EFFECT OF AUTOLOGOUS PLATELET RICH PLASMA ON NON-SURGICAL PERIODONTAL THERAPY FOR PERIODONTITIS IN SELECTED PAKISTANI PATIENTS WITH UNCONTROLLED TYPE-II DIABETES MELLITUS

YOUSAF ATHAR

UNIVERSITI SAINS MALAYSIA

2023

EFFECT OF AUTOLOGOUS PLATELET RICH PLASMA ON NON-SURGICAL PERIODONTAL THERAPY FOR PERIODONTITIS IN SELECTED PAKISTANI PATIENTS WITH UNCONTROLLED TYPE-II DIABETES MELLITUS

by

YOUSAF ATHAR

Thesis submitted in fulfilment of the requirement
for the degree of
Doctor of Philosophy

December 2023

ACKNOWLEDGEMENT

I would first like to thank Allah, the most gracious the most merciful, I relied on him completely, and he guided me throughout my life to a level, I could not have reached without his will.

I express my appreciation to my main supervisor; Dr. Siti Lailatul Akmar, for her persistent motivation, support, and leadership throughout my research project. My sincere and special gratitude to my co-supervisors; Dr. Basarudin and Prof. Dr. Qasim Saeed, for guidance and continual support and advice throughout my study.

My sincere special thanks to all staff at the Periodontics department and Diabetic clinic, oral pathology laboratory and other administrative staff at Institute of Dentistry, CMH, Lahore medical college and School of Dental Science, Universiti Sains Malaysia.

The successful completion of this research project owes credit to my family and friends. To my parents, wife and my children who loved and supported me through the course of my degree. This project owes heaps of gratitude Dr Bilal Mirza and Dr Anas Imran, Dr Ahmed Zia and Mr Shaukat, as their support and encouragement was invaluable in achieving this goal.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

ABL	Alveolar bone loss
AGE	Advanced glycation end products
APC	Autologous platelet concentrate
BFGF	Basic fibroblast growth factor
BT-LAB	Bio test Laboratory
CBCT	Cone beam computerized tomography
CEJ	Cemento enamel junction
CGF	Concentrated growth factor
CMH	Combined Military Hospital
CRP	C-reactive protein
DFU	Diabetic foot ulcer
DM	Diabetes mellitus
DP	Diabetes associated periodontitis
EDGF	Epithelial derived growth factor
EGF	Epidermal growth factor
ELISA	Enzyme linked immunosorbent assay
GCF	Gingival crevicular fluid
GF	Growth factor
GI	Gingival Index
GR	Gingival recession
GTR	Guided tissue regeneration
HbA1c	Glycated Heamoglobin
HMT	Host modulatory therapy
IGF	Insulin like growth factor
IL-1β	Interleukin 1-beta
IOD	Institute of Dentistry
i-PRF	Injectable platelet rich fibrin
LDL	Low density glycoproteins
LPS	Lipopolysaccharides
mg	milligram
ml	Milliliter

m-RNA	Messenger ribonucleic acid
NDSP	National Diabetes survey of Pakistan
NF-κB	Nuclear factor kappa
NK	Natural killer
NSPT	Non-surgical platelet therapy
PD	Pocket depth
PDAF	Platelet derived angiogenesis factor
PDEGF	Platelet derived epithelial growth factor
PDGF	Platelet derived growth factor
PDT	Photo dynamic therapy
PGE2	Prostaglandin E2
P-Gingivalis	Porphyromonas Gingivalis
PI	Principal investigator
PMNs	Polymorphonuclear leukocytes
PPD	Periodontal pocket depth
PPD	Periodontal pocket depth
PPP	Platelet poor plasma
PRF	Platelet rich fibrin
PRP	Platelet rich plasma
RAGE	Receptor of advanced glycation end products
RANKL	Receptor activator of nuclear factor Kappa-B ligand
RBC	Red blood cells
SD	Standard deviation
SRP	Scaling root planing
T2D	Type 2 diabetes
TGF-β	Transforming growth factor
TNF-α	Tumor necrosis factor-α
USM	Universiti Sains Malaysia
VEGF	Vascular endothelial growth factor

LIST OF APPENDICES

KESAN PLASMA KAYA PLATELET AUTOLOGOUS PADA TERAPI PERIODONTAL TANPA PEMBEDAHAN UNTUK PERIODONTITIS DENGAN DIABETES MELLITUS JENIS-II TIDAK TERKAWAL DALAM PESAKIT PAKISTAN TERPILIH

ABSTRAK

Penyakit periodontium yang merupakan "komplikasi keenam diabetes mellitus", sekali gus menjadikannya komplikasi oral diabetes yang paling biasa dalam kalangan pesakit diabetes. Rawatan untuk penyakit periodontium merangkumi pelbagai pendekatan iaitu melalui kaedah pembedahan dan tanpa pembedahan. Gangguan metabolik dalam pesakit diabetes boleh menurunkan daya tahan immuniti mereka terhadap jangkitan dan juga boleh memberi kesan kepada pembentukan dan perkembangan penyakit periodontium. Penggunaan platelet autologous pekat berpotensi digunakan sebagai terapi konservatif terhadap rawatan periodontium. Objektif kajian adalah untuk menilai kesan penggunaan plasma kaya platelet (PRP) dalam poket periodontium yang dalam, berikutan terapi periodontium tanpa pembedahan (NSPT) pada pesakit yang menghidap penyakit diabetes jenis II yang tidak terkawal. Kumpulan kajian terdiri daripada dua kumpulan, iaitu pesakit yang menerima terapi periodontium tanpa pembedahan dengan penggunaan plasma kaya platelet (PRP) dalam dua kepekatan berbeza dan kumpulan kawalan yang menerima NSPT sahaja. Perbandingan antara kumpulan tersebut telah dilakukan pada selang 45 dan 90 hari. Perbandingan tersebut mengambil kira parameter periodontium seperti kedalaman poket periodontium, kehilangan tulang alveolar dan indeks gingival serta sitokin pro-inflamasi, Tumor Necrosis Factor Alpha (TNF-α), tahap interleukin 1β (IL-

1 β) dan paras protein C-reaktif. Hasil kajian menunjukkan umur purata keseluruhan adalah 50.17 dengan SD = 7.13. Kedua-dua kumpulan intervensi menunjukkan peningkatan yang ketara secara statistik pada bahagian ketinggian tulang alveolar. Kumpulan PRP 1 menunjukkan peningkatan maksimum pada paras tulang alveolar. Paras HbA1c yang lebih rendah dicatatkan selepas NSPT dan penggunaan PRP tambahan. Kumpulan PRP didapati telah mengurangkan paras protein C-reaktif. Pengurangan tahap sitokin pro-inflamasi dalam plasma dan peningkatan parameter periodontium ini menunjukkan persekitaran yang kondusif bagi penyembuhan tisu periodonttium dan meningkatkan pengawalan diabetes.

EFFECT OF AUTOLOGOUS PLATELET RICH PLASMA ON NON-SURGICAL PERIODONTAL THERAPY FOR PERIODONTITIS IN SELECTED PAKISTANI PATIENTS WITH UNCONTROLLED TYPE-II DIABETES MELLITUS

ABSTRACT

The susceptibility to periodontal disease is often cited as the "sixth complication of diabetes mellitus" making it the most common oral complication of diabetes. Treatment for periodontal disease includes various non-surgical and surgical approaches. The metabolic disturbances in diabetic patients may lower their resistance to infections and may help in both initiation and progression of developing inflammatory periodontal disease. The use of autologous platelet concentrates has exhibited considerable promise as a conservative therapy towards periodontal treatment. The objective of the study was to evaluate the effects of platelet rich plasma placement in deep periodontal pockets, following non-surgical periodontal therapy (NSPT) in patients with standardized uncontrolled type II diabetes. The study groups comprised two patient groups receiving non-surgical periodontal therapy and platelet rich plasma in two different concentrations and a control group receiving NSPT only. An inter and Intra group comparison of Periodontal parameters including periodontal pocket depth, alveolar bone loss and gingival index along with pro-inflammatory cytokine, Tumour Necrosis Factor Alpha (TNF-α), interleukin 1β (IL-1 β) levels and C-reactive protein levels was conducted at 45- and 90-day interval. Overall average age was 50.17 with SD = 7.13. Both interventional groups exhibited statistically significant gain in bone height. PRP 1 group showed maximum gain in alveolar bone levels. A trend towards lower HbA1c levels was shown after NSPT and adjunctive

PRP placement. The PRP groups exhibited lower the levels of circulating C- reactive protein. This reduction in levels of pro-inflammatory cytokines in plasma and improved periodontal parameters indicated a conducive environment towards periodontal tissue healing and improved diabetic control.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Diabetes mellitus (DM) and periodontal disease, attacks the host tissue cell through inflammatory pathways. The disease alters the functions of immune cell, by increasing the pro-inflammatory cytokines in monocytes and Polymorphonuclear leukocytes (PMNs) and decreasing the growth factors in macrophages, leading to long-term inflammation, accelerating tissue breakdown and decreased tissue repair ability (Iacopino (2001); Portes *et al.*, 2021). The resulting susceptibility to periodontal disease is often cited as the "sixth complication of diabetes mellitus" making it is the most common oral complication of diabetes (Matos *et al.*, 2018). Periodontal disease starts with gingivitis initially but due to the poor glycaemic control and altered immune function progresses to advanced stage (Chapple *et al.*, 2013).

Treatment for periodontal disease includes various non-surgical and surgical approaches which has exhibited a decrease in the dosage of insulin required among the subjects with type I and II DM (Moeintaghavi *et al.*, 2012).

Between 2.8 to 3.4 times higher risk of developing periodontitis has been reported in Bahrain for diabetic patients compared to non-diabetics (Abdulla, 2018). The advanced phase of periodontal therapy in cases of Periodontitis includes surgical interventions like open flap debridement procedures, but consequently the risk of infection is immense in uncontrolled diabetics (Baeza *et al.*, 2020), along with a delayed post-surgical healing with higher chances of tissue necrosis (Jain *et al.*, 2020).

Metabolic disturbances in diabetic patients may lower their resistance to infections and may help in the initiation and progression of developing inflammatory

periodontal disease. Several factors such as defective leukocyte chemotaxis, T-cell immunity, bactericidal capacity, phagocytosis and disruption of epidermal and fibroblasts cell functions causes delayed healing and insufficient bacterial clearance in the diabetic wound (Cruz-Pineda *et al.*, 2022; Guo and DiPietro, 2010). The addition of hypoxia also contributes to the impair healing causing inadequate production of angiogenesis in diabetic wounds (Patel *et al.*, 2019)

In many clinical regenerative applications such as dermatology (Emer, 2019), hair rejuvenation and transplant (Elghblawi, 2018), diabetic foot ulcer and maxillofacial surgery (del Pino-Sedeño *et al.*, 2019) (Fan *et al.*, 2020), the use of autologous platelet concentrates (APC) in high concentration has become a successful source of multiple growth factors. The growth factors stored in the granules of blood-derived platelets are released and contribute in the healing of bone when activated. Several randomized control trials successfully reported the post-operative soft and hard tissue healing, bone formation and gingival attachment with the use of APC's (Del Fabbro *et al.*, 2011; Panda *et al.*, 2019; Picciolo *et al.*, 2020). Soft and hard tissue healing, and regeneration is initiated by blood clots after surgery. Platelets present in the blood clot release the growth factor which initiate the wound healing (Opneja *et al.*, 2019). Due to autologous nature of APCs, there is no risk of immune reaction or disease transmission to the patient. In addition, it also decreases the post-operative bleeding and promotes a faster vascularization in the soft and hard tissues. With the use of platelet-rich plasma, body's natural mechanism of wound healing is accelerated (Feigin and Shope, 2019). Treatment of intra-bony defects (Hanna *et al.*, 2004) as well as treatment of mandibular continuity defects (Marx, 2004) in tumour cases has been shown to benefit from the use of platelet rich plasma (PRP) applications in the past. Peri-implant defects have been known to receive treatment in the form of PRP preparation (Song *et al.*, 2019). PRP has

anti-inflammatory properties which lead to a decreases in the inflammation and accelerates the healing process resulting in a decrease the pocket depth and increase the attachment gain (Ameer *et al.*, 2018).. PRP application in periodontal regenerative therapy possesses definite potential although the concept has not been investigated as an adjunct to non-surgical periodontal therapy in diabetics.

The aim of this study was to assess the practical benefits or side effects of a platelet rich plasma placement in residual deep periodontal pockets following non-surgical periodontal therapy (NSPT) in patients with moderately uncontrolled type II diabetes and periodontitis.

This study involved a comparison between selected uncontrolled type II diabetes patients suffering from periodontitis, receiving scaling and root planning (SRP) and patients receiving SRP along with autologous PRP injections. The study investigated the possible positive influence PRP therapy can exert over periodontal tissues as well as glycaemic control of the patient. Periodontal parameters including periodontal pocket depth (PPD), alveolar bone loss (Amable *et al.*), and gingival index (GI) were noted to study the health of periodontal tissues. Pro-inflammatory cytokine, Tumour Necrosis Factor Alpha (TNF-α), interleukin 1-β (IL-1 β) levels, along with Glycated haemoglobin (HbA1c) and C-reactive protein (CRP) levels were assessed over specified time periods to check for shift between health and disease.

Since the mechanisms of action of wound healing is still not fully understood leaving a wide scope for new discoveries to be made in the branch of transfusion medicine. Therefore, more clinical research is needed to investigate the long-term effects and role of PRP preparations used as an adjunct in the treatment of wound healing.

Neither PRP infiltration nor application of PRP for better healing is unheard of but its application in a setting with uncontrolled type II diabetics suffering from Periodontitis has yet to be studied. These two distinct conditions possess a well-documented two way relationship (Preshaw *et al.*, 2012). The development of such innovations requires the following of ideal framework of surgical innovation whereby such regimens need to pass through idea and development stage before entering exploration and development stage (McCulloch *et al.*, 2013).

1.2 Justification of study and problem statement

The use of platelet driven growth factors for improved periodontal healing in uncontrolled diabetics undergoing non-surgical periodontal therapy had yet to be studied. The bi-directional relation of periodontitis and type II diabetes recognizes the synergy of these two conditions towards tissue destruction. Present study finds its justification in the same bi-directional relation with the aim of treating one condition with the other condition improving consequently. An investigation into such innovations ideally requires conduction of clinical trials, following the ideal framework of surgical innovation to pass through such ideas and exploration stage before entering exploration and clinical trial stage (McCulloch *et al.*, 2013).

1.3 General objective

To determine the impact of autologous platelet rich plasma application following non-surgical periodontal therapy for Periodontitis in selected uncontrolled type-II diabetes mellitus patients.

1.3.1 Specific objectives

1. To compare the changes in clinical periodontal parameters in selected uncontrolled type II diabetic patients receiving non-surgical periodontal therapy and patients receiving Platelet rich plasma application as an adjunct to non-surgical periodontal therapy.

2. To compare the changes in plasma levels of biochemical markers HbA1c and C-reactive protein in selected uncontrolled type II diabetic patients receiving non-surgical periodontal therapy and patients receiving Platelet rich plasma application as an adjunct to non-surgical periodontal therapy.

3. To compare the changes in plasma levels of pro-inflammatory cytokines IL-1 and TNF-α in selected uncontrolled type II diabetic patients receiving non-surgical periodontal therapy and patients receiving Platelet rich plasma application as an adjunct to non-surgical periodontal therapy.

4. To compare the changes in periodontal parameters, biochemical markers and pro-inflammatory cytokine levels between two different concentrations of PRP preparations when applied in selected uncontrolled type II diabetic patients following non-surgical periodontal therapy.

1.4 Research question

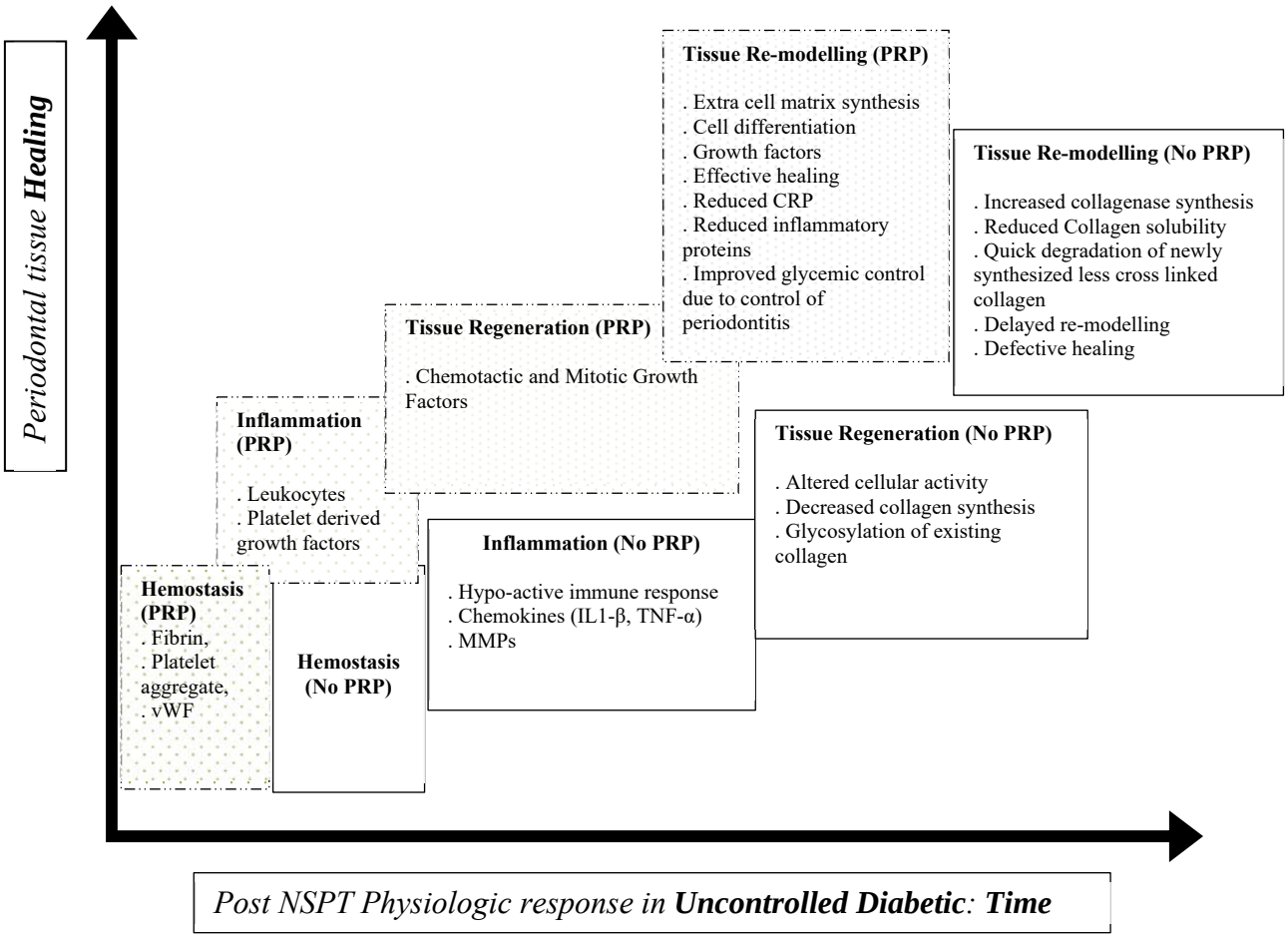
1. Does platelet rich plasma application exhibit better healing of periodontal tissues when employed in selected uncontrolled type II diabetic patients following non-surgical periodontal therapy for Periodontitis?

2. Would the positive effects from use of PRP following non-surgical periodontal therapy impact glycaemic control of the moderately uncontrolled diabetic patient through resolution of periodontal disease?

1.5 Research hypothesis

Non-surgical periodontal therapy followed by PRP application to healing periodontal tissues would result in improved periodontal healing for patients suffering from moderately un-controlled type II diabetes compared to non-surgical therapy alone.

1.6 Conceptual framework



CHAPTER 2

LITERATURE REVIEW

2.1 Diabetes and periodontium

The state of chronic hyperglycaemia due to diabetes disorder causes long term damage to several organs like heart (Rajbhandari *et al.*, 2021), eyes (Harman-Boehm *et al.*, 2007), kidneys (Gäckler *et al.*, 2013), nerves (Yagihashi *et al.*, 2011), and vascular system (Yamawaki, 2011). Due to the destruction of pancreatic b-cells by body's own self-defence system causes cessation of insulin production resulting in type I diabetes in patients. Type II diabetes occurs due to the resistance of insulin at the target cells, that is being produced by the body (Mealey, 2006; Ozougwu *et al.*, 2013).

In periodontal disease, the presence of bacterial microflora in subgingival level is necessary to initiate the disease, and the research found no or insignificant difference between the bacterial flora of a diabetic and non-diabetic patient (Ganiger *et al.*, 2019). Therefore this insignificant difference suggest that the major role in the mechanism of disease is caused by the alteration in the host-inflammatory system that aggravates the destruction in the periodontium seen in diabetes patients (Deshpande *et al.*, 2010). Alteration in the mechanism of neutrophils, macrophages and monocytes is seen in diabetic patients (Deshpande *et al.*, 2010). Due to the defective function of immune cells like neutrophils incomplete adherence, faulty chemotaxis, and phagocytosis bacterial cell are not killed in the periodontal pockets and causes destruction in the periodontium of a diabetic patient (Nicu and Loos, 2016). Mostly in diabetes where the role of neutrophil is declined, the function of monocyte/macrophage cell line is seen exaggerated, by significantly escalated production of pro-inflammatory cytokines and cell mediators in response to bacterial antigens (Naguib *et al.*, 2004; Salvi *et al.*, 1997). Monocytes found in the peripheral bool samples of diabetic patients have also observed

to produced high levels of tumour necrosis factor-alpha (TNF-a) in reaction to antigens from *Porphyromonas gingivalis* in contrast to samples from non-diabetic subject group (control) (Dina *et al.*, 2012). This TNF stimulation was directly proportional to the inflammatory response and independent of pathogenic inoculation in the periodontium. The increased level of these inflammatory mediators was also seen in the gingival crevicular fluid of diabetic patients (Barros *et al.*, 2016). The quantity of inflammatory mediators in the gingival crevicular fluid was directly proportional to the glycaemic control level of diabetes.

Diabetic patients have also exhibited the high levels of periodontal attachment and bone loss due to the alteration in the function of connective tissue metabolism that impair the resorptive and formative process of connective tissue. Defect in the osteoclastic and osteoblastic activity is also seen in association to the increased level of glucose in diabetic patients (Wongdee *et al.*, 2017). The osteoblastic cell proliferation and collagen production decreases and results in less bone formation and weak mechanical properties of new bone (Jiao *et al.*, 2015).

2.2 Impact of Diabetes Mellitus type II on periodontal disease

Diabetes and periodontal disease are believed to possess a bi-directional relationship. The influence of Diabetes on immune cells and resulting pro-inflammatory products increase the susceptibility of inflammation-based diseases like periodontitis. A patient suffering from uncontrolled diabetes possesses an even greater risk of developing periodontal disease, which initiates with gingivitis and compounded with poor glycaemic control, progresses to advanced periodontal disease. An analysis of multiple variables confirms diabetes as a risk factor for periodontitis with subjects having a 2.8 - 3.4 fold higher chance of presenting periodontitis compared to non-

diabetic subjects post adjustment for the impact of confounding variables for example age, gender, and oral hygiene measures (Abdulla, 2018).

Even though there is sufficient literature present stating the relationship of chronic systemic disease with oral disease conditions but the association between the periodontal disease and DM is the most recorded (Acharya *et al.*, 2010; Grover and Luthra, 2013; Mealey and Oates, 2006).

The relationship of diabetes and periodontitis has been previously studied specifically the vascular variation such as in gingival microangiopathy (Listgarten *et al.*, 1974), granulocyte hypo function (Manouchehr-Pour *et al.*, 1981), changes in oral microflora (Zambon *et al.*, 1988), and increased gingival collagenase activity (Sorsa *et al.*, 1992). However, these studies were able to highlight the basic changes at a local tissue level but a greater understanding of the two conditions at a systemic level needed to be comprehended.

Research conducted by Williams and colleagues revealed a decrease in insulin requirements after gingivectomy in 7 diabetic patients, these results have aroused the interest among young researchers (Williams and Mahan, 1960). The type, duration and the metabolic control are the significant variants for diabetic patients, while for periodontal patients bleeding from the gingiva, calculus deposition, probing depth, and attachment loss are the important factors.

The state of diabetes disrupts the gingival fibroblast production of the collagen and glycosaminoglycan, increases the collagen breakdown activity in crevicular fluid resulting in the loss of periodontal fibres, alveolar bone support and finally loosening and shedding of the tooth from the socket (Choi, 2011). It also impairs the functions of immune cell, by increasing the production of pro-inflammatory mediators from

monocytes and polymorphonuclear leukocytes and decreasing the growth factors from macrophages, predisposing the host system to chronic inflammation, increasing the tissue breakdown and decreasing the tissue repair capacity (Iacopino 2001).

Research suggests that patients with well-controlled diabetes are at no risk of having gingivitis or periodontitis similar to the people with no diabetes at all, however patients with poorly controlled diabetes are at high risk of inflammation of gingiva and destruction to supporting bone levels (Llambés *et al.*, 2015; Nazir, 2017). It comes as no surprise that the diabetic individuals have macroscopic as well as microscopic changes, resulting in compromised wound healing, increased monocyte response to microbial antigens and impaired neutrophil chemotactic reaction, leading to exaggerated local tissue destruction (Wassall, 2011). The composition of bacterial biofilm is significantly the same in individuals with and without diabetes with the exception of *P. Gingivalis* (Casarin *et al.*, 2013).

Additional evidence emerged linking a decrease in the production of fibroblastic and osteoblastic cells due to the increased activity of apoptosis in a hyperglycaemic state in reaction to *P. gingivalis* bacterial infection (He *et al.*, 2004; Liu *et al.*, 2006). These matrix-producing cells are an integral part of periodontium repair system. The combination of decreased quantity of proliferation and differentiation and exaggerated levels of apoptotic cell death can be the reason of severe periodontal attachment loss in diabetic patients. This could be due to the imbalance in the connective tissue metabolism and degradation of the attachment apparatus of the periodontium. Due to high glucose levels in the blood, the glucose level in the crevicular fluid of gingiva also increases, suggesting it to be directly proportional to each other (Barros *et al.*, 2016). Periodontal pockets are a consistent site of injury due to bacterial infiltration, and a

balanced wound-healing system is an essential part to maintain the tissue health. However, the elevated plasma glucose levels impaired the wound-healing capabilities of the fibroblasts in the periodontal pockets, resulting in the attachment loss of the periodontium (Chiquet *et al.*, 2015).

The microvascular structural changes in the vasculature of blood vessels by the impaired growth and regeneration of blood vessels is known as diabetic angiopathy, in diabetic patients. The diabetic angiopathy specially targets the end organs like retina, and glomerulus, also effect the periodontium in a similar way, making it a complication associated with diabetes (McCrimmon *et al.*, 2012; Mealey and Rose, 2008).

2.3 Impact of periodontitis on diabetic control

The periodontal tissue holds a very distinctive position in the human body. For example, in patients with edentulous jaws the bacterial microbiota which is present in the oral cavity does not pose any risk to the host tissue, when compared to the dentate patients (de Waal *et al.*, 2014).

The teeth alter the unique ecological system present in the oral cavity. This situation is similar to the situation when a catheter is placed intravenous through the skin surface. Previously, without the catheter, the intact skin surface poses no risk but when the catheter is placed, it becomes a sight of microbial species, and pose a high risk for infections. In the oral cavity the space between gingiva and tooth is known as gingival sulcus when healthy, but during inflammation, it's known as periodontal pocket. This space is critical for the balance between bacterial microbiota and host defence system. The presence of bacterial colonization residing in this gingival sulcus along the surface of tooth is mostly Gram-negative and anaerobic bacteria with causes Periodontitis (How *et al.*, 2016). There can be more than 400 bacteria species present

in the oral cavity in gingival sulcus during the diseased state (How *et al.*, 2016). Therefore, a balanced host defence system capable of wound healing is essential for the preservation of the host tissue and prevent the systemic invasion of these bacteria. Other bacterial products are also disseminated into the pockets of periodontium which include bacterial endotoxins, organic acids and chemotactic peptides (Kinane and Bartold, 2007). If this situation is not treated on time, acute periodontal disease can result in the ulceration of the epithelium of periodontal pockets which can range from 8 to 20 cm^2, approximately an adult hands size proportion (Hujoel *et al.*, 2001). It also increases the risk of invasion of bacterial products to access the systemic calculation.

There has been reported cases in the literature of Bacteraemia and endotoxemia which has been initiated by dental treatments and also by daily routine activities, like tooth brushing and chewing (Forner *et al.*, 2006; Hujoel *et al.*, 2001). 40% of patients with periodontal diseases have suffered systemic endotoxemia through the simple act of chewing, when compared to periodontally healthy patients which was only 12%. Moreover, the level of endotoxin was five-fold increase in the blood samples of patients with periodontal diseases (Geerts *et al.*, 2002). These studies provide enough evidence that periodontitis can lead to systemic distribution of bacteria and its products during routine life.

Previously, the treatment for periodontitis included systemic antibiotics, scaling and root planning, gingivectomy and extraction of hopeless teeth resulting in decreased need for insulin in type-1 DM patients (Taylor and Borgnakke, 2008). However more recently, scaling and root planning have significantly shown better glycaemic control in type 2 DM patients (Jain *et al.*, 2019). Whereas, other studies have only shown better periodontal improvement but no significant improvement in glycaemic control in both

type 1 and 2 DM patients (Tervonen *et al.*, 2009). Another study has shown that the combination of systemic antibiotics, specially doxycycline with scaling and root planning have enhanced glycaemic control in both type 1 and 2 DM patients (Gaikwad *et al.*, 2013). The reason for the increased use of systemic Tetracycline antibiotics in combination with scaling and root planning is the decreased production of matrix metalloproteinases, especially the collagenase enzyme which is found in abundance in DM patients with periodontal disease (Preshaw *et al.*, 2004). These studies have shown reduced presence of glycated haemoglobin (HbA1c) in DM patients with decreased periodontal inflammation. The significant results clinically and statistically were observed in DM patients with advanced periodontitis and with worst glycaemic control. Recently a meta-analysis of 10 clinical trials have shown that mechanical therapy without systemic antibiotics decreased HbA1c levels by 0.4% when compared with baseline, whereas, the combination of antibiotics with periodontal therapy showed a decrease of 0.7%, both of which are statistically insignificant (Janket *et al.*, 2005). Therefore, there are conflicting results present where it has been observed that periodontal therapy may have the capability to impact diabetic control in some patients with worst condition and advanced periodontitis (Navarro-Sanchez *et al.*, 2007).

The studies to date have not well established the exact mechanism of decreased periodontal inflammation after treatment which affects glycaemic control and insulin resistance, but it has been observed that the dissemination of inflammatory mediators, locally as well as systemically is most notable for cytokines TNF-α and IL-6 which can increase insulin resistance. Additional insulin resistance occurs through the stimulation of TNF-α production through IL-6 itself. The up regulation of these mediators can contribute to the insulin resistance in periodontal infections (Moritz and Mealey, 2006). Moreover, monocytes cells in DM patients have been observed to produce higher

amounts of TNF-α when compared with monocytes in non DM patients after stimulation by periodontal bacteria leading to great levels of systemic mediators (Torres-Castro *et al.*, 2016).

Due to the periodontal pathogens there is an overproduction of inflammatory cytokines due to the shift in monocyte/macrophage phenotype in diabetic patients (Sczepanik *et al.*, 2020). Patients with diabetes as well as periodontitis may display an increased level of serum levels of IL-6, TNF-α and CRP above normal levels which lead to increase insulin resistance worsening the glycaemic control (Aspesi, 2021). This can explain the reason behind the deteriorating condition of glycaemic control in diabetic patients with type 2, and as well as the improvement observed after the periodontal therapy in many studies (Li *et al.*, 2015).

In a 2-year longitudinal clinical trial the impact of metabolic state of diabetic patients with and without periodontitis was studied and the result indicated that there is a significant increase in the levels of worsening glycaemic control up to six-fold over time with increase in the periodontitis, when compared with diabetic patients without periodontitis (Taylor *et al.*, 1996). The addition of periodontitis has also elevated the risk of early onset of other complications, such as cardiovascular, cerebrovascular and peripheral vascular issues up to 82% in diabetic patients as compared to only 21% in diabetic patients without severe periodontitis (Stanko and Izakovicova Holla, 2014; Thorstensson *et al.*, 1996).

When there is an increase in in cellular energy demands, the insulin receptors are displayed on the surface of all cell except brain cell, which helps in the uptake of glucose from the systemic blood into the cells. But in certain bacterial or viral infections the cells become insulin-resistant, causing the pancreas to produce increased levels of

insulin to force the uptake of glucose by the cells (Olefsky and Glass, 2010). This is one of the major underlying pathophysiologic abnormalities of diabetic patients with type-II diabetes.

2.4 Factors influencing the bi-directional nature of periodontitis and Diabetes Mellitus

There appears to be a definitive relationship between serum glucose levels and systemic health especially tissue repair capacity and immune cell function (Genco and Borgnakke, 2013). In terms of the potential relationship, it is possible that periodontitis-induced changes in immune cell function cause metabolic dysregulation of glucose metabolism through mechanisms involving pro-inflammatory cytokines.

In type 2 diabetes, chronic dysregulation of glucose metabolism is seen. Pancreatic beta cells, responsible for secretion of insulin, fall short in their ability to compensate for insulin resistance by peripheral cells. This causes hyperglycaemia, which disrupts the physiological activity of blood vessels, and enhanced production of reactive oxygen species, resulting in oxidative stress (Monea *et al.*, 2014). Because of insulin resistance, the pancreas ineffectively ends up secreting higher amounts of insulin to compensate for the failure of peripheral cells in the muscles, adipose tissues, and liver to absorb glucose. The liver hence starts releasing glucose into the blood, increasing blood sugar levels even more (Perry *et al.*, 2014; Saponaro *et al.*, 2015).

Type II (non-insulin dependent or adult-onset), diabetes is a condition which often develops over a period and involves reduced responsiveness of tissues to circulating insulin (Itariu and Stulnig, 2014). Majority of clinical and epidemiological evidence demonstrates that individuals with diabetes (type I and type II) tend to have a higher prevalence and more severe/rapidly progressing forms of periodontitis than non-

diabetics (Grover and Luthra, 2013). Several studies have indicated that circulating monocytes from diabetic patients exhibit an exaggerated inflammatory response to Gram-negative bacterial lipopolysaccharides (LPS) (particularly P. gingivalis LPS) releasing large amounts of inflammatory mediators and pro-inflammatory cytokines such as Interleukin 1-Beta (IL-1β) and Tumour necrosis factor alpha (TNF-α) (Saini *et al.*, 2011b). This systemic monocytic hyper-response trait is significant in that it may cause diabetic patients or patients with high serum lipids to maintain continually high levels of serum pro-inflammatory cytokines predisposing them to tissue breakdown and/or certain systemic diseases.

Protein molecules are irreversibly glycated to produce advanced glycation end products (AGEs) in individuals with persistent hyperglycaemia. AGEs are stable carbohydrate-containing proteins that influence numerous cell-to-matrix and cell-to-cell interactions and are generally termed as the chief cause of various diabetic complications. AGEs production is also observed in the periodontium, with higher levels of periodontal AGEs accumulation being found in diabetic compared to non-diabetic subjects (Hashim and Zarina, 2011). AGEs formation on collagen leads to augmentation of collagen cross-linking and consequent formation of highly stable collagen macromolecules. These molecules are resistant to tissue turnover and normal enzymatic degradation which leads to accumulation in tissues (Delgado *et al.*, 2015). This AGE-modified collagen has a tendency to accumulate in larger blood vessel walls, leading to thickening of the vessel wall as well as causing a narrowing of the vessel lumen. In addition to its effects on collagen, AGE-modified vascular collagen exhibits an affinity for low-density lipoprotein (LDL), influencing the accumulation of LDL in the vessel wall, leading to atherosclerotic changes that are characteristic of macrovascular complications of diabetes (Bhat *et al.*, 2017).

An increase in the production of vascular endothelial growth factor (VEGF) is observed in conjunction with formation of AGE. VEGF is a multifunctional cytokine that stimulates neovascularization and plays an essential part in microvascular complications of diabetes (Chawla *et al.*, 2016). In diabetic individuals, elevated VEGF has been found in serum as well as all major tissues affected by diabetic vasculopathies (Nardi *et al.*, 2020). The effect of AGE-collagen levels on the duration of diabetes, glycaemic control and diabetic complications has been well instituted (Gurav and Jadhav, 2011). A reduction in AGE-collagen formation is linked with an improvement in glycaemic control (Gurav and Jadhav, 2011).

Mechanically, AGE–bone collagen may lead to aberrations in bone metabolism due to alterations in cellular, structural, and functional properties (Wang *et al.*, 2002). Adjusted level of glycation in bone collagen is likely to influence bone turnover in a negative manner where elevated levels of AGE collagen bring about less bone formation (Morgan and Gerstenfeld, 2021). This effect is attributed to extracellular matrix production and altered osteoblastic differentiation (Gunczler *et al.*, 2001; Santana *et al.*, 2003).

As the effect of AGEs during the resorption phase of bone metabolism is established, they are probably relevant to the inflammatory response. AGEs are responsible for activation of ‘‘receptor for AGEs’’ (RAGE) that are observed on the surface of endothelial cells, neurons, smooth muscle cells and monocytes/macrophages (Chuah *et al.*, 2013). These receptors can be found in the periodontal tissues, and during studies, 50% increase in RAGE , messenger RNA (mRNA) was observed in the gingival tissue of type 2 diabetic individuals when compared to non-diabetic controls (Katz *et al.*, 2005).

Hyperglycaemic conditions lead to a rise in RAGE production as well as an increase in AGE-RAGE interaction on the cellular endothelium, bringing forth an increase in vasculature (Paul *et al.*, 2020). When this AGE-RAGE interface takes place in monocytes, it amplifies cellular oxidant stress and triggers nuclear factor kappa (NF-κB), a transcription factor. These changes cause an increase in production of pro-inflammatory cytokines such as TNF-a and IL-1b, by essentially altering the phenotype of these cells (C Tobon-Velasco *et al.*, 2014). These pro-inflammatory cytokines play a significant part in the chronic inflammatory process, leading to atheromatous lesions forming in larger blood vessels (Poznyak *et al.*, 2020). Elevated level of AGEs in the periodontium of diabetic subjects has been associated with an increase in oxidation stress in the gingiva (Patil *et al.*, 2016). It is this AGE-RAGE interaction that is thought to explain, up to some extent, the noteworthy increase in TNF-a, IL-1b and prostaglandin E2 (PGE2) levels in the gingival crevicular fluid in diabetic subjects, compared to non-diabetic controls (Engebretson *et al.*, 2004). The increase in production of these pro-inflammatory cytokines play a vital role in the pathogenesis of periodontal diseases especially in subjects with diabetes, particularly those with poor glycaemic control. Studies conducted on diabetic animal models, show that inhibition of RAGE receptors leads to a diminishment in IL-6, TNF-a and matrix metalloproteinase (MMP) levels in the gingiva, subsequently leading to a decrease in AGE accumulation in periodontium and alveolar bone loss due to P. gingivalis (Lalla *et al.*, 2000). Alterations in collagen synthesis and maturation, in addition to homeostatic turnover are frequent in diabetic subjects and, since collagen is a major structural protein in the periodontium, these changes play a part in the pathogenesis of periodontal diseases.

In environments with high glucose concentrations, human gingival fibroblasts generate diminished amounts of collagen and glycosaminoglycans (Buranasin *et al.*, 2018). A decrease in collagen production rate is seen in animal models with diabetes, which can be resolved with the administration of insulin which leads to balancing of plasma glucose levels (Apikoglu-Rabus *et al.*, 2010). Parallel to decrease in synthesis, this newly formed collagen is flawed in composition and hence susceptible to degradation at the hands of MMPs such as collagenase, which has elevated levels in the periodontium of diabetic subjects (Oswal *et al.*, 2011). A greater proportion of active tissue collagenase is present in diabetic compared to non-diabetic individuals (Gurav and Jadhav, 2011). In divergence to the impact of elevated MMPs on newly produced collagen, existing collagen becomes especially cross-linked due to the presence of AGEs, which decreases its solubility (Sunny *et al.*, 2014).

These alterations in collagen metabolism lead to an incongruity in the normal homeostatic collagen turnover in which newly synthesized collagen is swiftly obliterated by raised levels of active MMPs, while the flawed AGE-modified collagen with highly cross-linked macromolecules keep collecting in the tissues. This difference in homeostasis lead to changes in the normal wound healing responses to chronic microbial injuring of periodontal tissue.

Realizing that only clinical measures alone very limited as disease predictors, attention is placed on development of a reliable diagnostic test which can be clinically applicable to evaluate the risks of active periodontal disease in patients. Periodontitis has characteristic cycles of active tissue destruction and quiescence. Like other diseases, periodontitis is characterized by periods of active destruction of tissue, followed by quiescence. Consequently, a reliable, predictive and practical diagnostic test would

prove to be a vital clinical advance (Wolf and Lamster, 2011). Numerous elements have been assessed as potential diagnostic markers for periodontal disease progress (Ghallab, 2018). These markers can be categorized into three groups: inflammatory mediators and host-response modifiers, host-derived enzymes and their inhibitors and by-products of tissue breakdown. Non-surgical periodontal therapy has been an accepted 'gold standard' to which more recently developed therapeutic modalities must be compared (Cobb, 2002). NSPT in diabetic patients has been met with variable levels of success for the improvement of periodontal status with authors like Cruz *et al.* (2008) reporting an insignificant improvement versus Mauri-Obradors *et al.* (2018) showing a significant improvement of periodontal clinical parameters in their clinical trial with type 2 diabetics following same intervention. A similar correlation was also expressed by both studies in relation to glycated haemoglobin levels. More comprehensive assessment of this concept was laid down (Chatzopoulos and Doufexi, 2016; Vardhan, 2018) investigated the potential of NSPT itself or with adjunct antimicrobial, photo-dynamic therapy (PDT) or laser therapy etc. Their results could only bring forth a significant difference between for NSPT coupled with PDT and antimicrobials, whereas NSPT alone could not exhibit significant change. A combination which exhibited significant gains in improvement of diabetic status. Mauri-Obradors *et al.* (2018) and El-Makaky and Shalaby (2020) both reported a significant improvement in both periodontal parameters and glycaemic control.

2.5 Role of pro inflammatory cytokines (Interleukin 1 β and Tumour Necrosis factor-α)

Cytokines are cell signalling proteins secreted by cells as a way of communication for human body regulation. A large and diverse family of cytokines has been identified which encompasses immunomodulating agents such as interleukins and interferons. Without exception, all the nucleated cells play a pivotal role in the regulation of immune as well as inflammatory response, especially the endo/epithelial cells and macrophages that are potent producers of TNF-α and IL-1 cytokines that are in turn crucial for regulating immune as well as inflammatory responses. There is over whelming evidence that pro-inflammatory cytokines primarily involved in host defense are also involved in the pathogenesis of various manifestations of human inflammatory diseases (Cardoso *et al.*, 2018). Interleukin-lβ (IL-1β) and Tumour necrosis factor-alpha (TNF-α), which arbitrate both local and systemic effects accompanying many diseases (Gomes *et al.*, 2016), are produced rapidly by monocytes and macrophages in response to several stimuli and can induce wide-ranging changes in a variety of cells. Both cytokines are monocyte/macrophage-derived mediators, although virtually all nucleated cell types are capable of producing IL-lβ. TNF-α can overlap with IL-1β in many of its inflammatory properties (Yücel, 2015).

2.5.1 IL-1β in periodontitis and Diabetes Mellitus

Upon secretion, IL-1β trigger a cascade of inflammatory responses and is enrolled in the pathology of periodontal disease (Faizuddin *et al.*, 2003). Generally, IL-1β present within the site of inflammation is responsible for increased local blood flow, neutrophil infiltration and leucocyte recruitment (Schett *et al.*, 2016). IL-1β also acts as a potent stimulator of bone resorption, thus making it a member of the cytokine family in periodontitis. IL-1β is responsible for enhancing the expression of collagenolytic

enzymes such as matrix metalloproteinases (MMPs), which play a part in the degradation of extracellular matrix and essentially result in bone resorption and tissue destruction (Schett *et al.*, 2016). MMP-9 plays an important part as an indicator of the severity and progress of periodontitis (Rai *et al.*, 2008) and IL-1β is responsible for upregulating the expression of MMP-9 in numerous cell types that are tied to periodontal inflammation, including osteoblasts, osteoclasts, cementoblasts, and neutrophils (Du *et al.*, 2019). In human periodontal ligament cells and gingival fibroblast cells, IL-1β is also responsible for upregulating the production of other MMPs such as MMP-1 and/or MMP-3 (Cheng *et al.*, 2020),

Osteoclastogenesis is regulated by the receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) systems (Liu and Zhang, 2015). Increase in RANKL and a decrease in OPG is a characteristic of periodontitis (Belibasakis and Bostanci, 2012). IL-1β is responsible for upregulation of RANKL and hence promotes osteoclastogenesis (Huynh *et al.*, 2017). RANKL system is observed in various cells such as osteoblasts, activated T cells, bone marrow stromal cells, periodontal ligament fibroblasts, endothelial cells and cementoblasts (Yang *et al.*, 2018). RANKL, when expressed on precursor osteoclastic cells causes the activation of osteoclasts and controls their activation, differentiation, and proliferation. Apart from its direct effects, certain indirect effects take place as it increases PGE2 synthesis in fibroblast cells (Walsh and Choi, 2014) which in turn induces the expression of RANKL. In brief, IL-1β has an enduring effect on osteoclastogenesis, which ultimately leads to bone resorption (Bloemen *et al.*, 2011).

Type 2 (T2D) diabetes is regarded as an immune mediated disease which leads to defects in insulin signalling as well as selective destruction of β-cells that produce

insulin, in which cytokines play an essential role (Imai *et al.*, 2013). Disruption of the anti-inflammatory response could be an integral factor in chronic inflammation, resulting in T2D. The IL-1 family of cytokines is potentially able to impact endocrine functions and regulate the associated inflammatory stress responses (Banerjee and Saxena, 2012). T2D occurs when the beta-cell activity is unable to offset the body's insulin resistance (Basukala *et al.*, 2018). Beta-cell function increasingly deteriorates with the progression of diabetes, to an extent, because of beta-cell death through apoptosis (Cernea and Dobreanu, 2013). Sub-clinical inflammation occurs in insulin resistance and T2D. The diseases associated with this metabolic disorder are characterized by atypical cytokine production which includes higher circulating IL-1β, elevated acute phase proteins like CRP, and triggering of the inflammatory signalling pathways (Dinarello, 2011). Pro-inflammatory cytokines may lead to insulin resistance through inhibition of insulin signal transduction. In insulin-resistant states, the insulin target tissues themselves are sources of cytokine production. Primary among these is the liver but to a larger extent the activated tissue-resident macrophages (Khodabandehloo *et al.*, 2016).

Interleukin-1β has been observed being produced by beta cells in pancreatic sections retrieved from patients with T2D (Hull *et al.*, 2018). High glucose level elevates beta-cell production and secretion of interleukin-1β resulting in functional impairment and apoptosis (Larsen *et al.*, 2007). These findings indicate that inflammatory mediator production plays a crucial role in the pathogenesis of T2D, thus making interleukin-1β a likely therapeutic target for maintenance of beta-cell mass as well as function in T2D patients (Dinarello *et al.*, 2010).