.29, 9.05)	<0.001	1.73(1.55, 1.91)	<0.001	2.49(2.23, 2.75)	<0.001
CGP+DM	17.0(15.8, 18.2)	<0.001	1.39(1.15, 1.63)	<0.001	3.00(2.64, 3.34)	<0.001
Age	-0.08(-0.15, -0.001)	0.049	0.003(-0.01, 0.02)	0.687	-0.01(-0.03, 0.01)	0.397
Sex						
Female	Ref.		Ref.		Ref.	
Male	0.08(-0.58, 0.66)	0.795	0.02(-0.09, 0.14)	0.679	-0.08(-0.25, 0.10)	0.389
Arterial hypertension						
Absent	Ref.		Ref.		Ref.	
Present	-1.49(-2.87, -0.10)	0.035	0.03(-0.25, 0.31)	0.818	-0.21(-0.61, 0.20)	0.311
Hypercholesterolemia						
Absent	Ref.		Ref.		Ref.	
Present	1.92(0.69, 3.15)	0.002	-0.13(-0.38, 0.11)	0.284	0.08(-0.28, 0.44)	0.652
Education						
Low education	Ref.		Ref.		Ref.	
High education	-0.41(-1.44, 0.62)	0.975	0.06(-0.15, 0.26)	0.578	0.13(-0.17, 0.43)	0.389
BMI category						
Normal	Ref.		Ref.		Ref.	
Overweight	0.01(-0.89, 0.92)	0.975	0.08(-0.10, 0.26)	0.375	0.05(-0.21, 0.31)	0.708
Obese	0.34(-0.64, 1.32)	0.497	0.15(-0.05, 0.35)	0.134	0.28(0.001, 0.57)	0.049

A multiple regression model was used to estimate the p-value.

ascendancy the apelin concentration by modulating its expression and secretion.

Additionally, the adipose tissue levels in the body can serve as an independent parameter that can influence the apelin levels. The authors proposed that apelin might have arole in the pathogenesis of IR and T2DM with these diverse evidence [31]. Our previous study also reported similar results, where the serum apelin levels were evaluated. The group with T2DM and periodontitis had the highest apelin levels compared to other groups [32].

Another review explained the correlation of polymorphisms in genes encoding inflammatory cytokines and the increased level of these pro-inflammatory markers associated with chronic pathologic conditions in T2DM [33]. One Egyptiancase-controlstudy reported that Apelin levels in people with diabetes were raised when associated with the renal disorder, pre-diabetic cases, and compromised lipid profile. Thereby holding up the tie-inlinking the apelinergic network and diabetic nephropathy [34]. Multiple studies reported that apelin accumulation was raised in diabetic patients, especially among patients with poor kidney physiology, prolonged disease span, insulin resistance, elderly community, and BMI [35-37]. The jump-up of apelin was higher in type 1 than in T2DM patients. The negative interdependence with glycosylated hemoglobin (HbA1c) in patients with T2DM is able to stipulate that apelin takes part inresponsivity in glycemicequilibrium and even insulin sensitivity [35] (Figure 4). Activated AMPK stimulates energy-generating processes such as glucose uptake and fatty acid oxidation [38]. Apelin increases glucose transport in vitro inside muscle cells via the adenosine monophosphate-activated protein kinase pathway through nitric oxide synthase (NOS) inhibitors [39]. NOS inhibitors can reduce glucose uptake in vivo in muscle cells but not in vitro [40]. Another study utilizing in vivo, ex vivo, and in vitro methods revealed that apelin increases myocardial glucose uptake through an AMPK pathway [41]. In the intestinal lumen, apelin has been found to promote AMPKα2 phosphorylation and an increase in the synthesis of GLUT2/SGLT-1 proteins

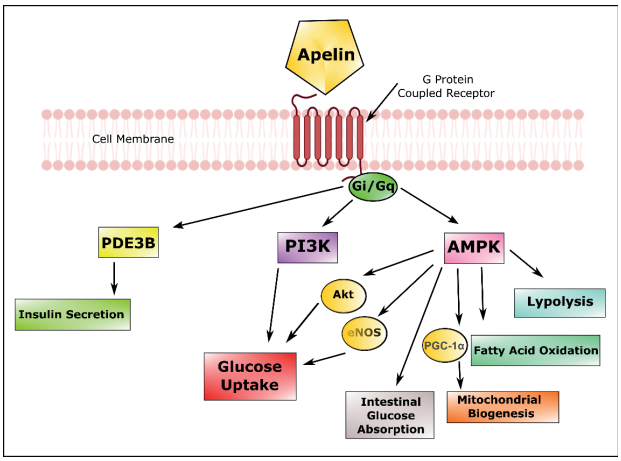

Figure 4: Metabolic Effects of Apelin. Gi/Gq- G protein; PDE3B- Phosphodiesterase 3B; PI3K- Phosphoinositide 3-kinases; AMPK- Adenosine monophosphate-activated protein kinase; Akt- Ak strain transforming (Protein kinase B); eNOS- Endothelial nitric oxide synthase; PGC-1α- Peroxisome proliferator-activated receptor gamma coactivator 1-alpha. ***Image Credit**: Susmita Sinha.*

in the brush border membrane of the gut leading to an altered ratio of SGLT-1: GLUT2 hence enhancing the carbohydrate flux through enterocytes [42]. An additional study suggested that glucose entry in the intestine induces apelin secretion [43].

Another study reported that apelin is the kind of adipokine crucial in regulating glucose levels. Apelin is released in reduced levels or without insulin, increasing the glucose uptake in vital tissues and thus decreasing the glucose level [44]. Hence, in patients with DM, higher levels of apelin are documented. Plenty of research work in recent years shows that periodontitis is an inflammatory response to periodontal pathogens present in oral biofilm - host response to these toxins from microorganisms in oral biofilm results in the destruction of the periodontium. Periodontitis is a polymicrobial disease, so mixed species likely stimulate carcinogenesis in the oral cavity and various extra oral diseases like cancer [45]. As a principal pathogen of periodontitis, Porphyromonas gingivalis can damage local periodontal tissue and evade the host immune system, affecting systemic health sooner or later [46, 47].

We tried to relate a significant salivary apelin level in periodontitis inflammatory disease with inflammatory conditions. Koguchi et al., in their study, postulated a possible anti-inflammatory role of apelin and observed that apelin could decrease the expression of pro-inflammatory cytokines like TNF-α and IL-1β protein as demonstrated in the preclinical setting [48]. It has been observed that apelin levels are significantly elevated in inflammatory diseases. Another research showed that apelin infusion reduces aneurysm formation by decreasing the macrophage burden and pro-inflammatory cytokines [49]. Further, it has been demonstrated that apelin is a potent vasodilator and can be used as a therapeutic agent in chronic lung disease [50]. Apelin has also been found to be crucial role in cardiac physiology concerningcontractility, vasodilation, and minimizing vascular wall inflammation [51]. A systematic review and meta-analysis conducted by Akbari H et al. observed that the levels of apelin in patients with cardiovascular diseases were found to be significantly lower than the normal individuals [52]

Apelin, a multisystem regulator peptide, modulates the function of cardiovascular, gastrointestinal, hypothalamus axis, and immune pathways. It also regulates the lipid metabolism and functioning of adipose tissue by enhancing the mRNA levels of uncoupling protein 1 (Ucp1), which is a marker of energy spending in brown adipose tissue, and mRNA levels of Uco3, which is a modulator of fatty acid transport in skeletal muscle [53, 54].

Contrary to all the studies above, other research revealed that apelin might not be a significant marker for inflammation and cardiovascular diseases [55]. If further research is conducted the outcome and performance of apelin association with DM and CGP may found more complicated. We suggest new studies to find a possible correlation between apelin and inflammation.

In our investigation, apelin was correlated to all the groups, but salivary apelin level was highest in individuals in group three (CGP+T2DM). Periodontal pathogens and the susceptible host are required to initiate periodontal diseases. Inflammatory mediators also play an essential role in T2DM, adding to hyperglycemia-induced IR and leading to diabetic complications [56].

The current study also reported that the mean PPD was 3.9±0.55 in CGP patients and 3.6±0.49 in CGP with DM patients. CAL was found more among CGP patients with DM (4.5±0.50). In support of the current study, Abdul-Wahab et al. reported that T2DM and uncontrolled sugar levels have a negative impact on periodontal health, and clinical periodontal parameters like PPD and CAL were found to be

significantly higher in the group of CGP and T2DM as compared to other groups [57].

Chronic pathologic conditions in T2DM and increased levels of IL 1 β, IL 6, TNF α, and IL 18 pro-inflammatory markers are observed because of polymorphisms in genes encoding these inflammatory cytokines [33]. An increase in the production of these inflammatory cytokines contributes to the rise in PPD and ultimately leads to bone loss. Disruption of the balance between bacteria and host response leads to the initiation of periodontal disease and, eventually, bone loss and tooth loss [58-60]. Periodontal ligament (PDL) is a crucial source of progenitor cells which can convert into bone-forming cells like osteoblast; hence, the loss of these progenitor cells of PDL by apoptosis is responsible for an increase in the risk of disease development. Periodontitis is considered a sixth complication [61] of diabetes, and its prevalence is higher in diabetic patients compared to non-diabetic subjects [62]. In diabetes, following bacterial infection, increased bone loss is seen as bone resorption overpowering bone formation because of the apoptosis of bone lining cells [63, 64]. New bone formation in periodontal disease is hampered because of the apoptosis of osteoblastic cells [65, 66]. Concomitant immune inflammatory responses by periodontal pathogens are responsible for severe periodontal disease in the hyperglycemic state of diabetes. Hence, diabetes has been very closely related to the development of periodontal diseases with a simultaneously increased propensity of infection by the periodontal microbes (Figure 5).

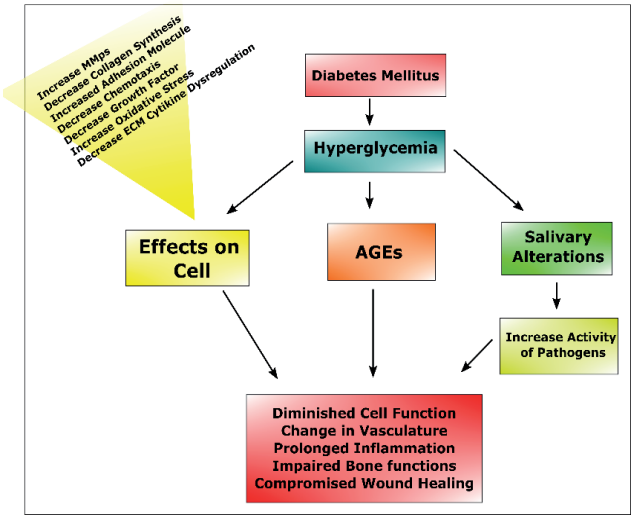

Figure 5: Consequences caused by altered salivary apelin in diabetes mellitus. ECM- Extracellular matrix; MMPs- Matrix metalloproteinase; AGEs- Advanced glycation end-products. ***Image Credit**: Susmita Sinha*.

Conclusion

In conclusion, the salivary apelin level was expressed highest in patients with CP+T2DM compared to patients without diabetes and healthy controls. Apelin might be a crucial modulating factor in IR and T2DM. Apelin induced anti-inflammatory and increasing insulin sensitivity effects, tends to modulate inflammation and glucose levels in patients with uncontrolled blood glucose. Hence, apelin can be used for the assessment of the risk of the development of periodontal disease.

Authorship Contribution

All authors made a substantial input to the assignments reported, regardless that in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted, and agreed to be accountable for all aspects of the work, including any issues related to accuracy or integrity.

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Institutional Review Board Statement: This research obtained Institutional Review Board (IRB) approval of Karnavati Research Ethics Committee, Karnavati School of Dentistry (Reference No.: KU/20/KSD247, Date: February 18, 2021), Uvarsad-Adalaj Road At. & PO.: Uvarsad, District: Gandhinagar, Gujarat 382422, India.

Informed Consent Statement: The subjects verbally explained the aim and purpose of the study. Additionally, patients who participated in this were volunteersand possessed the right not to participate in the study. Those cases only provided written informed consent were included in the study.

Data Availability Statement: Further data regarding the study is available upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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