MMP-8, IL-6 AND IL-1 *B*: THE MOST PROMISING SALIVARY BIOMARKERS FOR EARLY DIAGNOSIS OF PERIODONTITIS

DR MARIA ALI

06-114212-002

BAHRIA UNIVERSITY ISLAMABAD

PAKISTAN

MMP-8, IL-6 AND IL-1 β: THE MOST PROMISING SALIVARY BIOMARKERS FOR EARLY DIAGNOSIS OF PERIODONTITIS

DR MARIA ALI

06-114212-002

Thesis submitted in fulfillment of the requirement for the degree of Master of Philosophy (MPhil) Pathology (Microbiology)

DEPARTMENT OF PATHOLOGY

BAHRIA UNIVERSITY HEALTH SCIENCES CAMPUS

SEPTEMBER 2024

APPROVAL FOR EXAMINATION

Scholar's Name: **Dr. Maria Ali** Registration No. **37725**

Program of study: **MPhil Pathology (Microbiology)**

Thesis Title: **"MMP-8, IL-6 AND IL-1 β: The most promising salivary biomarkers for early diagnosis of periodontitis."**

It is to certify that above mentioned student thesis has been completed to my satisfaction and to my belief. Its standard is appropriate for submission and evaluation. I have also conducted plagiarism test of this thesis using HEC prescribed software and found similarity index at 9% that is within the permissible limit set by HEC for the MPhil degree thesis. I have also found the thesis in format recognized by the Bahria University for MPhil thesis.

Principle Supervisor's Seal and Signature:

PROF. DR. YASMIN TAJ
Sr. Professor & HOD
Pathology
BU Health Sciences
Karachi

Date: **2nd July 2024**

Name: **Prof. Dr Yasmeen Taj**

THESIS COMPLETION CERTIFICATE

Scholar's Name: **Dr. Maria Ali** Registration No. **37725**

Program of study: **MPhil Pathology(Microbiology)**

Thesis Title: **" MMP-8, IL-6 AND IL-1 β: The most promising salivary biomarkers for early diagnosis of periodontitis "**

It is to certify that above mentioned student thesis has been completed to my satisfaction and to my belief. Its standard is appropriate for submission and evaluation. I have also conducted a plagiarism test of this thesis using HEC prescribed software and found similarity index at **9%** that is within the permissible limit set by HEC for the MPhil degree thesis. I have also found the thesis in format recognized by the Bahria University for MPhil thesis.

Principle Co-Supervisor's Seal and Signature:

Date: **2nd July 2024**

Name: **Prof. Dr Farzeen Tanwir**

AUTHOR'S DECLARATION

I, Dr Maria Ali, hereby state that my MPhil thesis title ***"MMP-8, IL-6 AND IL-1 β: The most promising salivary biomarkers for early diagnosis of periodontitis."*** is my own work and has not been submitted previously by me for taking any degree from this university, Bahria University Health Science Campus Karachi, or anywhere else in the country/world.

At any time if my statement is found to be incorrect even after my graduation, the university has the right to withdraw/cancel my MPhil degree.

Name of Scholar: **Dr. Maria Ali**

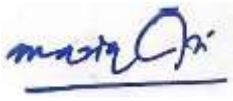
Date: **2nd July 2024**

PLAGIARISM UNDERTAKING

I, solemnly declare that research work presented in the thesis titled **"MMP-8, IL-6 AND IL-1 β: The most promising salivary biomarkers for early diagnosis of Periodontitis"** is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero-tolerance policy of the HEC and Bahria University towards plagiarism. Therefore, I as Author of the above-mentioned thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of formal plagiarism in the above title thesis even after ward of MPhil degree, the university reserves the right to withdraw/ revoke my MPhil degree and that HEC and the University has the right to publish my name on the HEC/University website on which names of scholar are placed who submitted plagiarized thesis.

Scholar/Author's Sign: 

Name of Scholar: **Dr. Maria Ali**

Dedicated to My Beloved parents And My Dear Husband

Dr. Shariq Nadeem

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to Allah (SWT) for blessing me with the opportunity to complete this research and for guiding me throughout my academic journey. I am grateful for the knowledge, wisdom, and strength that He has bestowed upon me. I acknowledge and thank Allah (SWT) for His infinite mercy, grace, and blessings that have enabled me to overcome challenges and reach this milestone. I would like to express my deepest gratitude to my supervisor, Prof Dr. Yasmeen taj for her unwavering guidance, support, and encouragement throughout my research journey. Her expertise, valuable feedback, and patience have been instrumental in shaping my research and helping me reach my full potential. I am forever grateful for her dedication and commitment to my academic growth, for believing in me and for being an exceptional mentor and role model. I am also grateful to my co-supervisor, Professor Dr. Farzeen Tanwir, for her expert guidance. It has been an honor to work alongside such a knowledgeable and experienced researcher. I am incredibly grateful to the BUHSCK administration, especially for Professor Dr. Naseem Karim's efforts as the head of PGPTM. My humble thanks to Professor Dr. Mehreen Latif, principal BUHSCK and in charge MDRL for helping me out in analysis of ELISA tests for my research project. I am indebted to my esteemed teachers Dr Hina Wasti, Dr Shaista bakhat, Prof. Dr Summayya Shawana and Prof. Dr Naveed Faraz for their consistent assistance. They have helped me to develop my skills and knowledge. I am particularly grateful to Dr Kiran Saleem, Dr Amna Farrukkh and Dr Hadia Khursheed for their unwavering moral support. Special thanks to my dearest husband Dr. Shariq Nadeem, my son Sarim Shariq and my sister Javeria Ali for providing me the will to keep moving forward and my parents for their love, support, and prayers for my success. Their encouragement and belief in me have been a constant source of strength and motivation. I am grateful for their presence in my life and for the countless ways they have helped me achieve my goals. Completing this thesis has been a significant milestone in my academic journey, and I could not have reached this point without this support. Thank you all from the bottom of my heart.

Dr. Maria Ali

ABSTRACT

Periodontal disease is a chronic inflammatory condition affecting the supporting structures of teeth, which if left untreated, worsens gradually and can have serious consequences on the periodontium. There are several risk factors for periodontitis but smoking or tobacco use, and poor oral hygiene are the leading cause. Early diagnosis of periodontal disease is essential for successful treatment outcomes. Traditional diagnostic methods rely on clinical assessment and radiographic imaging, but these methods have limitations, particularly in identifying early-stage disease. According to W.H.O, it was reported that 18% population of Pakistan has some form of periodontal problems and out of these 31% has periodontitis. The purpose of this study is to evaluate salivary levels of MMP-8, IL-1β and IL-6 in healthy and periodontitis patients and to assess the association between these biomarkers' levels and clinical parameters. This was a case control study which was conducted at the Dental OPD of Bahria University Medical Health Sciences Karachi over a period of six months. 76 participants were included in the study after randomization, 38 subjects for control group and 38 subjects for case group. In control group a healthy periodontium is defined as the absence of either gingivitis or periodontitis history (BOP < 10%, PPD ≤ 3 mm, no proximal clinical attachment loss (CAL), and no recession).In case group periodontitis patient defined as a patient who had at least two nonadjacent teeth with interdental clinical attachment loss (CAL) ≥ 2 mm, probing pocket depth (PPD) > 3 mm, and radiographic evidence of bone loss. Periodontal examination and saliva sampling were performed in all patients. Saliva sample was taken for measuring different parameters like Plaque Index (PI), Calculus Index (CI), Periodontal Probing Depth (PPD), Clinical Attachment Loss (CAL) and Bleeding on Probing (BOP), after taking ethical approval from Bahria University Health Sciences Karachi (BUHSCK). Levels of salivary cytokines including MMP-8, IL-1β and IL-6 were evaluated by sandwich ELISA test kit. For Statistical analysis: Data analysis was done by using IBM SPSS Statistics v27.Mean and standard deviation were calculated for quantitative variables whereas frequency and percentage were reported for qualitative

variables. Mean comparison of IL-1β, IL-6 and MMP-8 according to study groups were done by using independent t test. P- value less than 0.05 were considered as significant. Results: IL-1β, MMP-8 and IL-6 levels were significantly higher in periodontitis patients. We found significant association for gender(p=0.022), age groups(p=0.000), any co-morbid (p=0.012), plaque (p=0.001), calculus(p=0.002), bleeding on probing (p=0.001) and clinical attachment loss (p=0.001) whereas no significant association were found with dietary habits, and medication use (p=0.108). Concentration of these proteins in saliva showed significant association with plaque index, calculus index, periodontal pocket depth and clinical attachment loss and it reflect the clinical status of diseased periodontium. In conclusion, salivary biomarkers have emerged as a promising tool for the early diagnosis and monitoring of periodontal disease by providing valuable information about disease status.

KEY WORDS: Periodontitis, Saliva, Salivary biomarkers, Matrix metalloproteinases (MMPs), Human interleukin 1β (IL-1β), Human interleukin 6 (IL-6)

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	APPROVAL OF EXAMINATION	i
	THESIS COMPLETION CERTIFICATE (CO-SUPERVISOR)	ii
	AUTHOR'S DECLARATION	iii
	PLAGIARISM UNDERTAKING	iv
	DEDICATION	v
	ACKNOWLEDGEMENT	vi
	ABSTRACT	vii
	TABLE OF CONTENTS	ix
	LIST OF TABLES	xii
	LIST OF FIGURES	xv
	LIST OF ABBREVIATIONS	xviii
	LIST OF ANNEXURES	xx
1	INTRODUCTION	
	1.1 Background	1
	1.1.1 Prevalence and Epidemiology	2
	1.1.2 Stages or Periodontitis	5
	1.1.3 Types of Periodontitis	6
	1.1.4 Staging and Grading of periodontitis	7
	1.1.5: Etiology	11
	1.1.5.1 Modifiable risk factors	11
	1.1.5.2 Non-modifiable risk factors	11

	1.1.6 Pathophysiology of Periodontitis	14
	1.1.6.1 Structure of the dental biofilm	16
	1.1.6.2 Formation of the dental plaque biofilm	16
	1.1.6.3 Maturation of biofilm	17
	1.1.7 Need for a biomarker	23
	1.1.8 Immune Response in Periodontitis	26
	1.1.8.1 Activation of (PMN)	26
	1.1.8.2 Activation of Macrophages	27
	1.1.8.3 Activation of osteoclasts	27
	1.1.8.4 Salivary Products by Immune Response	27
	1.1.9 Saliva: a reliable predictor for diagnosis	28
	1.1.10 Metalloproteinase-8, Interleukin1beta and Interleukin 6	29
	1.2: Research Gap/Rationale of Study	31
	1.2.1 Theoretical Gap	31
	1.2.2 Contextual Gap	32
	1.2.3 Methodological Gap	32
	1.3 Problem Statement	32
	1.4 Hypothesis of Study/ Research Question	33
	1.5 Objectives of Study	33
	1.6 Significance of the Study	33
2	LITERATURE REVIEW	34
	Operational Definitions	
3	METHODOLOGY	
	3.1 Study design	56
	3.2 Subjects / Animals	56
	3.3 Setting	56

	3.4 Inclusion criteria	56
	3.5 Exclusion criteria	57
	3.6 Duration of study	57
	3.7 Sample size estimation	58
	3.8 Sampling technique	59
	3.9 Human subjects and consent	59
	3.10 Materials used (Drugs/ Chemicals/Performa / Questionnaire/any other)	59
	3.11 Parameters of study	76
	3.12 Protocol of the study	80
	3.13 Algorithm of study	85
	3.14 Statistical Analysis	86
4	RESULTS	87
5	DISCUSSION AND CONCLUSION	
	5.1 Discussion	109
	5.2 Implication of the study	127
	5.2.1 Theoretical Implications	127
	5.2.2 Practical Implications	127
	5.2.3 Policy Implications	128
	5.3 Limitations and strength of the study	128
	5.4 Recommendation	129
	5.5 Conclusion	131
6	REFERENCES	132
7	ANNEXURES	151

LIST OF TABLES

TABLE	TITLE	PAGE
1.4.1	Staging and Grading of Periodontitis	11
4.1	Frequency distribution of gender among cases and controls	93
4.2	Descriptive statistics of age (years) among cases and controls	93
4.3	Frequency distribution of co-morbid among cases and controls	94
4.4	Frequency distribution of co-morbid type among cases and controls	94
4.5	Descriptive statistics of plaque index among cases and controls	95
4.6	Frequency distribution of plaque among cases and controls	95
4.7	Descriptive statistics of calculus index among cases and controls	96
4.8	Descriptive statistics of calculus index among cases and controls	96
4.9	Descriptive statistics of bleeding on probing among cases and controls	97
4.10	Frequency distribution of bleeding on probing among cases and controls	97
4.11	Descriptive statistics of probing depth (mm) among cases and controls	98
4.12	Descriptive statistics of clinical attachment loss (mm) among cases and controls	98
4.13	Frequency distribution of clinical attachment loss among cases and controls	99
4.14	Normality testing for IL-1β, IL-6 and MMP-8	100
4.15	Descriptive statistics of IL-1β (pg/mL) among cases and controls	100

4.16	Descriptive statistics of IL-6 (pg/mL) among cases and controls	101
4.17	Descriptive statistics of MMP-8 (pg/mL) among cases and controls	101
4.18	Mean comparison of IL-1β according to study groups	102
4.19	Mean comparison of IL-6 according to study groups	103
4.20	Mean comparison of MMP-8 according to study groups	104
4.21	Association of demographic and clinical parameters according to study groups	105
4.22	Area under curve (AUC) for IL-1β, IL-6 and MMP-8	108
4.23	Optimal cut-off for IL-1β, IL-6 and MMP-8 in detection of periodontitis	108

LIST OF FIGURES

FIGURE	TITLE	PAGE
1.1	Periodontitis showing the inflammatory process	3
1.2	Periodontal infection and systemic condition	4
1.3 (a)	A diagrammatic depiction of periodontitis	9
1.3 (b)	Periodontitis showing clinical features and radiographic findings	10
1.4	Components of dental biofilm	18
1.5	Biofilm formation and development in the oral cavity	19
1.6	The association of periodontitis among subgingival species	20
1.7	Inflammation-Mediated Polymicrobial Emergence and Dysbiotic Exacerbation (IMPEDE) model	22
1.8	Blood coming out during probing	25
1.9	Functional diagram to evaluate the patient's risk for recurrence of periodontitis	25
2.1	A change of paradigm in periodontal diagnosis is desirable	52
3.1 (a)	Examination Set	59
3.1 (b)	Disposable Cup	59
3.1 (c)	CPITN Probe	59
3.1 (d)	Polypropylene Tube	59
3.1 (e)	Eppendorf Tube	60
3.1 (f)	ELISA washer	60
3.1 (g)	ELISA Reader	60
3.2 (a)	Matrix Metalloproteinase 8 ELISA Kit	64
3.2 (b)	Human Interleukin 6 ELISA Kit	64
3.2 (c)	Human Interleukin 1 beta ELISA Kit	64

3.3	Reagent Storage Refrigerator	65
3.4	Sandwich ELISA	65
3.5	Saliva sample in polypropylene tube	70
3.6	Samples were transferred in Eppendorf tubes for centrifugation	70
3.7 (a) & (b)	Sample centrifugation	70
3.7 (c)	Sample stored in -80°C	71
3.8 (a)	Buffer solutions were thawed at room temperature	71
3.8 (b)	Samples were thawed at room temperature	72
3.8 (c)	Sample mixing on vortex	72
3.8 (d)	Standard Antigen Diluent	73
3.8 (e)	Adding streptavidin-HRP	73
3.8 (f)	Adding Substrate Solution A	73
3.8 (g)	Adding Substrate Solution B	73
3.8 (h)	Adding Stop Solution	74
3.8 (i)	Microplate Reader	74
3.8 (j)	Optical Density Determined	74
3.9 (a)	Clinical Attachment loss	78
3.9 (b)	Plaque Index	78
3.9 (c)	Calculus Index	78
3.9 (d)	Bleeding on Probing	79
3.9 (e)	Periodontal Probing Depth	79
3.10	OPG (Orthopantomagram)	84
4.1	Receiver Operating Curve (ROC)	107

LIST OF ABBREVATIONS

CI	Calculus Index
PI	Plaque Index
PPD	Periodontal Probing Depth
CAL	Clinical Attachment loss
MMP-8	Matrix Metalloproteinase-8
aMMP-8	Active Matrix Metalliproteinase-8
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
SRP	Scaling and Root Planing
PISF	Peri-Implant Sulcus Fluid
PICF	Peri-Implant crevicular fluid
AAP	Asymptomatic apical periodontitis
OPG	Osteoprotegerin
TRAP-5	Tartrate-resistant acid phosphatase-5
ON	Osteonectin
TNF-α	Tumor necrosis factor-alpha
IFN-γ	Interferon gamma
PMN	Polymorphonuclear leukocyte
P. gingivalis	Porphyromonas gingivalis
A. actinomycetem Comitans	Aggregatibacter actinomycetemcomitans
C. rectus	Campylobacter rectus
F. nucleatum	Fusobacterium nucleatum
P. intermedia	Prevotella intermedia
T. forsythia	Tannerella forsythia
T. denticola	Treponema denticola
PCR	polymerase chain reaction
GCF	Gingival crevicular fluid

IFMA	Immunofluorometric assay
ELISA	Enzyme-linked immunosorbent assay
TIMP-1	Tissue inhibitor of matrix metalloproteinase
AgP	Aggressive periodontitis
CP	Chronic Periodontitis
GAgP	Generalized aggressive periodontitis
G	Gingivitis
PD	Periodontal Disease
MIP-1α	Macrophage inflammatory protein
PI	Peri implantitis
PGE2	Prostaglandin E2
CRS	Cumulative risk score
PoC	Point-of-care
POCID	Point-of-care immunoflow device
APD periodontal	Peri-implant degeneration
STPs	Smokeless tobacco products
AUC	Area under the curve
WHO	World Health Organisation
OP	Optical Density
SPSS	Statistical Prograam for Social Sciences

LIST OF ANNEXURES

ANNEX	TITLE	PAGE
A	BUHS IRB APPROVAL LETTER	189
B	BUHS FRC APPROVAL LETTER	189
C	Consent Form (ENGLISH VERSION)	190
D	Consent Form (URDU VERSION)	191
E	Subject Evaluation Form	192
F	Hospital / Institute Card	193
G	Turnitin Plagiarism Check report	194

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Periodontitis is an infection of periodontium, whereas the word ‘Perio’ means gingiva and other tissues surrounding teeth, ‘dont’ mean tooth and ‘itis’ means inflammation. The word "periodontium" refers to the supporting structure that surrounds the tooth, which consists of the cementum, gingival tissue, alveolar bone, and periodontal ligament. (Kinane, D. F.,2017). Thus," Periodontitis" denotes persistent gingival inflammation, PDL ligaments, alveolar bone and dental cementum (Preshaw et al.,2013).

WHO states that it is a chronic illness and its widely spreading around the globe (Chapple et al.,2018). As it is a chronic inflammatory disease it is caused by pathogenic bacteria that destroys alveolar bone and connective tissue, (Slots, J. 2017) (Fig 1.1) The mildest form of periodontal disease is gingivitis, and it can be found in the population of more than 90%. Gingivitis is a reversible inflammatory condition of the gingival tissue, caused by bacterial infection. Unlike periodontitis, there is no attachment loss and therefore no migration of the junctional epithelium. The condition is restricted to the soft-tissue area of the gingival epithelium and connective tissue. (Marchesan, et al.,2020). Periodontitis is established when periodontal disease has developed into a chronic, destructive, irreversible inflammatory condition following gingivitis. (Kinane et al., 2017)

Dental calculus is a chief contributing factor in the development of periodontal diseases. Individuals often present with yellowish and brownish discoloration which is commonly called calculus. The prerequisite for calculus is dental plaque. Calculus can be defined as a hard deposit that is formed by mineralization of dental plaque on the surfaces of natural teeth and dental prosthesis which are usually covered by a layer of

unmineralized plaque. (Aghanashini, S et al.,2016).A series of factors are related to calculus formation. Among them the prominent factors are bacterial plaque retention, biochemical factors (characterized by saliva or crevicular fluid), microorganisms and dietary factors. (Hidaka, S., & Oishi, A. 2007).

Calculus can be classified as supragingival, which is found coronal to the gingival margin, and subgingival calculus, located apical to the gingival margin, thus not visible on routine clinical examination. The subgingival calculus is affected by hemorrhagic components from the GCF, and the mineralization of anaerobic microorganisms brings the characteristic black pigmentation of this type of calculus. The supragingival calculus is influenced by saliva, food pigments, and tobacco, presenting a rather claylike consistency. Supragingival calculus is mainly observed on the buccal surfaces of the maxillary molars and the lingual surfaces of the mandibular anterior teeth, as the flow from the salivary gland in these areas favors the deposit of calcium and phosphate ions derived from saliva. By contrast, subgingival calculus is mainly found on the interproximal and lingual tooth surfaces, being randomly distributed throughout the mouth. (Whittaker, D. K et al.,1998).

1.1.1 Prevalence and Epidemiology:

It is the most prevalent oral inflammatory illness in adults; according to Richards (2013), 3.9 billion people worldwide have moderate to severe periodontitis, affecting up to 90% of the population. According to cross-sectional studies conducted in the United States alone, up to 80% of individuals have had periodontal disease at some point in their lives, and about 50% of persons presently have gingivitis. It has been demonstrated that some populations are more likely to develop periodontal diseases. These demographics include men, African Americans, and the elderly. (Nazir et al.,2017). A systematic review of periodontitis in Pakistan in 2022 reported the prevalence of periodontitis at 37% in Punjab, 40% in Sindh, 20% in Khyber Pakhtunkhwa and 3% in Baluchistan. (Fahim, A et al 2022). A severe case of periodontitis has also been linked to lower levels of education and income. Periodontitis is mostly brought on by inflammation of the tooth's supporting tissues (Kinane et al, 2017). If left untreated, this inflammation can result in tooth loss and leads to systemic inflammation. (Fig 1.2)

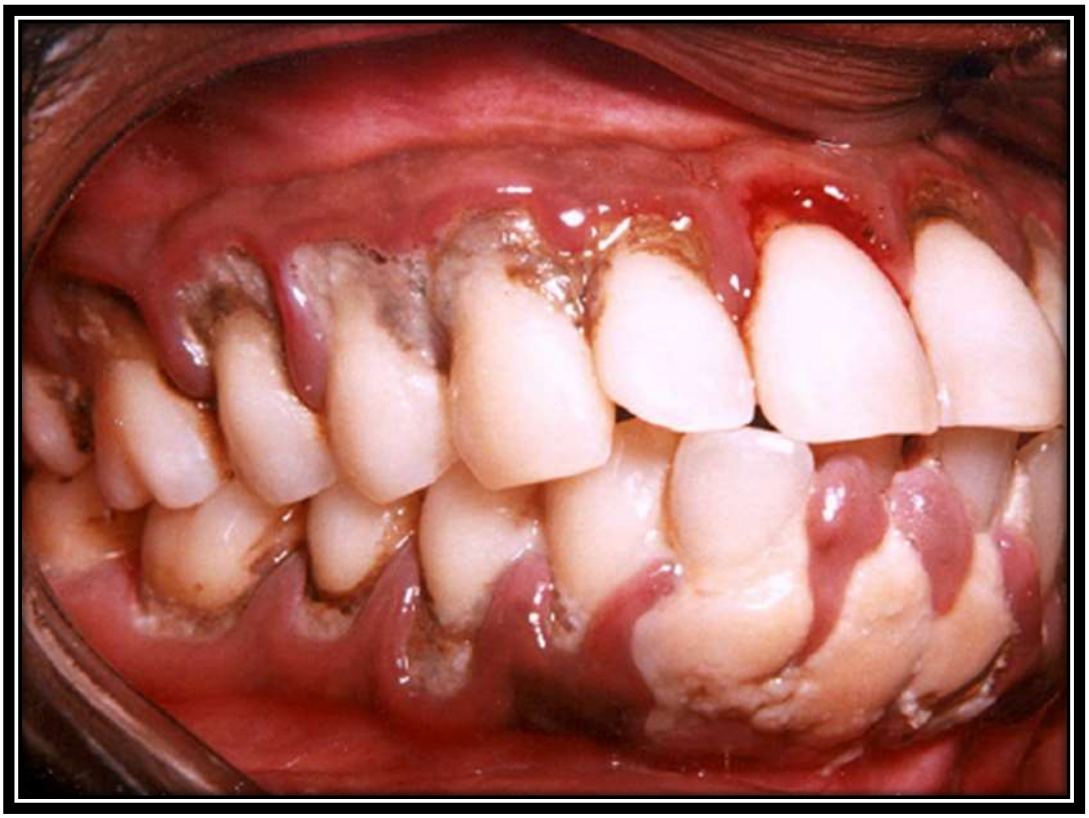


Fig 1.1: A case of periodontitis showing the inflammatory process and destruction of the supporting tooth structures. (Anil, S., Varma, 2012).

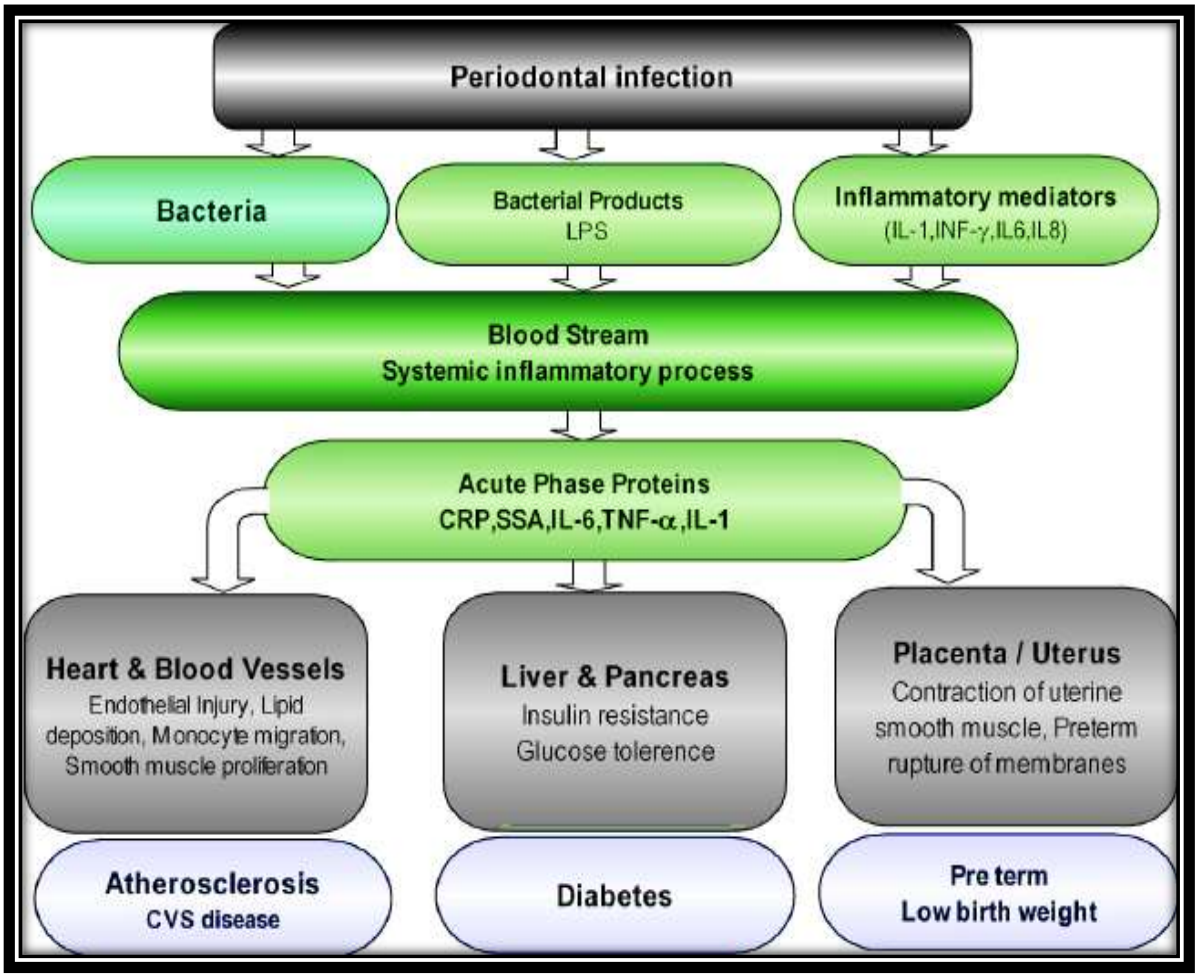


Fig 1.2: Periodontal infection and systemic conditions - Potential linkage and possible pathogenic mechanisms (Anil, S., Varma, 2012)

1.1.2 Stages of Periodontitis:

There are four phases of periodontal disease, each phase has its own distinct clinical signs and symptoms, histopathological appearance of tissue as well as radiological findings. These stages are as follows: (Fine et al., 2018; Graetz et al.,2019; Dietrich, 2019).

Initial Lesion (Gingivitis):

The *initial lesion* is marked by a plaque that results in vascular changes and intercellular gap formation that leads to increased amounts of gingival crevicular fluids (GCF). Adhesion molecules attract polymorphonuclear neutrophils to the site of the lesion. T lymphocytes specifically alter the fibroblasts of the affected area. Clinically, this stage of the lesion is benign. Halitosis, swollen, bright red gums, and bleeding during brushing and flossing are the most common painless symptoms at this point. By keeping up with routine exams and dental cleanliness, it can be reversed. Clinical attachment loss typically ranges from 1-2 mm, bone loss of <15% occurs around the root, and probing depths of 4 mm or less. (Kang et al., 2016)

Early Lesion:

As redness at the site develops, it is known as the *early lesion.* Polymorphonuclear neutrophils (PMN) infiltrate the area and clear the fibroblasts that are undergoing apoptosis. The infiltration also causes collagen fiber breakdown leading to an increased amount of space for infiltrates. There is a degradation of the marginal connective tissue matrix. Clinical symptoms include bleeding from gums during brushing or flossing, severe foul breath, and noticeable gum irritation. The space between teeth also steadily widens. This results in CAL of 3–4 mm, <15–33% of bone loss which surrounds the root, and a probing depth of 5 mm or less. (Kang et al., 2016)

Established Lesion:

The *established lesion* is predominantly dominated by leukocyte aggregation and B cells, either plasma cells or lymphocytes, that initiate the transformation of the site by changing both the junctional epithelium and sulcular epithelium into the pocket epithelium. Pocket epithelium is extremely permeable and vulnerable. Clinically, this

manifests as bleeding upon gentle probing of the gingival tissues. Radiographically, CAL of 5mm, 33% of tooth loss of four teeth or fewer, and complicated problems such Class II–III furcation's, substantial ridge abnormalities, and/or probing depths of six millimeters or more. (Kang et al., 2016)

Advanced Lesion (Periodontitis):

The final stage, known as *advanced lesion*, is a transition to periodontitis. The advanced lesion is created by the migration of biofilm to the pocket, which gives an ideal niche for anaerobic bacteria to proliferate. There is an irreversible loss of attachment and bone loss that can be seen histologically and clinically. Loss of gingival fibers and loss of the alveolar bone are the hallmarks of this stage. This lesion is highly influenced by the microbial factors themselves and can cause several changes depending on the host and organism. At this phase the periodontal tissue of 50–90% are lost at this last stage of periodontal disease. Additional symptoms include pus-filled, swollen gums, mobile teeth and tooth loss, cold sensitivity, painful chewing, and severe halitosis. (Kang et al., 2016) Fig 1.3(a), (b) shows pathogenesis and different stages of periodontitis.

1.1.3 Types of Periodontitis:

Gingivitis:

Gingivitis is an inflammatory condition of the gingival tissue most commonly caused by bacterial infection, it is caused by the microbial plaque deposits located in or close to the gingival sulcus. Unlike periodontitis, there is no attachment loss and therefore no migration of the junctional epithelium. The condition is restricted to the soft-tissue area of the gingival epithelium and connective tissue. (Marchesan, J. T et al., 2020)

Chronic Periodontitis:

Chronic periodontitis (CP) is a polymicrobial infection initiated by plaque biofilm that triggers a chronic immunoinflammatory lesion, which results in progressive damage to tooth supporting structures and leads to tooth loss. (Eke, P. I et al.,2012).

Aggressive Periodontitis:

Aggressive periodontitis is a severe form of periodontal disease characterized by rapid destruction of the supporting structures of the teeth, including the periodontal ligament and alveolar bone. It generally affects younger patients, often under the age of 30, and is known for its rapid progression compared to other forms of periodontitis The condition can be either localized or generalized, and it often affects otherwise clinically healthy individuals without systemic diseases that could explain the extent of periodontal damage. This disease typically has familial aggregation, indicating a significant genetic predisposition. (Demmer, R et al., 2010)

Necrotizing Ulcerative Gingivitis:

Acute necrotizing ulcerative gingivitis (ANUG) is a non-communicable microbial disease of the gums that causes a significant and drastic damage when the host is immunocompromised. It is distinguished by the appearance of typical "punched-out" crater-like lesions of the papillary gingiva. People with HIV, immune suppressants, and malnutrition are the major populations affected. (Aaron, S. L.,2020).

Peri implant mucositis:

Peri-implant mucositis is defined as an inflammatory condition confined to the soft tissues surrounding a dental implant, without any accompanying loss of supporting bone. The condition is primarily caused by the accumulation of dental plaque around the implant site. Clinically, it is characterized by signs of inflammation such as redness, swelling, and bleeding on probing, like gingivitis around natural teeth. (Zitzmann, N. U et al.,2008)

Systemic Chronic Periodontitis:

Systemic chronic periodontitis is a long-standing inflammatory disease that affects the periodontium (the tissues surrounding the teeth) and can have systemic implications. The persistent inflammation and infection associated with chronic periodontitis can lead to a dysregulated immune response, potentially contributing to systemic conditions such as cardiovascular disease, diabetes, and other chronic inflammatory disorders. It is

primarily caused by the buildup of bacterial plaque, leading to the destruction of the supporting structures of the teeth, including the gums, periodontal ligament, and alveolar bone. (Martínez-García et al., 2021).

1.1.4 Recent 2017 Staging Guidelines of Periodontitis: Classification of periodontitis into staging and grading of periodontitis.

Staging is based on the severity and extent of the management required and is given a stage depending on factors such as clinical attachment loss, radiographic bone loss, and tooth loss.

Stage I: Initial periodontitis

Stage II: Moderate periodontitis

Stage III: Severe periodontitis with the potential for additional tooth loss

Stage IV: Severe periodontitis with the potential for loss of dentition

Grading is a measure used to describe the rate of progression of the disease based on the evidence for associated risk factors such as smoking and diabetes mellitus:

Grade A: low rate of progression

Grade B: expected progression

Grade C: high risk of progression

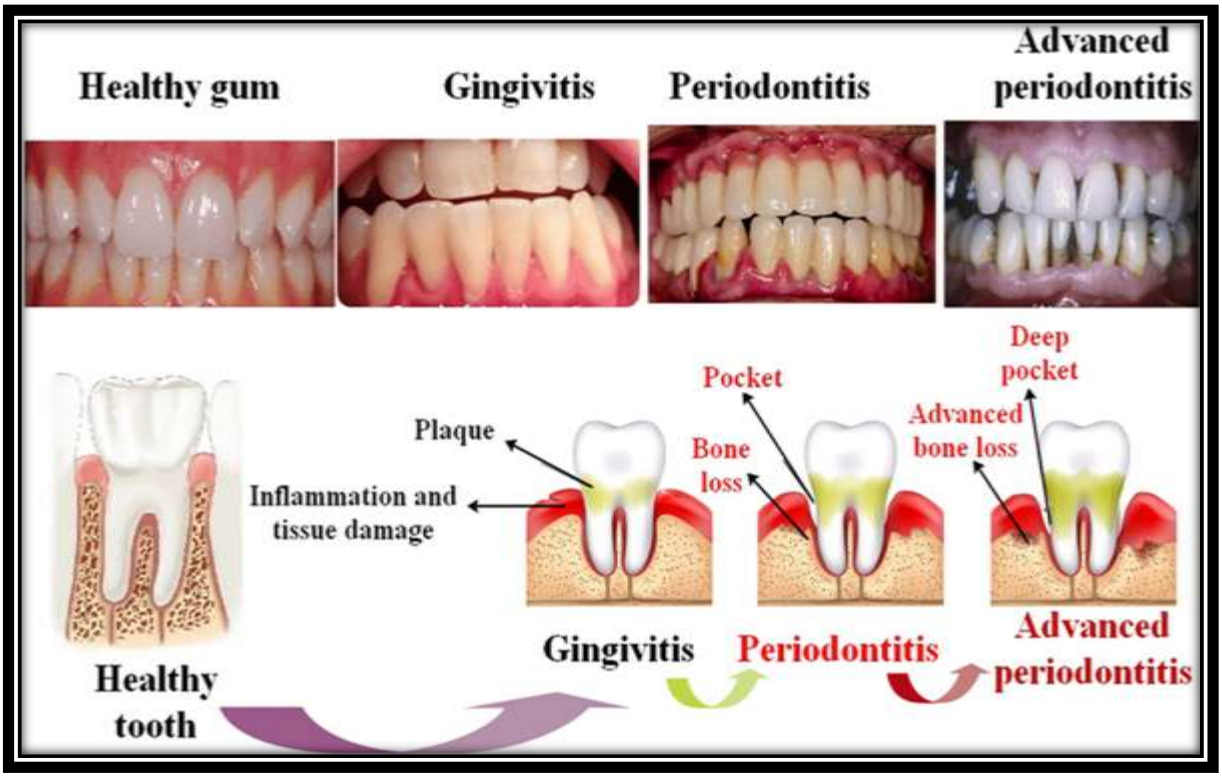


Fig 1.3 (a): A diagrammatic depiction of periodontitis. Gingivitis is distinguished by the presence of inflamed, red, and oozing gums that encircle the teeth. Although periodontal disease exhibits similar symptoms, it additionally manifests as bone loss. A viscous substance called plaque, which is produced in the oral cavity by food, saliva, and bacteria, irritates the gum tissue by coating the tooth both above and below the gumline. Plaque, if not eliminated, solidifies into calculus, a substance that becomes exceedingly challenging to remove. Plaque and calculus microorganisms have the potential to eventually obliterate the bone and gingival tissue that surround the teeth. This results in the formation of deep fissures, bone atrophy, and potential tooth loss. (Kiarashi, M. 2024).

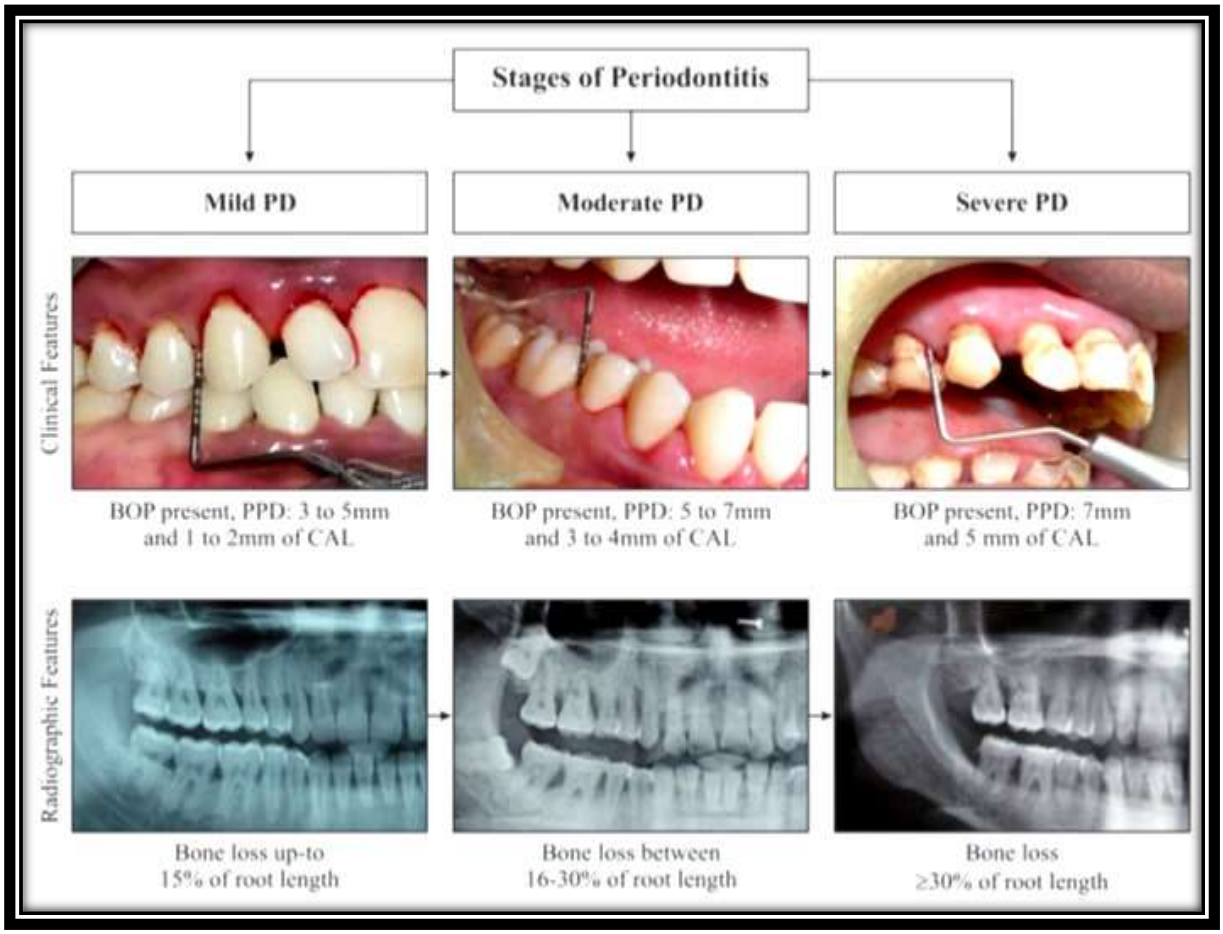


Fig 1.3 (b): Various stages of periodontitis showing clinical features and radiographic findings (Dubey, P.,2020)

Table 1.14 shows Staging and grading of Periodontitis (American Academy of Periodontology (AAP) 2017)

	Periodontal Stage	Stage I	Stage II	Stage III	Stage IV
Severity	Greatest interdental clinical attachment loss	1-2 mm	3-4 mm	≥ 5 mm	
	Percent bone loss	Coronal third (>15%)	Coronal third (15% - 33%)	Middle third or apical third	
	Teeth lost to periodontitis	No tooth loss		≤ 4 teeth	≥ 5 teeth
Complexity		• Probing depths ≤ 4mm • Mostly horizontal bone loss	• Probing depths ≤ 5mm • Mostly horizontal bone loss	• Probing depths ≥ 6mm • Vertical bone loss ≥ 3 mm • Class II or Class III furcation	In addition to Stage III complexity: • Need for rehabilitation • Masticatory dysfunction • Tooth mobility ≥ 2 • < 20 remaining teeth
Extent	• Localized (<30% of teeth involved) • Generalized (>30% teeth involved) • Molar/incisor pattern				

	Progression		Grade A: Slow rate	Grade B: Moderate rate	Grade C: Rapid Rate
Primary criteria	Direct evidence	Bone loss or clinical attachment loss	No loss in 5 years	<2 mm in 5 years	≥2 mm in 5 years
	Indirect evidence	% bone loss/age	<0.25	0.25 to 1.0	>1.0
		Case phenotype	Heavy deposits with little destruction	Destruction correlates with deposits	Destruction disproportional to deposits
Grade Modifiers	Risk factors	Smoking	Non-smoker	<10 cigarettes/day	≥10 cigarettes/day
		Diabetes	No diabetes diagnosis	HbA1c <7.0% in diabetic patient	HbA1c ≥ 7.0% in diabetic patient

1.1.5 Etiology: There are two types of risk factors which are patient-specific (Genco, R. J.,2013).

1.1.5.1 Modifiable risk factors:

1. Microorganisms (specific pathogen)
2. Smoking
3. Poorly controlled diabetes mellitus
4. Stress
5. Poor self-care
6. Untreated human immunodeficiency virus or acquired immunodeficiency syndrome
7. Oral effects of some metabolism
8. Local factors
9. Obesity
10. Improper diet
11. Chronic inflammation

1.1.5.2 Non Modifiable risk factors:

1) Osteoporosis
2) Some hematological disorders
3) History of periodontitis
4) Age
5) Gender
6) Race
7) Genetic disorders

8) Bone level

Periodontitis initiated primarily and developed because of poor oral hygiene habits. Inadequate and poor dental hygiene can lead to accumulations of plaque calculus and debris around the teeth, causing swelling, redness and inflammation and which possibly leads to gingivitis and then periodontitis. The research has shown that there has been a link between severity, progression and prevalence of periodontal diseases. Insufficient or poor oral health can allow the microorganisms to cause periodontal illnesses, and it will spread and invading the deeper tissues of perioodntium, where they can conduct their damaging work. *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* are the principal bacteria implicated in periodontitis. These organisms cause inflammation when they are let penetrate far into the periodontium because they cause the host to release inflammatory mediators and other defensive substances. (Ridgeway, 2000) (Nazir, M. A. 2017) (Albandar JM. 2002) (Zee, K. Y. 2009) The main bacteria linked to periodontitis include *Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola,* and *Aggregatibacter actinomycetemcomitans.* When these organisms are allowed to go deeply into the periodontium, they trigger the production of defence chemicals and inflammatory mediators by the host. As stated by Ridgeway (2000) (Zed, K. Y. 2009; Albandar, J. M. 2002; Nazir, M. A. 2017)

Tobacco smoking is the most notable significant modifiable risk factor for periodontal disorders. The odds ratio between smoking and chronic periodontitis is 5.4, meaning that it might raise the risk of periodontal disorders by a factor of 5 to 20. Furthermore, compared to non-smokers, tobacco users had higher rates of bone loss, attachment loss, deep periodontal pockets linked to the illness, and tooth loss. Tobacco smoking is linked to a marked decline in treatment efficacy in addition to a heightened severity of periodontal disorders. (Bergström, J. 2004) (Hilgers, K. K.,2004) (Brothwell, D. J. 2001) (Grossi, S. G., 1996).

Another significant risk factor for periodontal diseases is diabetes mellitus. This illness is linked to specific pathologic processes, such slowed wound healing, that promote periodontal degradation. The complications category contains more relationships

between diabetes mellitus and periodontal disorders. Compared to individuals without or with mild periodontal disease, those with diabetes mellitus who have severe disease have a higher chance of dying. (Nazir, M. A. 2017) (Douglass, 2006).

Hormone changes brought on by pregnancy have been demonstrated to incite an inflammatory response that is connected to periodontitis and gingivitis. Maternal hormones have been demonstrated to positively link with *Porphyromonas gingivalis* levels, a crucial bacterium in the advancement of periodontal disease, for reasons that are yet unclear. It has been demonstrated that hyper- and hypoestrogenism both contribute. (Daalderop, L. A., 2018) (Uwitonze, A. M., 2018) (Carrillo-de-Albornoz, A., 2010) (Wu, M., 2015)

Age is a risk factor for periodontal diseases that cannot be changed and has been widely studied in the literature. It has been demonstrated that older people respond to plaque deposition with a more severe inflammatory response that includes more inflammatory cells. The deterioration of the periodontium is more likely to occur in elderly people due to this inflammatory cell accumulation. Furthermore, because ageing is linked to a decline in dexterity, oral hygiene habits are often less proficient in older adults. Higher levels of plaque are the consequence, and this is a known risk factor for the emergence of periodontal disorders. Moreover, studies have shown that people 60 to 90 years old have higher clinical attachment loss (CAL) than people under 50 years old. (Persson, G. R. 2018) (Grodstein, F., 1996) (Rheu, G. B., 2011).

In a research, periodontal disease was found in 52.1% of the population based on radiographic bone loss. In comparison, an earlier study carried out in Abha, Saudi Arabia, discovered an overall frequency of 36.68% (Zahid et al., 2018). A study found that 36% of patients had mild periodontitis, 13.9% had moderate periodontitis, and just 1.9% had severe periodontitis based on the amount of radiographic bone loss. By contrast, a greater frequency of mild, moderate, and severe periodontitis was reported by Zahid et al. (36.4 % of patients had mild, 36.6 % had moderate, and 4.95% had severe disease) The results showed that, in comparison to female patients, the male population had a greater frequency of alveolar bone loss and periodontal disease (Hossain, M. Z. et al 2018).

Finally, it has been demonstrated that several genetically associated systemic illnesses can present as periodontal diseases. The research has also established the etiology of the development of periodontal diseases within these systemic disorders. These conditions include Crohn's disease, Down syndrome, and Ehlers-Danlos syndrome (types IV and VIII). (Borgnakke, W. S. 2015) (Nualart Grollmus, Z. C., 2007) (Kim, J., 2006).

1.1.6 Pathophysiology:

The mechanisms behind the periodontal disease, a genetically connected pathology determined by anaerobic infection of microorganisms, have been revealed by an astounding evolution in dental research today (Cafiero, C. 2013). According to Cafiero (2013) and Paster (B. J. et al., 2001), the most prevalent genera of Gram-negative bacteria in the oral cavity and consider *Actinobacillus actinomycetemcomitans* as the primary causes of periodontal disease. These microorganisms continue to be a major focus of periodontal disease research (Paster, B. J. et al., 2001). The oral microbiome is a term used to describe the community of microorganisms that lives in the oral cavity and is thought to consist of between 500 and 700 common species. (Paster, B. J etal.,2001), (Dewhirst, F 2010).

Dental plaque is the primary cause of gingivitis and periodontitis. About 150 distinct and different kinds of bacteria have been detected in a single human, while 800 different types of germs have been found in dental calculus. The species include viruses, spirochetes, and anaerobic gram-negative bacteria. A "pathogenic unit" is formed when these microorganisms are out of balance, which occurs in cases of chronic periodontal disease. (Kato, T.,2020).The cause of gingivitis is microbial biofilm. Microbial biofilm development depends on the dysbiosis of the ecological shifts in harmful byproducts and an enzyme that breaks down the tissue. These biofilms are a type of matrix that are adhered to the surface of teeth by various microbial species colonies. (Berglundh, T.,2005). There are seven stages of plaque biofilm formation are given as:

	STAGES:	PLAQUE BIOFILM FFORMATION
1	Pellicle Formation	Occurs by adsorption of Host and bacterial molecules, salivary glycoprotein on tooth surface.
2	Transport	Occurs via natural salivary flow, transport of bacteria such as *Neisseria, Streptococcus Sanguis, S. oralis, S. mitis* and *Actinomyces* to the pellicle occurs.
3	Long Range Interactions	This stage leads to reversible adhesion with Vander Wall's and electrostatic forces between microbial cell surface and the pellicle.
4	Short Range Interactions	This stage leads to irreversible interaction between microbial cell surface and pellicle.
5	Co- Aggregation	Increased micro flora diversity due to co-adhesion of new microbes over already attached microbes.
6	Multiplication	Multiplication of adhered bacteria on tooth surface led to severity of periodontal disease.
7	Detachments	Detachment of colonies to the new site for confluent growth.

1.1.6.1 Structure of the dental biofilm:

Microbial cells encased in EPM, which is formed from the host and the invading microbiota, make up biofilms(Jakubovics, N. S et al 2021)Approximately 700 different species are involved in the bacterial component of dental plaque biofilms, according to research using molecular-based sequencing (Dewhirst, F. E et al 2010).The bacteria in biofilm are grouped both geographically and functionally, rather than randomly (Earle, K. A., 2015). Depending on their environment or location, different bacterial species and types of microbiotas can be found in dental biofilms. On a single tooth surface, the supragingival biofilm may contain more than 109 microorganisms. The number of germs in a periodontal pocket can vary, ranging from 103 in a healthy crack to more than 108 in a deep pocket. (Fig 1.4)

1.1.6.2 Formation and development of the dental plaque biofilm

Formation of acquired pellicle:

A condensed layer of macromolecules called the acquired pellicle at the base of the biofilm should exist prior to the colonization of organisms inside the dental biofilm (Listgarten, M. A. et al 1976) (Lai, C. H., Listgarten, M. A., et al 1975). This layer was previously believed to be mostly formed by salivary glycoproteins, but a recent study suggested that gingival crevicular fluid (GCF) had a significant role in its creation (Larsen, T. et al 2017) (Odanaka, H., et al 2020). (Fig 1.5)

Adhesion Of Bacteria:

The biofilm matrix is the primary means by which bacteria adhere at this stage. The bacteria's attachment within the biofilm is initiated by fimbriae or pili which is a specialized appendage, which are made up of subunits called fibrillin and possess adhesins that selectively adhere to pellicle-coated teeth or to other bacteria (Handley, P. S., et al 1984).The weak bonds that contribute to this attachment are Lifshitz-van der Waals, Lewis acid-base, and electrostatic interactions (Huang, R., et al 2011) (Hannig, C., et al 2009). Numerous bacterial species, such as *Streptococci, Actinomyces,* and P. *gingivalis,* are known to harbor fimbriae (Handley, P. S., et al 1984). Fibrils, which differ from fimbriae in shape and distribution and are shorter, aid in bacterial adhesion

as well. Certain strains of *Streptococcal bacteria, P. nigrescens*, and *Prevotella intermedia* are among the oral bacteria that contain fibrils (Handley, P. S. (1990) (Devine, D. A. et al 1989)(Hogg, S. D., et al 1981)

1.1.6.3 Maturation of biofilm and coaggregation of bacteria:

As late colonizers such as *F. nucleatum, T. forsythia, P. gingivalis, P. intermedia,* and A. *actinomycetemcomitans* recognize polysaccharide or protein-binding sites on the cell surface of primary colonizers, the dental plaque biofilm begins to mature. As a result, primary colonizing bacteria such *Neisseria* and *Streptococci* are reduced in quantity while the proportion of late colonizers in the dental plaque biofilm rises (Ritz, H. L. 1967).

An extensive knowledge of the bacterial populations of the subgingival microbiota associated with biofilms was made possible by the research of Socransky et al 1998. Five complexes of bacteria were created and each complex was given a distinct color(Socransky et al 1991). The red complex of pathogens includes *P. gingivalis, T. forsythia,* and *T. denticola,* which are primarily detected in patients with periodontitis. Furthermore, there is a common link between periodontitis and the orange complex pathogens, *Fusobacterium, Prevotella,* and *Campylobacter species*. (Fig 1.6)

The other complexes such as the yellow complex (composed of different *Streptococcus species*) and green complex (composed of *Capnocytophaga species*) were strongly associated with periodontal health. Yellow, green, and purple complexes are the primary colonizers and considered as a prerequisite for the appearance of the orange and red complexes (secondary colonizers)

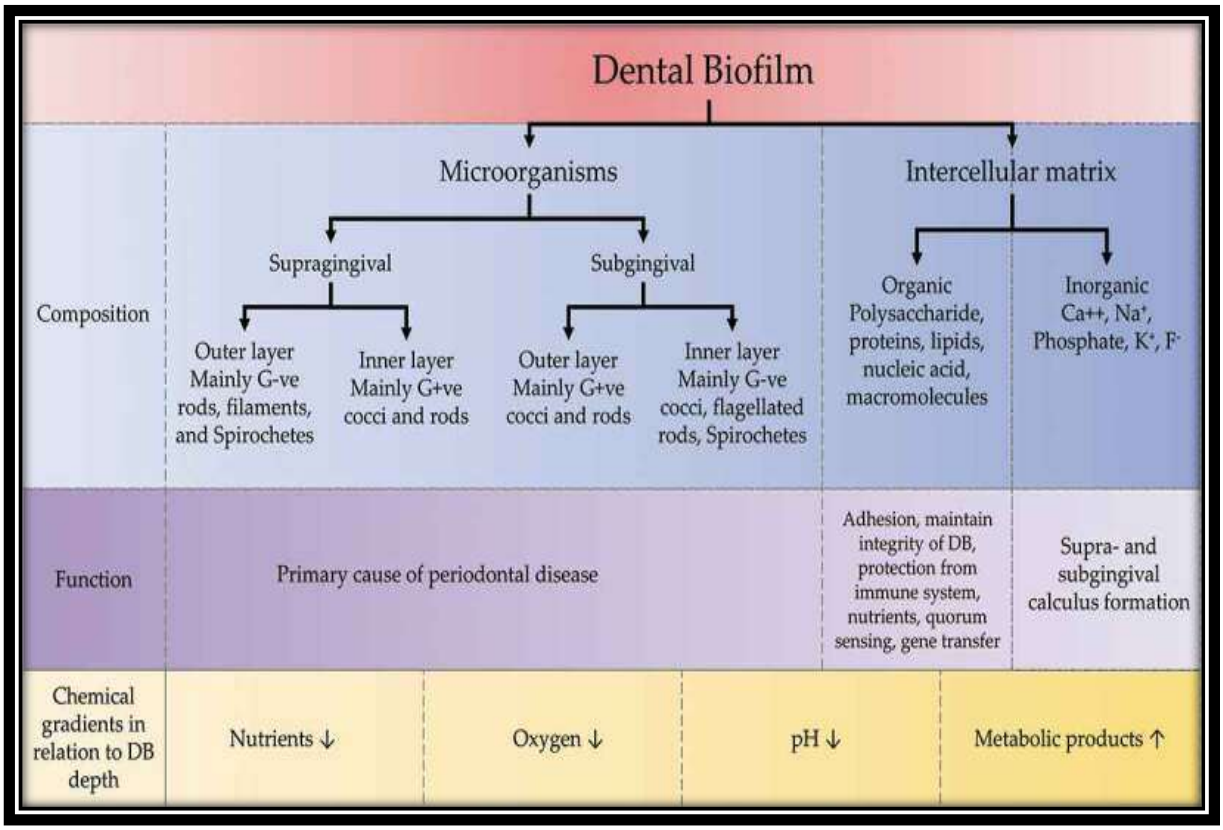


Fig 1.4: Components of dental biofilm with their functions and relation of chemical gradients to the depth of dental biofilm. (Abdulkareem, 2023)

DB: dental biofilm, G+ve: Gram-positive, G-ve; Gram-negative.

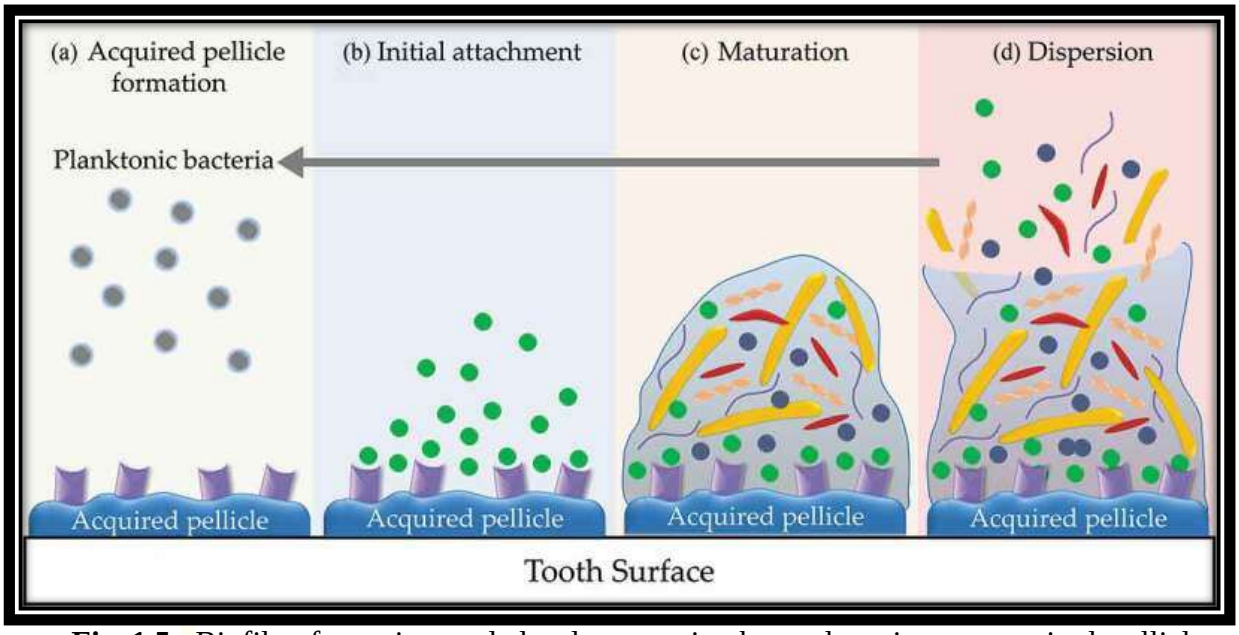


Fig 1.5: Biofilm formation and development in the oral cavity. a. acquired pellicle formation; b. initial attachment of early colonizers; c. maturation of biofilm and coaggregation of bacteria; D. dispersion of bacteria. (Abdulkareem, 2023)

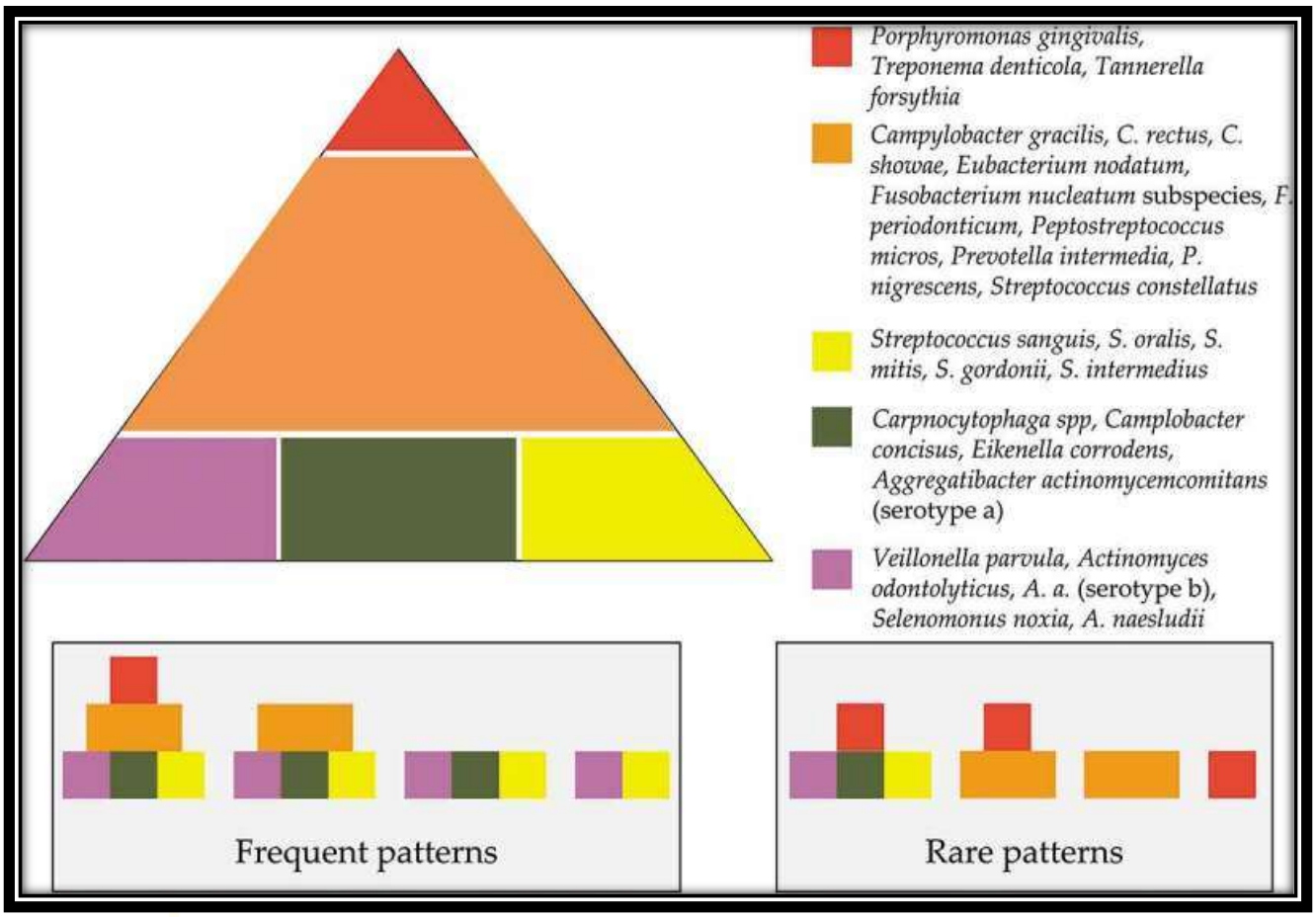


Fig 1.6: The association among subgingival species (adapted from Socransky et al). The base of the pyramid represents the early colonizers, followed by the orange complex, which bridges the early colonizers with the red complex that dominates the biofilm at the advanced stages of periodontitis.

To support the 2018 classification of periodontal diseases (Caton, J. G. et al 2018), a new theory known as the "Inflammation-mediated polymicrobial-emergence and disease exacerbation" (IMPEDE) model (Van Dyke, T. E., et al 2020) has emerged considering how dysbiosis and the associated aggravation of periodontal tissue destruction are driven by environmental changes. This categorization views the progression of periodontitis from a state of health to a disease in terms of four phases: severity, complexity, extent, and dispersion. A potential cause of the clinical problems that might appear at each stage of periodontitis is the IMPEDE model. The IMPEDE model describes five stages encountered, which are identical to the clinical categorization of periodontal disease: health, gingivitis, and periodontitis that may develop, progress, or remain confined.

The remaining four stages signify the progression of the illness, with the healthy condition being as:

Stage 0 (lack of clinical inflammation).

Gingivitis is the first stage, characterized by inflammation brought on by commensal bacteria growing in response to a non-specific dental plaque accumulation in susceptible people, which can result in gingival swelling and early pocket development.

Stage 2 is known as early periodontitis, during which dysbiosis and a rise in polymicrobial diversity coincide with rising inflammation.

Stage 3 is a self-sustained feedforward loop-driven worsening of inflammation-induced dysbiosis, wherein more pathobionts and symbionts tend to favor the growth of Gram-negative organisms. If the inflammation is brought under control, commensal bacteria can once again take center stage.

Stage 4 is late periodontitis, which in susceptible individuals is defined by the appearance of a polymicrobial infection with a decreasing diversity of polymicrobial species; anaerobic microbial species predominate in the associated environment, and this, combined with uncontrollable inflammation, leads to advanced tissue destruction.

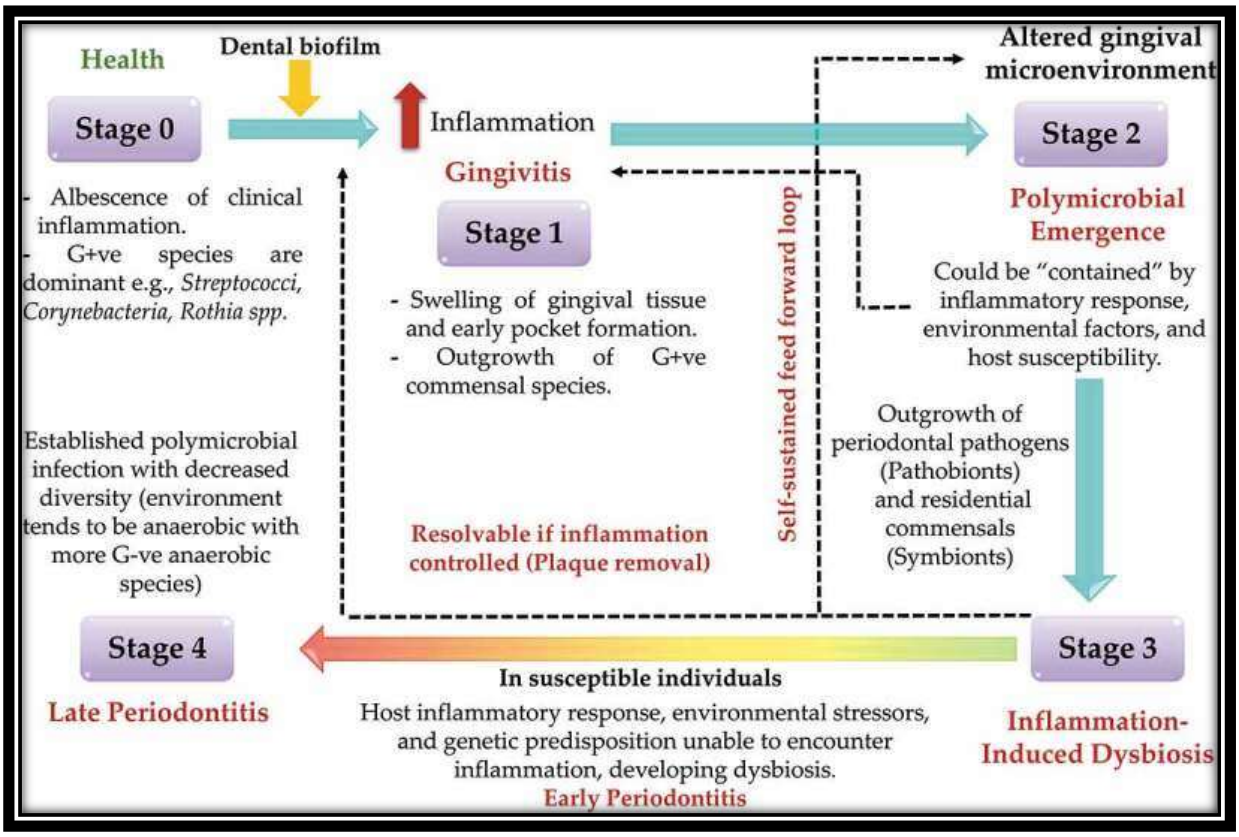


Fig 1.7: Inflammation-Mediated Polymicrobial Emergence and Dysbiotic Exacerbation (IMPEDE) model. According to this proposed model, plaque-induced periodontitis is mainly derived from inflammation. This model consists of 5 stages: stage 1: gingivitis, stage 2: emergence of polymicrobial diversity in early periodontitis, stage 3: inflammation mediated dysbiosis and opportunistic infection, and stage 4: late stage of periodontitis. (Van Dyke et al., 2020)

1.1.7 Need for a Biomarker:

Recent years have seen an incredible advancement in dental research that has revealed the intricate mechanisms underlying periodontitis, a genetically linked pathology characterized by composite immune responses in response to bacterial load and determined by gram-negative anaerobic infection. Currently, a full clinical status check of the patient's periodontal health is performed to diagnose periodontal disease. This includes measuring the patient's probing depth, clinical attachment level, bleeding on probing, recessions, mobility, and migration. The results are supplemented by digital photos and periapical X-rays. Periodontists only begin treating periodontal diseases once they have already started, which means that their approach to defense is now almost entirely reactive (Cafiero, C et al 2013).

"Bleeding on Probing, (BoP)" (Lang, N. P. et al 1986) is a special prediction test that periodontists use to regularly assess the stability or advancement of periodontitis (Fig 1.8). It is obtained by inserting a periodontal probe at the base of the gingival sulcus or periodontal pocket. Blood that emerges from the bottom of the pocket can be recorded during probing. BoP that is repeatedly negative (BoP−) is a predictor of periodontal health in 98% of cases (negative predictive value); in 30% of cases, BoP+ is a predictor of future loss of attachment (activity phase). Furthermore, a "Spider's web" functional diagram has been presented (Lang, N. P., et al 2003) to assess the patient's risk of recurrent periodontitis. As there are now relatively few "periodontal predictive" equipment available, periodontists mostly employ "periodontal reactive approach" instruments to diagnose periodontitis (Lang, N. P. et al 1996) (Lang, N. P., et al 1990).

The purpose of a “futuristic” periodontal diagnosis soon will be to diagnose periodontal disease before it comes clinically detectable in order to prevent its progression by the use of biomarkers. Traditionally, the diagnosis of periodontal disease has been made by using clinical and radiographic tests that show a prior history of the condition but are unable to identify active illness. The demand for early periodontal disease diagnosis is unfulfilled since periodontal disease progresses without symptoms, many patients wait until irreversible periodontal damage has already occurred before seeking professional dental care, and so on (Monje, A. et al., 2021). A full clinical status examination of the

patient's periodontal health, including the plaque score and bleeding, is performed to make the diagnosis of periodontitis. (Cafiero, C. 2013). To track the progression of the illness and to stop further damage, it might be helpful to identify periodontal tissue deterioration. (Lang, N. P., & Tonetti, M. S. *23*).Biomarkers are biological indicators that have a strong prognostic and predictive capacity and are related to the beginning or progression of a disease. These markers can be utilized as indicators of the progression of periodontal disease. And can be used for the early detection of periodontitis, according to several studies. They must have a strong prognostic or predictive value and be able to measure precise results quickly. If it is a disease marker, it must forecast the presence of the disease, or if it is a treatment response marker, it must indicate the right sort of medicine and response.

The use of salivary biomarkers for the early identification of periodontitis has gained popularity in recent years. According to Giannobile et al. (2009) and Sexton et al. (2011), saliva is a useful oral fluid for assessing the condition of the oral cavity, including the existence of periodontal disease. It is a cost-effective, non-invasive diagnostic technique that can offer important details about the existence and severity of periodontal disease. There are a lot of promising salivary biomarkers linked to PD that have been reported. (A. Monje et al., 2021). Salivary biomarkers are biomolecules that can be utilized as indications of the progression of periodontal disease. Salivary biomarkers may be used studies, the early detection of periodontitis, according to a number of studies.

Fig 1.8: Blood coming out during probing Cafiero, C. et al 2021).

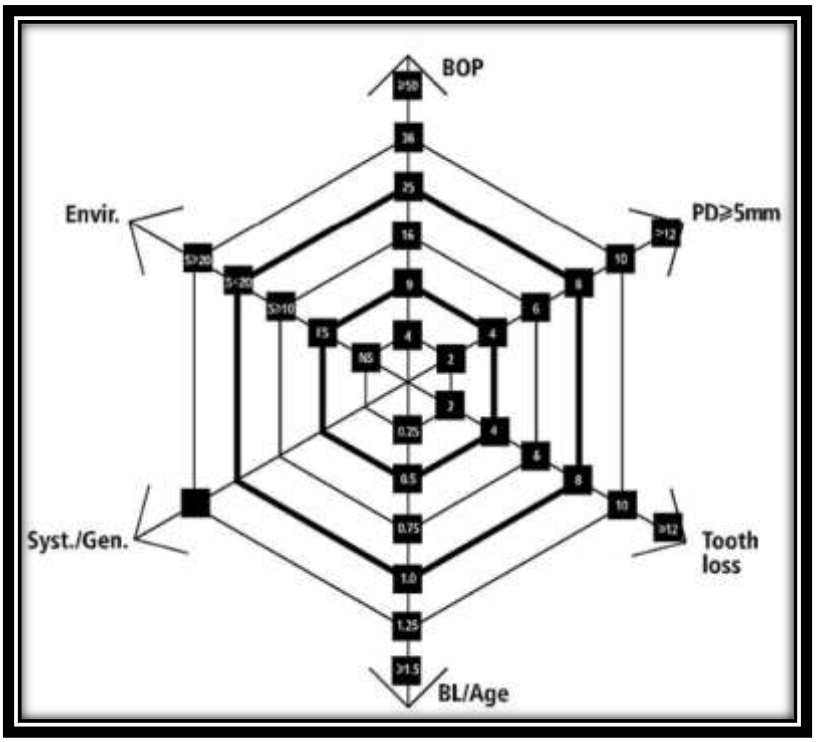


Fig 1.9: Functional diagram to evaluate the patient's risk for recurrence of periodontitis. Each vector represents one risk factor with an area of low risk, an area of moderate risk and an area of high risk for disease progression. (Lang, N. 2003)

1.1.8 Immune Response in Periodontitis:

Periodontists are expected to have enormous cultural influence on a global scale to transform the current "reactive" therapy perspective into a more advanced "predictive" one. Because of this, it is crucial to (i) diagnose a periodontal initial lesion before it becomes clinically apparent and (ii) to stop periodontitis in its so-called "active phase." Clinicians must use advanced diagnostic instruments to identify the precise biomarkers secreted in the early stages of the immune response to obtain this outcome. Clinically healthy periodontal tissue is characterized by the host-microbial balance; when plaque bacterial load arises, it triggers a significant immune response that produces a variety of chemicals in periodontal tissues, some of which may be suitable as biomarkers for the early diagnosis of the disease.

Using periodontal biomarkers, we may predict periodontal disease and treat the illness as soon as feasible even when periodontal tissues lesions are not clinically obvious. The primary cells implicated in the pathophysiology of periodontal disorders are osteoclasts, macrophages (Mθ), and polymorphonuclear leukocytes (PMN). (Cafiero, C., & Matarasso, S. 2013). The following is a brief description of their roles in the periodontal immune response. We concentrated on the immunological pathways and associated cells that result in the release of molecules suitable as biomarker candidates for an early diagnosis of periodontitis in this succinct explanation of the pathophysiology of the disease.

1.1.8.1 Activation of Polymorphonuclear Leukocytes (PMN)

Because PMN leukocytes accumulate in gingival epithelial cells, they can harm tissue while being the first line of defense for periodontal tissues. Several other enzymes and oxygen metabolites that PMN releases during the immunological response have the potential to induce further tissue damage. These actions lead to the formation of ulcers on the junctional epithelium, which permits bacteria to penetrate through connective tissue below. (Fig1.8) Matrix metalloproteinase-8 (MMP-8) or neutrophil collagenase is one of the most typical enzymes implicated in the extracellular matrix degradation in periodontitis. MMP-8's main job is to break down type I, II, and III collagens to assess the loss of periodontal attachment.

1.1.8.2 Activation of Macrophage(Mθ)

The majority of macrophages are members of the second line of defense. They are an important source of enzymes, cytokines, and inflammatory mediators like Prostaglandin E2 (PGE2), Tumor Necrosis Factor-α (TNF-α), Macrophage Inflammatory Protein-1 Alpha (MIP-1α/CCL3), and they play a decisive role in controlling the diffusion of bacteria in the connective tissue. The following lists these compounds' principal purposes: LPS-activated macrophages, lymphocytes, and fibroblasts produce IL-1 β. TNF-α is primarily released by LPS-stimulated macrophages and lymphocytes, which promotes osteoclastic differentiation and activation (Beutler, B., & Cerami, A. 1986); it also induces Mθ and fibroblasts to secrete PGE2 and causes osteoclastic differentiation and activation. (Dewhirst, F. E., 1987).

1.1.8.3 Activation of Osteoclasts:

Osteoclastic activation is mediated by many substances (PGE2, IL-1, IL-6, TNF-α) released by Mθ, fibroblasts, plasma cells, and T lymphocytes. Inhibiting osteoclast apoptosis and promoting osteoclastic differentiation are two functions of the receptor activator of NF-kB ligand (RANKL). (Yasuda, H. 2021).

1.1.8.4 Salivary Products Delivered by Immune Response Hosts as Biomarkers for Early Periodontal Diagnosis

Substances in biologic samples that have the potential to predict a patient's illness condition are referred to as "biomarkers." The term has, however, expanded to encompass proteomics or genomics, which may also be utilized to predict "a response to a drug (efficacy, toxicity, or pharmacokinetics) or indicate an underlying physiologic mechanism." (Ray, P.2010)

Numerous dental societies acknowledge the value of scientific research on oral fluid diagnostics, including the American Dental Association (ADA). (Cafiero, C., 2021). It will be ideal in regular dentistry soon to employ a chair-side lab-on-a-chip (LOC) to identify biomarkers for numerous dental problems. Together, industry and research should develop LOCs that enable operators to quickly identify biomarkers in very tiny amounts of entire saliva. This will help diagnose periodontal disease and other oral disorders at an early stage. Gingival crevicular fluid and glandular-duct saliva are two

types of oral fluid (whole saliva), in which several chemicals produced by the immune response may be found. (Taba, M., Kinney, 2005).

Recently, several research have established that host-delivered biomarkers may play a critical role in the early identification of periodontitis. (Ghallab, N. A. 2018). There are 1166 proteins in human saliva, according to reports on the total salivary proteome during the past ten years (Esteves, E. J. M. 2023). Some of which have been identified as potential biomarkers for periodontal disorders. (Armitage, G. C. 2004).

1.1.9 "Saliva: a reliable predictor for diagnosis– Need of an hour"

Obtaining saliva is a basic simple, non-invasive body fluid test that can be useful for diagnosis since saliva includes omics components (genomics, transcriptomics, proteomics, metabolomics, and metagenomics) have contributed to the identification and characterization of salivary components, including DNA, RNA, proteins, metabolites, and microorganisms, that might indicate the periodontal tissue's present physiological state. (Zhang, Y.,2016). Over the last several decades, a range of salivary indicators, including cytokines, Jaedicke, (K. M., 2016), host enzymes, (Ramseier, C. A.,2009) bacteria, (Belstrøm, D.,2018) and bone metabolic products, (Frodge, B. D 2008) have been studied as potential targets for distinguishing periodontitis sufferers from healthy individuals.

Patients have gingival inflammation without the loss of bone or connective tissue during the gingivitis stage of periodontal disease progression. Patients with periodontitis initially exhibit gingival inflammation and connective tissue degeneration, which subsequently advances to alveolar bone deterioration. We think that a biomarker combination might be more useful for determining the disease state since the changes in markers happen sequentially at different stages of periodontal disease. Currently, well-researched compounds found in oral fluid (whole saliva) and linked to host response variables have been suggested as periodontitis diagnostic biomarkers. (2005) (Taba, M. et al.). According to I. B. Lamster (1997), there have been over 65 oral components identified and tested as potential indicators of periodontitis progression. Three of the most promising biomarkers have been chosen from the group as possibilities for early periodontitis diagnosis. To create an efficient prediction panel for periodontal disease

diagnosis, the study will compare the diagnostic efficacy of these markers which stand for inflammation and tissue degradation among healthy and periodontitis individuals.

1.1.10(a)Metalloproteinase-8(MMP-8):

MMPs are important proteases linked to periodontal health and implicated in periodontitis. (Rathnayake,2015). Collagenases and gelatinases, such as MMP-8, MMP-13, MMP-2, and MMP-9, have received particular interest in periodontitis because type I collagen makes up a significant portion of the extracellular matrix in the periodontal cavity. Special focus has been given to collagenases because type I collagen makes up most of the periodontal extracellular matrix. This includes MMP-8, which is the primary collagenase in periodontitis and the source of 90% to 95% of the collagenolytic activity in gingival crevicular fluid. As a result, one of the most promising biomarkers for periodontitis in oral fluids now. (Franco, C., 2017). It is an enzyme that polymorphonuclear cells release during an immunological response (Zhang, L. et al., 2009). MMP8 levels in saliva seem to be an important biomarker for identifying periodontitis. (2018) Lahdentausta et al. (2011) (Buduneli, E. et al.) Recent reports have shown that local and systemic levels of aMMP-8 can reflect the grading and staging of periodontitis. (Sorsa, T.2020). A study conducted in Brazil and researchers investigated the levels of MMP-8 in periodontal patients. They utilized enzyme-linked immunosorbent assay (ELISA) to quantify MMP-8 levels in gingival crevicular fluid (GCF) samples collected from the participants. The findings of this study contribute to understanding MMP-8 as a potential biomarker for periodontal disease. (Räisänen, I.2023).

1.1.10(b)Interleukin-1beta (IL-1*β*):

As a cytokine that promotes inflammation, IL-1β has a role in immunological control, bone resorption, and inflammation in periodontitis. Its relevance is well supported by the clinical data. The biological impact of IL-1β is contingent upon its concentration in tissue, which is heightened in cases of periodontitis. Saliva and gingival crevicular fluid (GCF) from people with periodontitis are often shown to have higher amounts of IL-1β in comparison to healthy controls (Rangbulla, 2017).

GCF IL-1β levels are higher in patients with greater pocket depths and more severe bleeding on probing (BOP) (Kinney et al ,2014). MMPs, which are collagenolytic enzymes that aid in the breakdown of extracellular matrix and ultimately cause bone resorption and tissue damage, are expressed more often when L-1β is present (Schett, G. et alv2016). It's a key marker of the severity and course of periodontitis is MMP-9(Rai, B., et al 2008). MMP-9 expression is upregulated by IL-1β in a variety of periodontal inflammation-related cell types, such as neutrophils, cementoblasts, osteoblasts, and osteoclasts (Du, M et al 2019). Osteoblastic cells, gingival fibroblasts, gingival lymphocytes, and macrophages activated by IL1β that have been stimulated by lipopolysaccharide as stated in Czuszak (1996). It causes these fibroblasts and macrophages to release prostaglandin E2, which will influence how much bone is destroyed (Cheng, R., et al 2020).

1.1.10(c)Interleukin-6 (IL-6):

A pro-inflammatory cytokine released by osteoblasts to promote osteoclastic activity and by macrophages in response to certain bacteria.Periodontal disease develops because of localized inflammation, tissue damage, and the presence of tooth plaque bacteria and their byproducts. As demonstrated, by promoting osteoclast development and bone resorption and suppressing bone formation. (Apolinario Vieira, 2021) When present at high quantities, IL-6 mostly increases the activation of mature osteoclasts, however it can also stimulate the maturation of osteoclasts from precursors. (Kudo, O.,2003). Important cytokines like IL-6 control how the body reacts to bacterial infections (Balta, M. G et al 2021). The typical invasion of neutrophil cells is followed by monocyte cells whenever an inflammatory process is started because of a traumatic injury or inadequate hygienic care. Numerous cytokines are created during the inflammatory process after the formation of inflammatory cells, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). However, IL-6 is one of the most well-known interleukins (Iwashita, M. (2023). Research has indicated that IL-6 is linked to MMP release and activation, which may lead to severe extracellular matrix (ECM) degradation in periodontal disease (PD) patients with elevated blood levels of IL-6. (Isola G., 2021)

1.2 RATIONALE OF THE STUDY

Salivary biomarkers have become an important focus of research in the diagnosis and management of periodontitis. Periodontitis is a chronic inflammatory disease affecting the supporting structures of the teeth, leading to progressive bone loss and, eventually, tooth loss if left untreated. Traditional diagnostic methods for periodontitis include clinical measurements such as probing depth, clinical attachment level, and radiographic assessment. However, these methods are invasive and often detect the disease only after significant damage has occurred and these biomarker on initial stages offers a promising non-invasive alternative for monitoring the disease before it becomes clinically detectable.

1.2.1 THEORETICAL GAP

Identifying theoretical gaps in the research on salivary biomarkers for periodontitis is crucial for advancing the field and improving clinical outcomes. Understanding the cellular and molecular sources of salivary biomarkers and their regulatory mechanisms in the context of periodontal disease is essential. This includes studying how systemic conditions might influence these biomarkers. Many salivary biomarkers are not specific to periodontitis and can be elevated in other inflammatory or systemic conditions. Research is needed to identify biomarkers or combinations of biomarkers that can specifically differentiate periodontitis from other diseases. There is a need for biomarkers that can detect periodontitis at its very early stages and predict disease progression and treatment outcomes. Developing more robust and sensitive analytical techniques for the detection and quantification of salivary biomarkers is necessary. This includes advancements in technologies like mass spectrometry, ELISA, and next-generation sequencing. Most studies have been conducted on specific populations, often with limited diversity. Research involving diverse populations is needed to ensure that findings are generalizable and applicable to different demographic groups. Many potential salivary biomarkers have been identified in research settings, but few have been validated for clinical use. Rigorous

clinical trials are needed to confirm their diagnostic and prognostic utility. Assessing the cost-effectiveness of salivary biomarker testing and ensuring it is accessible in various healthcare settings is essential for widespread clinical adoption.

1.2.2 CONTEXTUAL GAP/ANALYSIS

A few studies on this topic have been conducted in Pakistan, and yet they have not combined all of these biomarkers together. No study in Pakistan has detected most promising salivary biomarkers in periodontitis patients.

1.2.3 METHODOLOGICAL GAP/ANALYSIS

Identifying methodological gaps in the use of salivary biomarkers for periodontitis can guide future research and improve the reliability and clinical utility of these biomarkers. There is a lack of standardized methods for saliva collection. Variability in collection techniques (e.g., unstimulated vs. stimulated saliva, time of day, pre-collection instructions) can affect biomarker levels. Differences in saliva processing (e.g., centrifugation, filtration) and storage conditions (e.g., temperature, duration) can lead to degradation or alteration of biomarkers, affecting the consistency and reliability of results. The selection of appropriate control groups (healthy individuals vs. patients with other inflammatory conditions) is crucial for distinguishing periodontitis-specific biomarkers. Most studies are cross-sectional, providing a snapshot of biomarker levels at a single point in time. Longitudinal studies are needed to track biomarker changes over time and correlate them with disease progression and treatment outcomes.

1.3 PROBLEM STATEMENT

Despite significant advances in the identification and analysis of salivary biomarkers for periodontitis, several challenges impede their effective clinical application. Periodontitis, a prevalent chronic inflammatory disease affecting the supporting structures of the teeth, requires early and accurate diagnosis to prevent severe complications such as tooth loss

and systemic health issues. Traditional diagnostic methods, although effective, are invasive and often fail to detect the disease at its initial stages and these biomarkers offer a promising non-invasive alternative.

1.4 RESEARCH HYPOTHESIS

Null Hypothesis: MMP8, IL-1β and IL-6 levels do not significantly increase in salivary sample of chronic periodontitis.

Alternative Hypothesis: MMP8, IL-1β and IL-6 levels significantly increase in salivary sample of chronic periodontitis.

1.5 OBJECTIVES

- To measure the levels of MMP-8, IL-1β and IL-6 in salivary samples of periodontitis and healthy participants.
- To associate the levels of MMP-8, IL-1β and IL-6 with clinicopathological parameters in salivary samples of periodontitis and controls.

1.6 SIGNIFICANCE OF STUDY

The study of salivary biomarkers for the detection of periodontitis is highly significant due to its potential to transform the diagnostic and management landscape of periodontal disease. By providing a non-invasive, efficient, and personalized approach to early detection and monitoring, biomarker research holds promise for improving patient outcomes, reducing healthcare costs, and advancing our understanding of the disease. Continued investment in this research area is essential for realizing these benefits and achieving better oral and systemic health for the population.

CHAPTER 2

LITERATURE REVIEW

Gingivitis and periodontitis together make up periodontal disease, a common oral infection that damages the tissues surrounding and supporting teeth. (Newman, M. G., 2011). A chronic inflammatory condition known as periodontal disease affects the alveolar bone, gingiva, and periodontal ligament as well as the tissues that support the teeth. One of the most important, prevalent illnesses and a public health concern is severe periodontitis. (Kassebaum, N. J., 2014). It is the sixth most common inflammatory gum disease and one of the most common oral disorders, impacting millions of individuals globally. The Global Burden of Disease (GBD) research from 2019 estimates that 1,087.37 million individuals worldwide now have severe periodontitis, with a 50% global prevalence. (Chen, M. X., 2021).

According to W.H.O, it was reported that 18% population of Pakistan has some form of periodontal problems and out of these 31% has periodontitis. A systematic review of periodontitis in Pakistan in 2022 reported the prevalence of periodontitis at 37% in Punjab, 40% in Sindh, 20% in Khyber Pakhtunkhwa and 3% in Baluchistan. (Fahim, A et al 2022).

Periodontitis and gingivitis are common in all age groups, although they are more common in people with risk factors such as long-term drug usage, poor dental hygiene, diabetes, smoking, anxiety, stress, and inherited causes (Lertpimonchai, A et al 2017). Any gum disease's major cause is plaque. Adolescents with significant calculus deposits varied from 35% to 70% in impoverished countries and from 4% to 34% in industrialized countries (Elías-Boneta, A. R. et al 2018).Periodontitis has been classified into many categories by the American Academy of Periodontology (AAP) (Sanz M., et al 2020)..Understanding the incidence of periodontitis in Pakistan is crucial since treatment options are mostly based on a patient's risk assessment. To stop the disease's progression, which can cause major problems like tooth loss, bone

loss, and systemic disorders, early detection and treatment are crucial. The use of salivary biomarkers for the early identification of periodontal disease has drawn more attention in recent years.

An amazing progression in dental research today has revealed the processes behind the condition known as periodontal disease, a genetically related pathology dictated by anaerobic infection of microorganisms (Cafiero, C. 2013). In the oral cavity, the most common species of gram-negative bacteria include *Actinobacillus actinomycetemcomitans*, which is thought to be the main cause of periodontal disease (Cafiero, 2013; Paster, B. J. et al., 2001). Research on periodontal disease is still heavily focused on these bacteria (Paster, B. J. et al., 2001). The population of microorganisms that reside in the mouth cavity is referred to as the oral microbiome, and it is estimated that there are 500–700 common species in it. (Dewhirst, F. 2010), (Paster, B. J. et al., 2001). The main cause of periodontitis and gingivitis is dental plaque. While 800 different forms of germs have been identified in dental calculus, about 150 distinct and diverse kinds of bacteria have been found in a single individual. Among the species are spirochetes, anaerobic gram-negative bacteria, and viruses. When these microbes are out of balance, as they are in cases of chronic periodontal disease, a "pathogenic unit" is generated. (T. Kato, 2020). The microbial biofilm is the cause of gingivitis. An enzyme that breaks down tissue and the dysbiosis of adverse ecological alterations in byproducts are necessary for the formation of microbial biofilms. These biofilms are a kind of matrix that different microbial species colonies have attached to the surface of teeth. (T. Bernglundh (2005).

The main cause of periodontitis is inadequate oral hygiene practices, which lead to its development. Inadequate and careless dental hygiene can result in debris and plaque buildup around teeth, which can induce swelling, redness, and inflammation. This can also develop to gingivitis and eventually periodontitis. Studies have demonstrated a correlation between periodontal disease severity, progression, and prevalence. Insufficient or poor dental hygiene can facilitate the growth of bacteria that cause periodontal diseases, which can then spread and invade

the deeper tissues of the periodontal wall, where they can perform their harmful job.

There are three basic types of periodontal disease: gingivitis, chronic periodontitis, and aggressive periodontitis. Since the host's reaction determines whether a case of gingivitis progresses to periodontitis, not all gingivitis cases do (Bartold, P. M., et al 2013). Two basic categories of periodontitis are aggressive and chronic. Cases of calculus and plaque are common in patients with chronic periodontitis (CP) (Armitage, G. C. 2010) .On the other hand, greater periodontal damage with little contribution from local variables is the defining trait of aggressive periodontitis (AgP), which is a familial aggregation of illness. AgP is further divided into two categories: generalized aggressive periodontitis (GAP) and local aggressive periodontitis (LAP). Epidemiological research by Susin et al. found that differences in geographic location and ethnicity affect the prevalence of LAP. In African Americans, the estimated percentage is 2.6%. 0.5 to 1% in North Americans, 0.3 to 2% in South Americans, 1 to 5% in Africans, and 0.2% in Asians. GAP has a prevalence of 0.13% overall, whereas LAP has a prevalence of less than 1% (Susin, C., et al 2014). When compared to wealthy countries, the prevalence of chronic periodontitis is higher in developing nations (Ababneh, K. T et al 2012). Fifty percent of American adults had gingival and periodontal disease, according to the National Health and Nutrition Examination Survey III (NHANES III) (JM, A. 1999).

According to Arias-Bujanda, N. et al. (2020), it is a complex condition that involves a cascade of consecutive inflammatory events involving bacterial pathogens and the host immune system. *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella* forsythia are the three main bacterial pathogens most linked to chronic periodontitis, while *Aggregatibacter actinomycetemcomitans* is the principal pathogen responsible for aggressive periodontitis. According to Wadia (2020) and Hajishengallis (2015), genetic, environmental, and behavioral risk factors are also thought to be important in the etiology of periodontitis.
The biofilm can contain facultative aerobes, capnophiles, and microaerophiles, whose abundance depends on the environment within the shaped biofilm and periodontal pocket. Anaerobes make up most periodontal infections. Most periodontal pathogens

are actually periodontal infections. *Actinomyces, specific Streptococcus,* and *Staphylococcus spp.* are abundant in the periodontal environment, and when the ecology is disturbed, they can cause opportunistic infections. According to some research, finding certain enterobacteria in the periodontal pockets may signal a superinfection linked to a damaging periodontal process. Recently, it has become widely acknowledged that periodontal disorders are complicated infections. (2002) (Mombelli, A. et al.).

Concurring to the conventional Koch hypothesizes, which Socransky improved the theory, the pathogenicity of specific bacterial species in connection to periodontal tissues is evaluated utilizing the taking after criteria: • relationship with illness, • treatment end, or the affect of successful pharmaceutical on clinical measurements and the related microbiota, taken after by illness reduction, • generation of destructiveness components with coordinate and circuitous impacts, which allow microbes a particular advantage to devastate host tissues or evade host defense pathogenicity in creature models, which has the capacity to deliver illness on testcreatures.

Aggregatibacter actinomycetemcomitans and *Porphyromonas gingivalis* are the two species categorized as periodontal pathogens that meet all of the requirements given above, according to published reports (Haffajee, A. D., & Socransky, S. S. (1994).

Treponema denticola, T. forsythia, *Fusobacterium nucleatum, Prevotella intermedia, Campylobacter rectus, Eikenella corrodens, Peptostreptococcus micros, and Selenomonas sp.* are among the other periodontal infections that may be present. According to scientific data, Socransky and Haffajee divided the bacteria in the bacterial complexes into categories based on their involvement in the pathophysiology of periodontal disorders, presence in the biofilm and subgingival region, and other factors (Socransky, S. S., & Haffajee, A. D. 2002).

A remarkable advancement in dental research has uncovered the principles behind periodontitis, a genetically related condition characterised by gram negative anaerobic infection (Cafiero, C. 2013). In the oral cavity, the most common species of Gram-negative bacteria include Actinobacillus *actinomycetemcomitans,* which is

thought to be the main cause of periodontal disease (Cafiero, 2013; Paster, B. J. et al., 2001). Research on periodontal disease is still heavily focused on these bacteria (Paster, B. J. et al., 2001). The microbial community that inhabits the mouth cavity is known as the oral microbiota, oral microflora, or oral microbiome. It is estimated that there are 500–700 common species in this population. (Dewhirst, F. 2010),

The use of salivary biomarkers for the early identification of periodontitis has gained popularity in recent years. According to Giannobile et al. (2009) and Sexton et al. (2011), saliva is a useful oral fluid for assessing the condition of the oral cavity, including the existence of periodontal disease. It is a cost-effective, non-invasive diagnostic technique that can offer important details about the existence and severity of periodontal disease. There are a lot of promising salivary biomarkers linked to PD that have been reported. A. Monje et al., 2021). Salivary biomarkers are biomolecules that can be utilized as indications of the progression of periodontal disease. Salivary biomarkers may be used studies, the early detection of periodontitis, according to a number of studies.

Matrix metalloproteinase-8 (MMP-8), an enzyme that is crucial in the destruction of extracellular matrix in periodontal tissues, is one of the most frequently researched biomarkers. The enzyme matrix metalloproteinase-8 (MMP-8) is essential for the decomposition of extracellular matrix in periodontal tissues. The potential of salivary MMP-8 as a biomarker for the detection of periodontal disease has been examined in several research.

MMPs are important proteases linked to periodontal health and implicated in periodontitis. (Rathnayake,2015). Collagenases and gelatinases, such as MMP-8, MMP-13, MMP-2, and MMP-9, have received particular interest in periodontitis because type I collagen makes up a significant portion of the extracellular matrix in the periodontal cavity. Special focus has been given to collagenases because type I collagen makes up the majority of the periodontal extracellular matrix. This includes MMP-8, which is the primary collagenase in periodontitis and the source of 90% to 95% of the collagenolytic activity in gingival crevicular fluid. As a result, one of the most promising biomarkers for periodontitis in oral fluids now. (Franco, C., 2017). It

is an enzyme that polymorphonuclear cells release during an immunological response (Zhang, L. et al., 2009). MMP8 levels in saliva seem to be an important biomarker for identifying periodontitis. (2018) Lahdentausta et al. (2011) (Buduneli, E. et al.) Recent reports have shown that local and systemic levels of aMMP-8 can reflect the grading and staging of periodontitis. (Sorsa, T.2020). A study conducted in Brazil and researchers investigated the levels of MMP-8 in periodontal patients. They utilized enzyme-linked immunosorbent assay (ELISA) to quantify MMP-8 levels in gingival crevicular fluid (GCF) samples collected from the participants. The findings of this study contribute to understanding MMP-8 as a potential biomarker for periodontal disease. (Räisänen, I.2023).

Both soft and hard tissues are destroyed by periodontitis and peri-implantitis, which are widespread infection-induced oral inflammatory illnesses of teeth and dental implants supporting soft and hard tissue. This process is known as active periodontal and peri-implant deterioration (APD). Type I collagen predominates in the tissues of the periodontal/peri-implant regions. Active periodontal/peri-implant soft and hard tissue degeneration (APD) is primarily caused by the proteolytic enzyme matrix metalloproteinase (MMP-8), which is also referred to as neutrophil collagenase or collagenase-2. MMP-8 belongs to the MMP family. MMPs are structurally related but genetically distinct endopeptidases that are dependent on Ca2+ and Zn2+. They can break down nearly all extracellular matrix and basement membrane protein components in both physiologic tissue repair and pathologic tissue destruction. For example, extracellular matrix can break down during embryonic development, wound healing, and tissue remodeling. (Kinane, D. F. 2000).

Degranulating triggered neutrophils are a major source of MMP-8 (neutrophil-type MMP-8) in humans, but non-PMN-lineage cells like epithelial cells, smooth muscle cells, fibroblasts, macrophages, and endothelial cells also de novo express and secrete MMP-8 (mesenchymal cell-type MMP-8) in trace amounts. (Owen, C. A., 2004) (Kiili, M., 2002).

Saliva, mouthwash, GCF, PISF, and other oral fluids have all been utilized as specimens(Sorsa, T., 2006) (Sorsa, T.,2010).Compared to the collection of GCF and

PISF, mouth-rinse samples can be obtained more rapidly, noninvasively, and with less effort. The primary utility of the mouth-rinse assay is for screening; it is not accurate in identifying or localizing the locations of clinically active illness. The results might be affected by whole saliva, variations in the salivary flow rate, the use of antimicrobial medications, and smoking behaviors. Site-specific information is provided by GCF and PISF, making them valuable for an individual's customized treatment strategy. (Gursoy, U. K.,2013). According to Johnson et al., 2016 saliva from periodontal patients had 4.1 times greater concentrations of MMP-8 when tested using lateral flow immunoassay than did saliva from healthy periodontal controls.

While locations with the progressive illness exhibit comparable or greater levels of aMMP-8 in both smokers and non-smokers, Mäntylä et al. 2006 reported that the mean aMMP-8 levels in smokers were found to be lower than in non-smokers.

Numerous investigations have shown that people with localized and generalized periodontitis had greater salivary and oral fluid levels of aMMP-8 than did healthy controls; however, the levels decreased following nonsurgical periodontal treatment, such as scaling and root planing (SRP). (Mauramo, M., 2018) (Marcaccini, A. M., 2010) (Özçaka, Ö., 2011) (Sexton, W. M., 2011).

Nwhator et al 2014, demonstrated that aMMP-8, measured by lateral flow chair-side/PoC immunoassay (PerioSafe®), is directly proportional to the oral hygiene status. It demonstrates a positive link with both BOP and chronic periodontitis, but only when two or more sites have a minimum of five mm of deeper PPD; these aMMP-8 PoC data suggest that these deepened sites are impacted by APD. It was discovered that immunoassay's sensitivity was lower for a single site afflicted by chronic periodontitis. It has been shown that levels of aMMP-8 in oral fluids correspond with clinical periodontal indicators, especially PPD, and represent the impact of therapy. Levels of aMMP-8 have been shown to significantly correlate with radiological parameters in addition to the clinical periodontal parameter status. It has been demonstrated that aMMP-8 levels may distinguish between individuals with severe bone loss and those with mild bone loss. (Salminen, A., 2014) (Gursoy, U. K.,

2013). In 2015 Izadi Boroujeni et al. showed that aMMP-8 has an 87% sensitivity and a 60% specificity in a point-of-care (PoC) diagnosis of generalized chronic periodontitis.

An MMP-8 PoC/chair-side immunoassay PerioSafe® mouth-rinse test can be used for online PoC detection of initial periodontitis or pre-periodontitis in adolescent patients with such a genetic predisposition. Heikkinen et al. report that genetic polymorphism of MMP-3 and vitamin D receptor was found to be linked to initial periodontitis in Finnish adolescents. This demonstrates the PerioSafe® ORALyzer®-aMMP-8 chair-side/PoC test's potential for prevention [70]. Consequently, three or more >4 mm pockets linked to the vitamin D receptor and MMP-3 single-nucleotide polymorphisms, as well as aMMP-8 mouth-rinse chair-side/PoC test positive. (Heikkinen, A. M., 2017).

Compared to adults with chronic periodontitis, children with aggressive periodontitis exhibit greater levels of MMP. (Alfant, B.,2008). According to Baeza et al.'s 2016 research, there was an increase in aMMP-8 levels in patients with chronic periodontitis. The cutoff value for aMMP-8 levels evaluated by ELISA was found to be 13 ng/ml in cases of chronic periodontitis.

Salivary MMP-8 levels were altogether higher in patients with periodontal infection compared to healthy people, concurring to a precise survey and meta-analysis by Preshaw et al. (2016), which raised the possibility that salivary MMP-8 could be a helpful biomarker for the diagnosis of periodontal disease.

Periodontal disease and blood MMP-8 levels did not correlate, according to (Passoja et al 2008). According to Özçaka et al.'s 2011 investigation, there was no significant difference seen in the blood levels of MMP-8 between patients with chronic periodontitis and periodontal healthy persons. Serum biomarker levels were shown by Kinney et al. to be insignificant in the diagnosis of periodontitis. (Kinney, J. S., 2014).

In the case of localized aggressive periodontitis, Gonçalves et al 2013 showed that SRP and systemic antibiotic usage successfully lowered local levels of MMPs.

Leppilahti et al 2015, demonstrated that MMP-8 levels in GCF are more stable and stay below a certain threshold level in patients who received azithromycin antimicrobial therapy. SRP improves all clinical periodontal markers evaluated, except for CAL,

According to research by Konopka et al 2012. Even yet, following treatment, the periodontitis patient's GCF levels of MMP-8 remained greater than those of the control group. On the other hand, Gonçalves et al 2013. discovered that the MMP-8 level in GCF was like that of healthy sites. most significant decrease in MMP-8.

Antibiotics and nonsurgical treatment together can lower the amount of total and active collagenase/MMP-8. The majority of the collagenase present at this point was in an active state since the overall collagenase activity at the start of the therapy was found to be comparable to that of active collagenase (Gangbar, S., 1990).A link between PPD and a proximal plaque index (PI), they were unable to establish any correlation between clinical parameters and the quantity of humoral components following medication (Konopka et al 2012).The change in level over a maintenance period is substantially predicted by baseline GCF MMP-8 levels. Smokers with elevated baseline levels of GCF MMP-8 have a poor response to treatment. (Leppilahti, J. M., 2014)

Zhang et al. (2020) also noted that salivary MMP-8 was a promising biomarker for periodontal disease diagnosis in their systematic review and meta-analysis. According to numerous studies, persons with periodontitis have much higher levels of salivary MMP-8 than healthy people do. According to some research, MMP-8 may be a sensitive and accurate biomarker for periodontitis early diagnosis.

According to recent studies, the progression and destruction of periodontitis can be reflected in the local and systemic levels of an MMP-8.(Keles Yucel, Z. P., 2020; Sorsa, T et al., 2020). Many authors have identified MMP-8 as the most accurate and strongest indicators for tissue degradation, with sensitivity ranging from 65% to 87% and specificity ranging from 48% to 87% (Ebersole, J. L et al., 2013).

One potent inducer of periodontal tissue degradation is IL-1β. It has the ability

to induce the creation of tissue-degrading proteinases and promote bone resorption. In periodontitis, IL-1β has a role in immune modulation, inflammation, and bone resorption as a pro-inflammatory cytokine. Strong evidence for its relevance is provided by the clinical results. Its tissue concentration—which is higher in periodontitis—determines the biological consequences of IL-1β. When compared to healthy controls, individuals with periodontitis usually have higher amounts of IL-1β in their saliva and gingival crevicular fluid (GCF).5,6 GCF IL-1β levels are higher in patients with more severe bleeding on probing (BOP) and deeper pocket depths. (Offenbacher, S., 2007) (Sánchez, G. A.,2013).

IL-1β is a cytokine that promotes inflammation, it has a role in immunological control, bone resorption, and inflammation in periodontitis. Its relevance is well supported by the clinical data. The biological impact of IL-1β is contingent upon its concentration in tissue, which is heightened in cases of periodontitis. Saliva and gingival crevicular fluid (GCF) from people with periodontitis are often shown to have higher amounts of IL-1β in comparison to healthy controls.(Rangbulla, 2017) GCF IL-1β levels are higher in patients with greater pocket depths and more severe bleeding on probing (BOP)(Kinney et al ,2014).Osteoblastic cells, gingival fibroblasts, gingival lymphocytes, and macrophages activated by IL1β that have been stimulated by lipopolysacchride as stated in Czuszak (1996). It causes these fibroblasts and macrophages to release prostaglandin E2, which will influence how much bone is destroyed (Cheng, R., et al 2020).

According to a study, the group with periodontitis had greater levels of IL-1B than the groups with gingivitis and the healthy control. A study comparing the salivary IL-1 levels of healthy individuals and patients with periodontitis found a similar tendency. They were much greater than those found in healthy controls, even though there was no statistically significant difference between the periodontitis groups. In this study, it was discovered that the levels of IL-B in gingivitis were higher than in healthy controls. However, there was no discernible difference in IL-1B levels between the gingivitis and periodontitis groups and the healthy controls. Additionally, this data matched the clinical measurements. The study's conclusions

confirmed the value of saliva as a sample technique in periodontal disease due to immunological factors. Increased amounts of IL-1β have been proposed as a potential host-response factor linked to periodontal disease clinical symptoms (Tobón-Arroyave, S. I., et al 2008).

Furthermore, individuals suffering from chronic periodontitis experience elevated blood levels of IL-1β, which might have a systemic effect (Kusuhara, M et al. 2006). This result suggests a potential causative relationship between systemic disorders including cardiovascular diseases and periodontitis through IL-1β. (Zhu, H et al. 2015). The inflammasome signaling pathway associated with nod-like receptor protein-3 (NLRP3) is implicated in periodontal disease by activating IL-1β. In both gingivitis and periodontitis, the NLRP3 inflammasome complex's mRNA expression was increased. (Bostanci, N et al. 2009).

In both aggressive and chronic periodontitis, there are notable elevations in the salivary concentrations of NLRP3, IL-1β, and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). (Isaza-Guzmán, D. M et al 2017).

A study demonstrated that, in comparison to healthy controls, naswar users' saliva had higher levels of IL-1β.It was also shown that the GCF and saliva of different types of STP users had elevated IL-1β levels, highlighting the fact that IL-1β is essential to the development of periodontitis and that its levels rise in correlation with risk factors like the use of STPs. This may be because those who use Naswar have worse periodontal health, as evidenced by higher PD and CAL values. (Gaphor, S. M et al. 2014) (Jacob, P. S et al 2014).

Interleukin-1β (IL-1β), a pro-inflammatory cytokine included within the resistant reaction to periodontal contaminations, has moreover been considered as a salivary biomarker for the discovery of periodontitis. It is implicated in the immune response to periodontal infections. Salivary IL-1β has been studied for its potential to serve as a biomarker for the detection of periodontal disease (Arias-Bujanda N et al), (Lahdentausta, L. S 2018). Salivary IL-1β levels were shown to be considerably

greater in patients with periodontal disease in comparison to healthy persons, according to a systematic review analysis by Guo et al. (2020), which raised the possibility that salivary IL-1 can be a helpful biomarker for the identification of periodontal disease.

According to numerous studies, persons with periodontitis have much higher amounts of salivary Interleukin-1β than healthy people do. In patients with early-stage or moderate periodontitis, IL-1β has been proposed as a potential biomarker for the early detection of the disease. (J. Clin. Med. 2021) (Detection of association between periodontitis and polymorphisms of IL-1beta + 3954 and TNF-alpha 863 in the Korean population after controlling for confounding risk factors) suggests that genetic variations of IL-1 + 3954 are linked to an increased risk of periodontitis in Koreans.

MMP-8 and IL-1β have been found to positively correlate in disease groups, according to research. Gingivitis and periodontitis can be diagnosed more accurately by combining salivary biomarkers such IL-1β and MMP-8. Salivary biomarkers have the potential to offer valuable supplementary diagnostic data and might be employed as screening, prognostic, and predictive tests for periodontal disease. Novel diagnostic approaches will become possible in the future due to advancements in proteomics analysis and personalized treatment. Its application in dentistry, however, will depend on how dentists incorporate technology into their routine clinical work. (Liu, Y., et al 2020) (Bartold, P. M. 2018).

Another most promising salivary biomarkers for the early detection of periodontitis is interleukin-6 (IL-6). Ahn et al. (2011) carried out the first study with the goal of assessing the diagnostic capacity of salivary IL-6 for the identification of periodontitis. 40 patients with periodontitis and 40 healthy controls were enrolled in the study. In comparison to healthy controls, periodontitis patients had considerably greater levels of salivary IL-6. Salivary IL-6, according to the study, may be used as a possible biomarker for the early detection of periodontitis. The findings showed that the periodontitis group had considerably greater salivary IL-6 levels than the control group. Salivary IL-6 may be employed as an easy-to-use, non-invasive diagnostic tool

for the early diagnosis of periodontitis, according to the study.

Immune cells, such as activated T-cells, B-cells, and myeloid immune cells, such as macrophages and dendritic cells, as well as pertinent non-immune cells, such as keratinocytes, endothelial cells, and fibroblasts, can all secrete interleukin 6 (IL-6), a lymphocyte chemoattractant factor. (Kang, S et al 2020). Salivary IL-6 levels were assessed in 40 patients with chronic periodontitis and 40 healthy controls in a cross-sectional study by Gürkan et al. (2013).

The diagnostic utility of salivary IL-6 levels was examined by Tiwari et al. (2016) in 60 chronic periodontitis patients and 60 healthy controls. According to the study, the periodontitis group had considerably greater levels of salivary IL-6 than the control group. Salivary IL-6, according to the study, may be a strong biomarker for the early detection of periodontitis.

According to a research it is an overreaction to IL-6 in conjunction with the production of active-phase reactants may lead to the formation of a chronic inflammatory lesion that eventually destroys alveolar bone and periodontal ligaments(Nibali et al.,2012)(Apolinario Vieira et al 2021).Interleukin 6 is significant because, through terminal B-cell differentiation, immunoglobulin production, and T-cell activation, it not only triggers active-phase responses but also contributes to the establishment of particular cellular and humoral immune responses. In light of these details, it also functions as a regulator of inflammation from the acute to the chronic stages(Kaur, S et al 2020).Elevated IL-6 levels are associated with more osteoclastic activity in the alveolar bone area and more periodontal-pathogenic bacteria, indicating a local destructive process (Ptasiewicz, M et al 2022).

IL-6 is a pro-inflammatory cytokine released by osteoblasts to promote osteoclastic activity and by macrophages in response to certain bacteria. Periodontal disease develops as a result of localized inflammation, tissue damage, and the presence of tooth plaque bacteria and their byproducts. As demonstrated, by promoting osteoclast development and bone resorption and suppressing bone formation. (Apolinario Vieira, 2021). When present at high quantities, IL-6 mostly

increases the activation of mature osteoclasts, however it can also stimulate the maturation of osteoclasts from precursors. (Kudo, O.,2003). Research has indicated that IL-6 is linked to MMP release and activation, which may lead to severe extracellular matrix (ECM) degradation in periodontal disease (PD) patients with elevated blood levels of IL-6. (Isola G., 2021) Compared to healthy controls, patients with Chronic Periodontitis appear to have higher amounts of salivary IL-6. According to Interleukin-6 research (Batool, H. et al., 2018), Zhao, B., Li, X., and Li, R. (2019), polymorphisms show up as hereditary hazard variables for periodontitis patients within the Asian population.

IL-6 levels in PD have been studied in a number of scientific research in conjunction with other illnesses, and they have been employed as a diagnostic factor in disease grading (Isola, G et al 2021).The relationship between PD and systemic conditions including diabetes , atrial fibrillation (AF) , heart disease , and oral cancer, as well as other variables like smoking and obesity , is assessed by measuring the levels of IL-6. Moreover, IL-6 levels, which are directly linked to periodontal disorders, may be raised or lowered by variables including radiation, vitamin C administration, tocilizumab application, and monoclonal antibodies. (Struppek, J. et al 2021) (Ross, J. H et al 2010) (Toraman, A. et al 2020) (Zhou, S. Y et al 2013) (Sever, E et al 2023) (Balli, U. M. U. T., et al 2016).

It has also been shown that periodontal disease and atherosclerosis development are related. Bacteria can cause inflammation in blood vessels and release pro-inflammatory cytokines, such as IL-6, which could be the cause of the connection between periodontal disease and atherosclerosis. Acute coronary syndromes are brought on by the development of inflammation in the artery walls, which also triggers the start of the atherosclerotic process and may lead to plaque instability. It has been demonstrated that IL-6 is crucial to the development of the atherosclerosis process, since it increases vascular inflammation, which in turn causes endothelial dysfunction and instability of the atherosclerotic plaque (Park, S et al 2023) (Sitompul, S. I et al 2023) (Nikiforov, N. G et al 2023). Heart conditions, particularly ischemic heart disease, are caused by the atherosclerosis process. Atrial

fibrillation (AF), a condition that is well-known to afflict PD patients and can result in heart failure and stroke, is another health issue. There is a connection between periodontitis and dental health, as shown by the pathophysiological processes of AF (Struppek, J et al 2021). The oral microbiota in periodontitis was investigated by Plachokova et al (2021) in connection to systemic inflammation and disease severity. Their findings indicate an inflammatory mechanism explaining the correlation between periodontitis and cardiovascular disease and imply that severe periodontitis may be influenced by the oral flora.

PD and diabetes mellitus (DM) type 2 are recognized to have a tight pathophysiological link, and IL-6 plays a key role in the development of both diseases. Diabetes and periodontal disease work together to increase the levels of inflammatory cytokines (Bahammam, M. Aet al 2018). There has been evidence of a sharp increase in IL-6 levels together with bone shrinkage, a deeper periodontal probing depth, and a greater clinical attachment loss. According to these findings, diabetes may increase the body's cytokine levels, which in turn may cause bone loss and tissue damage (Zhao, M et al 2023). In contrast, persons with diabetes who get appropriate treatment had lower amounts of IL-6 and a milder form of periodontitis. Complex connections between the microbiota, oxidative stress, inflammation, genetics, the host immunological response, and other variables affect both diabetes and periodontitis (Barutta, F et al 2022).

Salivary IL-6 levels were assessed in 60 patients with chronic periodontitis and 60 healthy controls in a study by Kumar et al. (2019). According to the study, the periodontitis group had considerably greater levels of salivary IL-6 than the control group. Salivary IL-6, according to the study, has potential as a biomarker for the early detection of periodontitis.

In a recent study, 50 patients with chronic periodontitis and 50 healthy controls were examined to determine the diagnostic utility of salivary IL-6 levels. According to the study, the periodontitis group had considerably greater levels of salivary IL-6 than the control group. The study concluded that salivary IL-6 may be a useful biomarker for the early detection of periodontitis. According to the studies that have

been examined, patients with periodontitis have significantly greater levels of salivary IL-6 than do healthy controls. A strong, non-invasive, and practical biomarker for the early detection of periodontitis may be salivary IL-6. Further research is necessary to assess the diagnostic efficacy of salivary IL-6 levels in bigger populations and at various stages of periodontitis, though.

Trevilatto et al. (2003) initially looked at the relationship between the IL-6 −174 G/C polymorphism and chronic periodontitis, and they found that the genotype and allele frequencies of periodontitis patients differed from those of controls. The question of whether the IL-6 −174 G/C polymorphism predisposes to periodontitis has been the subject of several published investigations, however the findings are conflicting (Babel et al., 2006; Moreira et al., 2007; Nibali et al., 2009). There is a comparable problem with the correlation between periodontitis and the −572 C/G polymorphism (Holla et al., 2004; Komatsu et al., 2005; Nibali et al., 2009).

Combining biomarkers to identify underlying illness or predict a binary outcome has attracted the attention of several researchers (Meisner, A., et al 2019). When combined, a few of the previously identified biomarkers seem to have a very high sensitivity and specificity for periodontitis diagnosis. Just a single study has examined the distinction between gingivitis and periodontitis groups; it found that IL-6 and MIP-1α combined had an 81% sensitivity and a 71% specificity. In contrast, IL-1β, IL-6, MMP-8, and MIP-1α combined had an excellent 78% sensitivity and 78% specificity. Combining IL-6 and MMP-8 demonstrated a high sensitivity of 94% and specificity of 100% when comparing periodontitis to healthy gingiva. Combining IL-1β, IL-6, and MMP-8 resulted in the highest level of diagnostic accuracy, with ranges for sensitivity and specificity of 78–94% and 77–97%, respectively. For the triple combination of IL-6, MMP-8, and IL-1β, as well as the paired combinatory analysis of IL-1β and IL-6, an exceptional predictive value of 98% was found. Ultimately, it was determined that the combination of IL-6 and MMP-8 had an optimal positive predictive value of 100 (Ebersole, J. L et al 2015) (Ebersole, J. L et al 2013).

The saliva of 209 individuals was found to include salivary markers in 2015,

including IL-1β, IL-6, MIP-1, and MMP-8. These biomarkers, according to the study, might distinguish between periodontal disease and healthy gingiva (Ebersole, J. L et al 2015). A study aimed at improving periodontitis diagnostic criteria to go beyond periodontal probe-based diagnosis examined and identified 15 biomarkers. The research used receiver operating characteristic curves to evaluate and compare the efficacy and accuracy rate of eight biomarkers in the diagnosis of gingivitis (Hong, I et al 2020).

Salivary samples include DNA, RNA, proteins, metabolites, and microorganisms that may provide information about the current physiological state of the periodontal tissue. As a result, collecting saliva is a basic, straightforward, non-invasive bodily fluid test that can be helpful for diagnosis. Salivary samples also include omics components (genomics, transcriptomics, proteomics, metabolomics, and metagenomics). Zhang, Yu (2016). Numerous salivary markers have been investigated as possible targets for separating periodontitis patients from healthy people over the past few decades. These markers include cytokines, host enzymes, bacteria, periodontal disease, and bone metabolic products (Ramseier, C. A., 2009; Belstrøm, D., 2018; Froggede, B. D. 2008). As of right now, well-studied substances connected to host response factors in oral fluid (whole saliva) have been proposed as diagnostic biomarkers for periodontitis. (Taba, M. et al. 2005) (I. B. Lamster (1997) states that more than 65 oral components have been found and examined as possible markers of the advancement of periodontitis.

Other proteins have recently been suggested as possible indicators of periodontitis: **Salivary neuropeptides:** Patients with periodontitis had considerably greater amounts of salivary neuropeptides (neuropeptide Y, or NPY, and vasoactive intestinal peptide, or VIP), which were also connected with bleeding on probing scores in periodontitis patients (Haririan, H. et al 2018)

Oxidative stress-related indicators (OS): Oxidative stress-related indicators (OS) in saliva and gingival crevicular fluid that are linked to chronic periodontitis. A substantial increase in total antioxidant capacity and a significant decrease in levels of malondialdehyde (MDA), nitric oxide, total oxidant status (TOS), and 8-hydroxy-de-

oxyguanosine in the saliva of CP patients have suggested a direct correlation between CP and OS-related biomarker levels in the local site (Chen,M.,et al 2019).

MicroRNAs (MiRNA-146a and miRNA155): Patients with and without diabetes can monitor the state of their periodontal health in their saliva by using microRNAs (miRNA-146a and miRNA-155), which are reliable, non-invasive, diagnostic, and predictive indicators (Al-Rawi, N.H et al 2020)

Salivary oxidative stress biomarkers and advanced glycation end products: Cross-sectional research involving patients with periodontitis, patients with type 2 diabetes who are in good periodontal health, and comparable controls who are in good systemic health. Potential non-invasive screening markers for periodontitis include salivary 8-hydroxy-2'-deoxyguanosine (8-OHdG) alone, or in combination with 4-hydroxy-2-nonenal (4-HNE), advanced glycation end products (AGE) and AGE receptor (RAGE) for diabetics, and salivary 8-OHdG alone, or in combination with malondialdehyde (MDA) and high sensitivity C-reactive protein (hsCRP) for those in otherwise healthy systems(Altıngöz, S. M., 2021).

Soluble Neuropilin-1 (sNRP-1): A glycoprotein that is strongly correlated with periodontitis and has angiogenic and immunological regulatory properties; it may also play a role in the pro-inflammatory pathways linked to periodontal clinical tissue inflammation (Prieto, D., et al 2021).

Alpha-amylase, an enzyme involved in the digestion of starch in saliva, and C-reactive protein (CRP), a measure of systemic inflammation, are additional salivary biomarkers that have been researched for the diagnosis of periodontitis. However, the outcomes of research into these biomarkers have been conflicting, and it is still uncertain whether or not they can be used to diagnose periodontitis. Alpha-amylase, lactoferrin, and myeloperoxidase are additional salivary biomarkers that have been researched for the identification of periodontal disease. Salivary alpha-amylase levels were altogether higher in patients with periodontal infection compared to healthy people, according to a systematic review and meta-analysis by Zhang et al. (2021), which raised the possibility that salivary alpha-amylase could be a helpful biomarker

for the diagnosis of periodontal disease. Finally, salivary biomarkers have demonstrated potential as early periodontitis diagnostic tools. The most promising biomarkers at the moment are MMP-8 and IL-1β and IL-6, although further research is required to substantiate their diagnostic use and demonstrate their therapeutic utility. Salivary biomarkers have the potential to completely change the way periodontitis is diagnosed and treated by enabling early detection and individualized treatment plans.

Saliva banking offers the alluring advantages of noninvasive, simple, and cost-effective sampling for the diagnosis of a range of illnesses. Furthermore, the application of salivary diagnostics provides point-of-care (POC) technologies, which may help in the early detection of genetic problems and systemic and oral illnesses like cancer(Khurshid, Z. (2018). POC technology is defined as a "medical device used to conduct testing outside the laboratory at or near the site of patient care, including the patient's bedside, the doctor's office, and the patient's home."(Song, Y., et al 2014). These technologies make it possible to identify analytes more quickly and precisely.

Proteomics, DNA analysis, and enzymatic analysis molecular biology protocols are being revolutionized by advances in microfluidics technology (Yager, P et al 2006)With chair-side Lab-on-a-chip technology that can identify periodontal biomarkers generated during the immune response, digital microfluidics seems promise for usage in the future to diagnose periodontal illnesses(He, W., et al 2018). The transition from a typical "reactive" strategy to a "predictive" one will take some time, but the route has already been established; in the case of dentistry, it is "time for new guidelines in advanced healthcare". (Fig 2.1

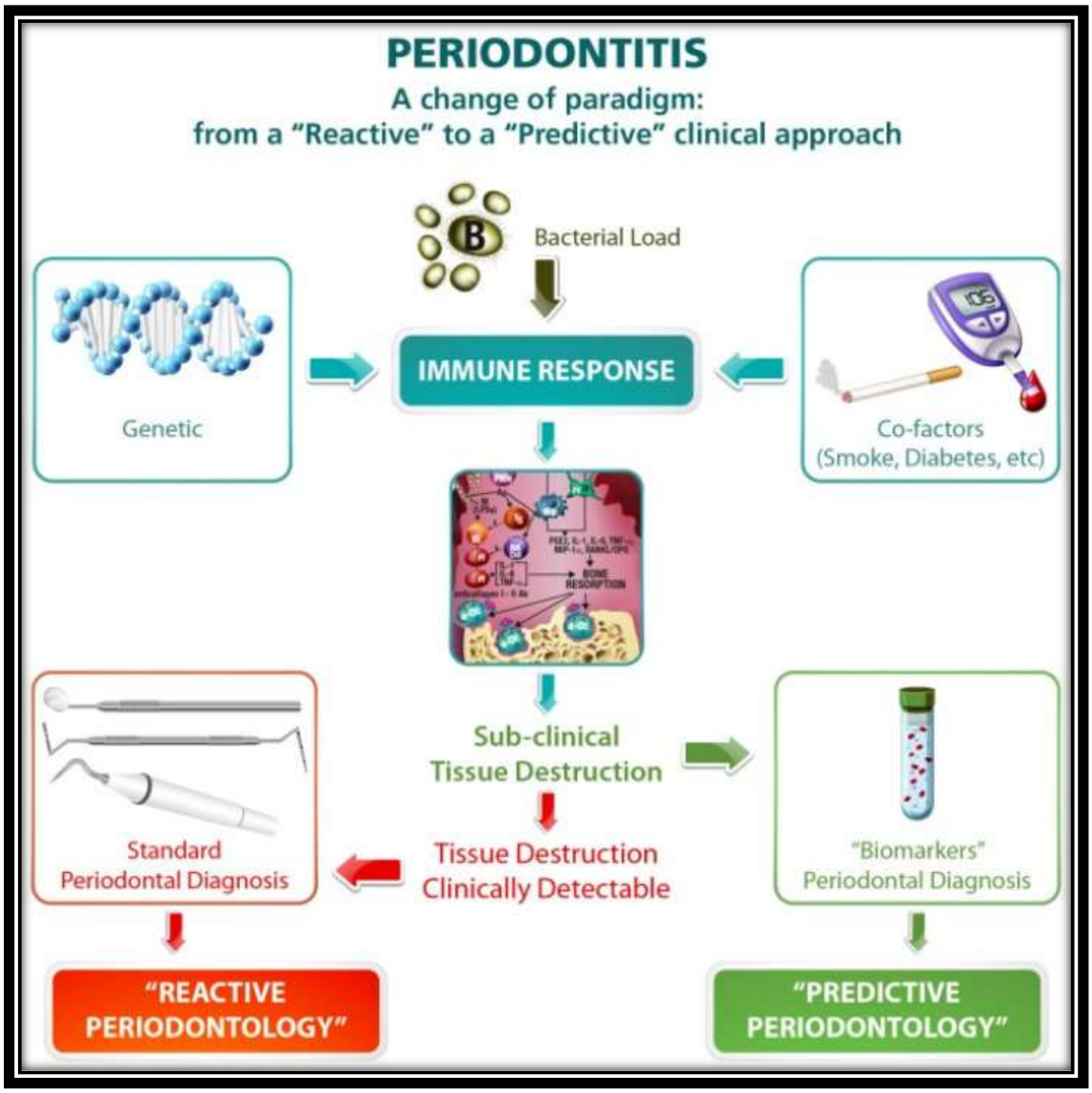


Fig 2.1: A change of paradigm in periodontal diagnosis is desirable. A shift from a "Reactive" approach, in which clinicians react to the presence of clinically evident periodontal damage, towards a "Predictive" approach, in which the disease is intercepted early when it is already in a sub-clinical phase, is the new objective in periodontal diagnosis.

OPERATIONAL DEFINITIONS

Periodontitis: Periodontitis is a multifactorial inflammatory disease of the periodontium which is associated with the accumulation of dental plaque, and it is characterized by the progressive destruction of the teeth and its supporting structures which includes the periodontal ligament and alveolar bone. The disease involves complex dynamic interactions among specific bacterial pathogens, and different environmental factors such as smoking. The most common features of periodontitis include gingival inflammation, clinical attachment loss, radiographic evidence of alveolar bone loss, sites with deep probing depths, mobility, bleeding upon probing and pathologic migration. (R. C., & Beck, J. D. 1997).

Gingivitis: Gingivitis is an inflammatory condition of the gingival tissue most caused by bacterial infection. Unlike periodontitis, there is no attachment loss and therefore no migration of the junctional epithelium (Rathee, M. et al 2022)

Saliva: Saliva (commonly referred to as spit) is an extracellular fluid produced and secreted by salivary glands in the mouth. In humans, saliva is around 99% water, plus electrolytes, mucus, white blood cells, epithelial cells (from which DNA can be extracted), enzymes (such as lipase and amylase), and antimicrobial agents (such as secretory IgA, and lysozymes) (Ombao, H (2016).

Salivary Biomarkers: Salivary biomarkers are indicators of many biological and pathological conditions and provide further information regarding the early detection of diseases, whether produced by healthy individuals or by individuals affected by specific diseases, they are sentinel molecules that could be used to scrutinize health and disease surveillance (Zhang, L., et al 2010).

Plaque Index (PI) : The Visible Plaque Index (VPI) was proposed by Ainamo and Bay 1975, to assess the quality of oral hygiene through clinical observation of the

presence of biofilm on dental surfaces by means of simple categorical definitions (presence or absence of plaque) the clinical plaque indices are used to evaluate the level and rate of plaque formation on tooth surfaces, and to test the efficacy of oral care products for removal and prevention of plaque deposits from these surfaces.

Calculus Index (CI): Calculus Surface Index (CSI) is used to calculate the presence of the calculus on the four mandibular incisors (supra and subgingival).The examined surfaces are the labial, lingual, mesial, and distal, according to the number of surfaces on which calculus is registered, each tooth is assigned number from 0 to 4. (Ennever, J., 1961).

Bleeding on Probing (BOP): Bleeding on probing indicates inflammation of soft tissue, whether around natural teeth or implants. It is an indicator of tissue inflammatory response to bacterial pathogens.The number of sites where bleeding is recorded is divided by the total number of available sites in the mouth and multiplied by 100 to express the bleeding index as a percentage. (Checchi, L., et al 2009).

Periodontal Probing Depth (PPD): The measurement of periodontal pocket depth is an indication of the depth of the gingival sulcus, which corresponds to the distance between the height of the free gingival margin and the height of the attachment apparatus below. In a healthy mouth, the pocket depth is usually between 1 and 3 millimeters (mm). Pockets deeper than 4 mm may indicate periodontitis. (Oxford)

Clinical Attachment Loss (CAL) : CAL represents the extent of periodontal support that has been lost around a tooth and is measured with the periodontal probe as the distance from the cemento-enamel junction (CEJ) to the base of the pocket (Highfield, 2009)

Metalloproteinase-8 (MMP-8): It is an enzyme which is released by Polymorphonuclear cells during immune reaction. Salivary levels of MMP8 appear to

be as a valuable biomarker in diagnosing periodontitis (Lahdentausta, et al.,2018), (Buduneli, E et al.,2011)

Interleukin-1beta (IL-1*β*): It is released by lipopolysaccharide activated macrophages, lymphocytes, and fibroblasts. It stimulates these macrophages and fibroblasts to secrete prostaglandin E2 and it will determine the bone destruction (Cheng, R.et al 2020).

Interleukin-6 (IL-6): This is produced by osteoblastic cells, gingival fibroblasts, gingival lymphocytes, and macrophages stimulated by IL1. It appears as a fundamental factor in the regulation of bone remodeling because it acts by increasing bone resorption determined by osteoclasts activated by IL1. (Cortellini, P., & Tonetti, M. S. 2011).

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN

This study was designed to be a case control study.

3.2 SUBJECTS

This study was consist of healthy and diseased individuals suffering from Chronic Periodontitis

3.3 SETTING

This case control study, conducted in Dental OPD, Periodontology department of Bahria University Health Sciences, Karachi.

3.4 INCLUSION CRITERIA

3.4.1 GROUPS:

- 20-60 years of age
- Having at least 16 teeth
- Underwent no medical treatment during the last 3 months before examination and sampling.

- **3.4.2 CONTROL GROUP:**

  The individuals for control group:

  Had no systemic condition, no sites with PL

  >3 mm or CAL > 3 mm and ≤10% sites with BOP and

  no radiographic findings of alveolar bone lose

- **3.4.3 CASE GROUP:**

  The individuals for case group:

  Presence of at least 16 natural teeth

  A minimum of 35% of sites with clinical attachment (CAL)≥ 3mm

  Probing depth (PD) ≥ 3mm

  Presence of ≥ 40% sites with bleeding on probing (BOP**)**

3.5 EXCLUSION CRITERIA

- Smokers
- Pregnant women
- Post radiotherapy salivary samples

3.6 DURATION OF STUDY

Individual study period: 3 months

Total period of study: 6 months

After approval from Bahria University Health Sciences Karachi Institutional Review Board (IRB)

3.7 SAMPLE SIZE ESTIMATION

For the calculation of the sample size, results are calculated from Open Epi, Version 3, open-source calculator—SS Proper are utilized. The required sample size was found to be 76 by using the equation

Sample size n = [DEFF*Np(1-p)]/ [(d2/Z21-α/2*(N-1)+p*(1-p)]

Sample Size for Unmatched Case-Control Study

For:

Two-sided confidence level(1-alpha)	95
Power(% chance of detecting)	80
Ratio of Controls to Cases	1
Hypothetical proportion of controls with exposure	10
Hypothetical proportion of cases with exposure:	40
Least extreme Odds Ratio to be detected:	6.00

	Kelsey	Fleiss	Fleiss with CC
Sample Size - Cases	33	32	38
Sample Size - Controls	33	32	38
Total sample size:	66	64	76

References

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15
Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19

CC = continuity correction
Results are rounded up to the nearest integer.
Print from the browser menu or select, copy, and paste to other programs.

3.8 SAMPLING TECHNIQUE

Consecutive sampling technique used to recruit samples for this study.

3.9 HUMAN SUBJECTS AND CONSENT

The total number of patients enrolled in the study were 76 subjects (38 patients in each group). Written informed consent was taken from each participant prior to enrollment in the study. (Appendix- C)

3.10MATERIALS

3.10.1 DRUGS

N/A

3.10.2 CHEMICALS

N/A

3.10.3 PROFORMA/ QUESTIONNAIRE

Subject evaluation proforma/ Questionnaire (Appendix -D)

3.10.4 EQUIPMENT

Examination Set, Disposable cups, CPITN Probe, Polypropylene tubes, Eppendorf tubes, ELISA Washer, ELISA Reader(Figure 3.1 a-g)

Figure 3.1(a): Examination Set (Mirror, Tweezer, Probe)

Figure 3.1(b): Disposable Cup

Figure 3.1 (c) : Polypropylene tube

Figure 3.1 (d) : CPITN Probe

Figure 3.1(e): Eppendorf Tube

Figure 3.1(f): ELISA washer

Figure 3.1(g): ELISA Reader

3.10.4.1 KITS USED

Human Matrix Metalloproteinase 8 ELISA Kit was procured from BT LAB. Figure 3.2 (a)

- CATALOGUE NO: E0903Hu
- Standard Curve Range: 0.05-10ng/ml
- Sensitivity: 0.021ng/ml
- Size: 96wells/ 48 wells
- Storage: Store the reagents at 2-8°C. For long term storage refer to the expiration date keep it at -20°C. (Figure 3.3) Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

Human Interleukin 6 ELISA Kit was procured from BT LAB. Figure 3.2 (b)

- CATALOGUE NO: E0090Hu
- Standard Curve Range: 2-600ng/L
- Sensitivity: 1.03ng/L
- Size: 96wells / 48 wells
- Storage: Store the reagents at 2-8°C. For over 6 months refer to the expiration date keep it at -20°C. (Figure 3.3) Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month

Human Interleukin 1β ELISA Kit was procured from BT LAB. Figure 3. 2 (c)

- CATALOGUE NO: E0143Hu
- Standard Curve Range: 20-6000ng/L
- Sensitivity: 10.07ng/L

- Size: 96wells / 48 wells
- Storage: Store the reagents at 2-8°C. For over 6 months refer to the expiration date keep it at -20°C. (Figure 3.3) Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

3.10.4.1.1 INTENDED USE

This Sandwich kit is for the accurate quantitative detection of MMP-8, IL-1ß and IL-6 in serum samples, plasma, cell culture super nates, Ascites, tissue homogenates or other biological fluids.

3.10.4.1.2 ASSAY PRINCIPLE

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with MMP-8, IL-1β and IL-6 antibody. MMP-8, IL-1β and IL-6 present in the sample is added and binds to antibodies coated on the wells. And then biotinylated MMP-8, IL-1ß and IL-6 Antibody is added and binds to MMP-8, IL-1β and IL-6 in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated MMP-8, IL-1β and IL-6 antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added, and color develops in proportion to the amount of MMP-8, IL-1β and IL-6. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. (Fig 3.4)

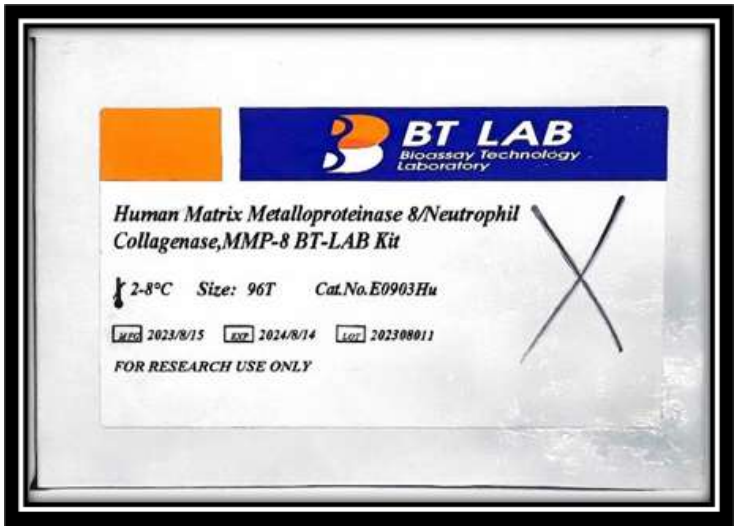


Figure 3.2 (a): Matrix Metalloproteinase 8 ELISA Kit

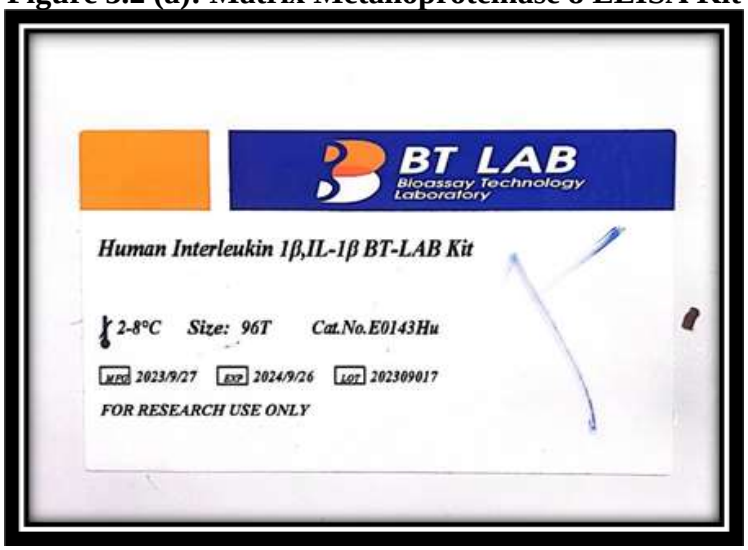


Figure 3.2 (b): Human Interleukin 6 ELISA Kit

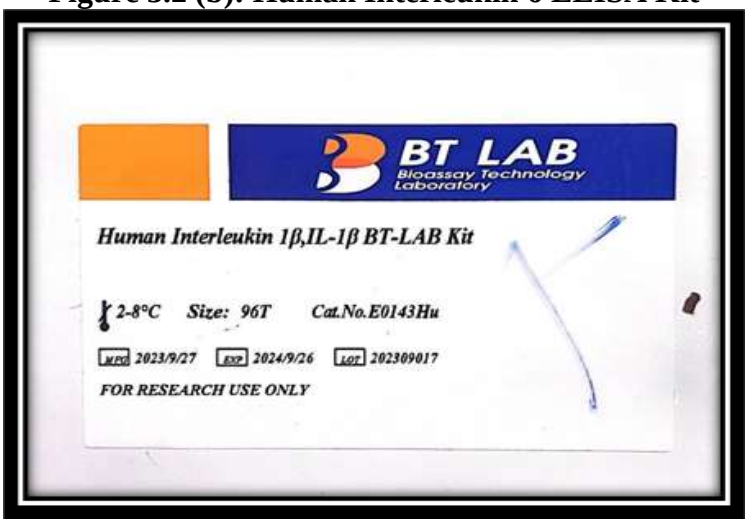


Figure 3.2 (c): Human Interleukin 1 beta ELISA Kit

Figure 3.3: Reagent Storage Refrigerator

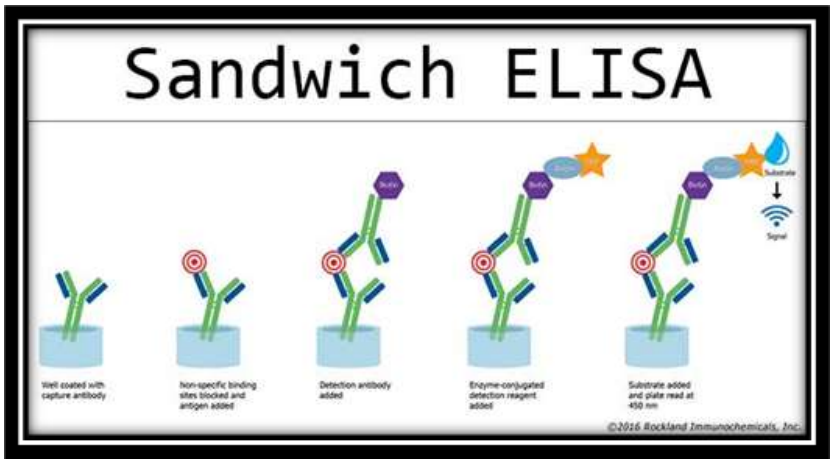


Figure 3.4: Sandwich ELISA

3.10.4.1.3 REAGENTS

Table 3.1: Components used for estimation of MMP8, IL-1ß and IL6levels

Component	Quantity
Standard Solution	0.5ml x 1
Pre-coated ELISA Plate	12 * 8 well strips x 1
Standard/Sample Diluent	3ml ×1
Streptavidin-HRP	6ml x 1
Stop Solution	6ml x 1
Substrate Solution A	6ml x 1
Substrate Solution B	6ml x 1
Wash Buffer Concentrate(25x)	20ml x 1
Biotinylated Human IL-1ß Antibody	1ml x 1
User Instruction	1
Plate Sealer	2 pics
Zipper Bag	1 pic

3.10.4.1.4 MATERIALS REQUIRED BUT NOT SUPPLIED

- 37°C ± 5°C incubator
- Absorbent paper
- Precision pipette and disposable tip
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

3.10.4.1.5 PRECAUTIONS

- Prior to use, the kit and sample should be warmed naturally to room temperature 30 minutes.
- This instruction must be strictly followed in the experiment
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Avoid using the reagents from different batches together.
- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date

3.10.4.1.6 ASSAY PROCEDURE

- Two- five ml of unstimulated saliva was collected in a polypropylene tube. (Figure 3.5)
- The samples were centrifuged for 15min and -4°C at 6000 rpm, separating the supernatant and pellet. (Figure 3.6 (a), (b))
- The samples were stored in Eppendorf at -80 ºC until analysis. (Figure 3.7 (a), 3.7 (b))
- Prior to use the kit, buffer solution and samples were thawed at room temperature for 30 minutes. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. (Figure 3.8(a), 3.8(b))
- Samples stored in Eppendorf were mixed on a vortex. (Figure 3.8c)
- Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
- Add 50ul standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody. (Figure 3.8d)
- Add 40ul sample to sample wells and then add 10ul Human IL16 antibody to sample wells, then add 50ul streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. (Figure 3.8 e)
- Cover the plate with a sealer. Incubate 60 minutes at 37°C. (Figure 3.8 f)
- Remove the sealer and wash the plate 5 times with wash

buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material. (Figure 3.8 g)

- Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark. (Figure 3.8 h and i)
- Add 50ul Stop Solution to each well, the blue color will change into yellow immediately. (Figure 3.8 j)
- Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution. (Figure 3.8 k and l)

Fig 3.5: Saliva sample in polypropylene tubes

Fig 3.6: Samples were transferred in Eppendorf tubes for centrifugation

Fig 3.7 (a and b): Sample Centrifugation

Fig 3.7 (c): Samples Stored at -80°C

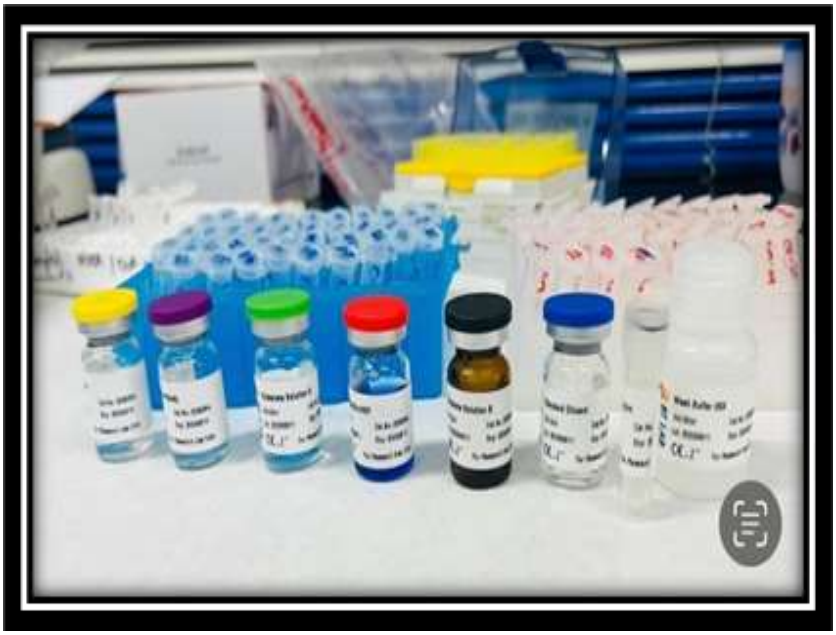


Fig 3.8 (a): Buffer solutions were thawed at room temperature

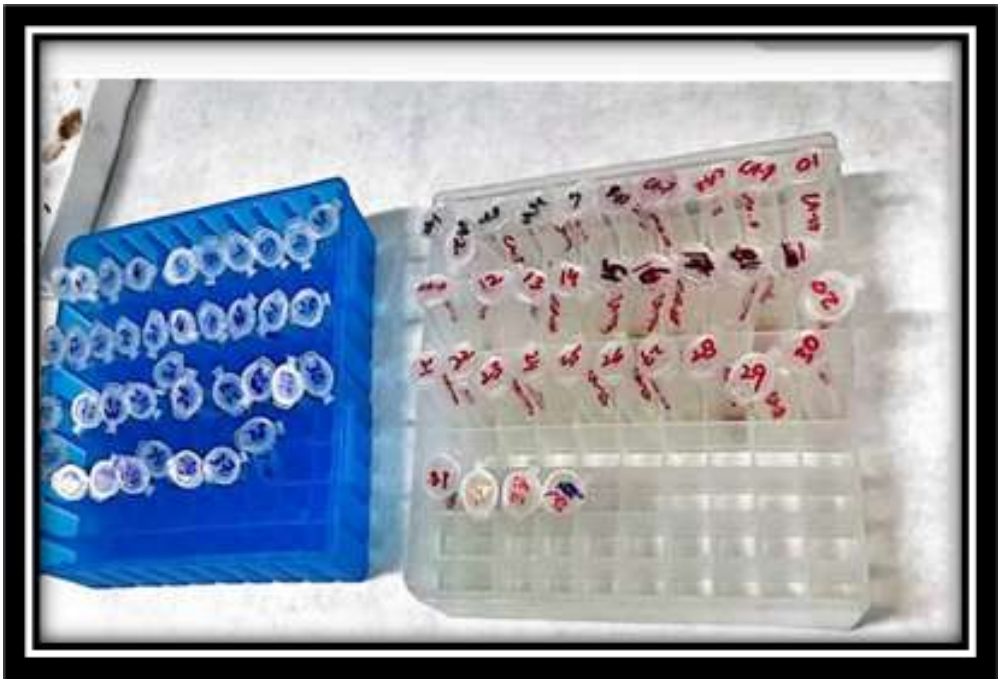


Fig 3.8 (b): Samples were thawed at room temperature

Fig 3.8 (c): Sample mixing on vortex

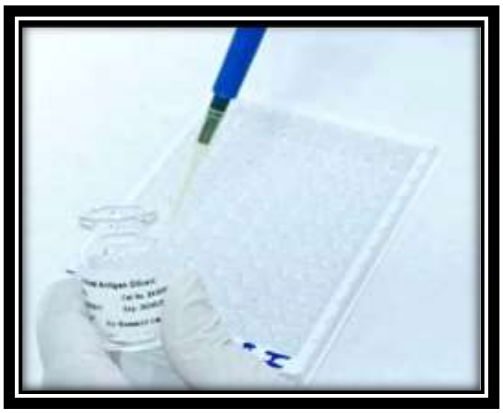


Fig 3.8 (d): Standard Antigen Diluent

Fig 3.8 (e): Adding streptavidin-HRP

Fig 3.8 (f):Incubation for 60min

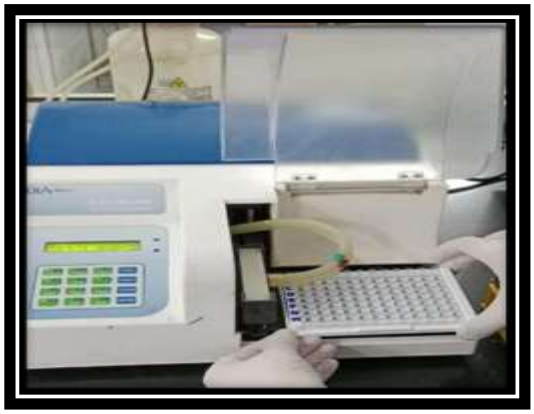


Fig 3.8 (g): Sealer Removed and washed

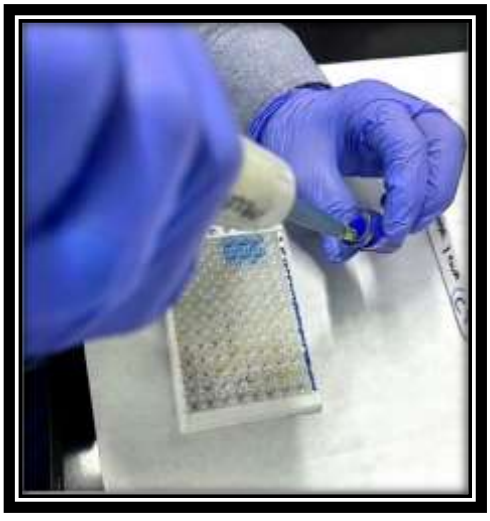


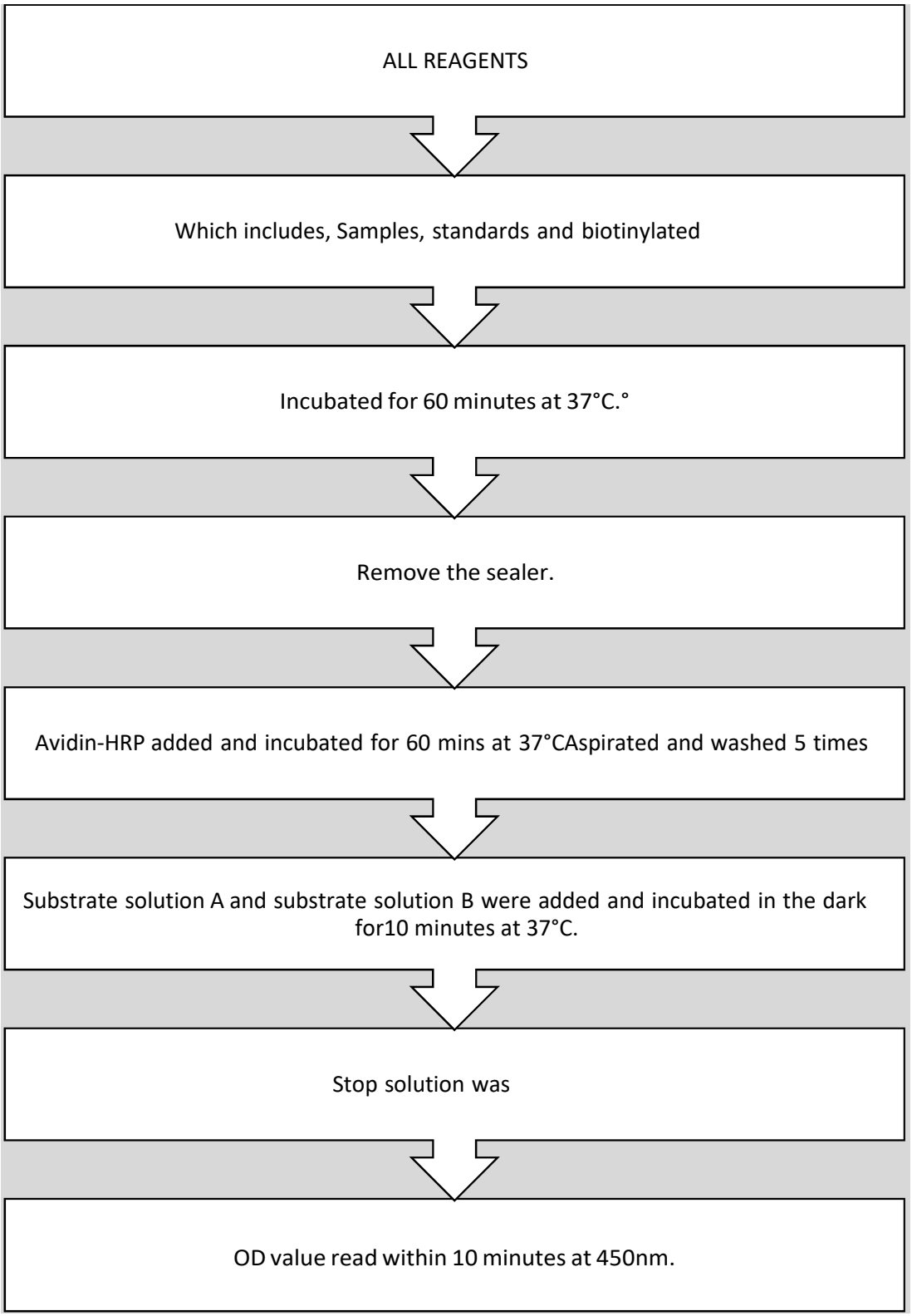
Fig 3.8 (h):Adding Substrate Sol A

Fig 3.8 (i):Adding Substrate Solution B

Fig 3.8 (j): Adding Stop Solution

Fig 3.8 (k): Microplate Reader

Fig 3.8 (l): Optical D density Determined


ALL REAGENTS
Which includes, Samples, standards and biotinylated
Incubated for 60 minutes at 37°C.°
Remove the sealer.
Avidin-HRP added and incubated for 60 mins at 37°CAspirated and washed 5 times
Substrate solution A and substrate solution B were added and incubated in the dark for10 minutes at 37°C.
Stop solution was
OD value read within 10 minutes at 450nm.

3.11 PARAMETERS OF STUDY

3.11.1 CLINICAL PARAMETERS

Signs of periodontal disease include active bleeding in response to mild or no tissue manipulation, pain, bad taste/odor, periodontal pocketing, radiographic bone loss, clinical attachment loss, and tooth loss.

3.11.1.1: CLINICAL ATTACHMENT LOSS: CAL represents the extent of periodontal support that has been lost around a tooth and is measured with the periodontal probe Clinical attachment loss measurements are made from the cement–enamel junction to the base of the periodontal pocket.

3.11.1.2: PLAQUE INDEX: Clinical plaque indices are used to evaluate the level and rate of plaque formation on tooth surfaces

3.11.1.3: CALCULUS INDEX: The CSI score is the total number of surfaces covered by calculus.

3.11.1.4: BLEEDING ON PROBING: Bleeding on probing (BOP) is an indicator of tissue inflammatory response to bacterial pathogens. The number of sites where bleeding is recorded is divided by the total number of available sites in the mouth and multiplied by 100 to express the bleeding index as a percentage.

3.11.1.5: PROBING DEPTH: The distance measured from the base of the pocket to the most apical point on the gingival margin.

3.11.2 ANTHROPOMETRIC PARAMETERS

N/A

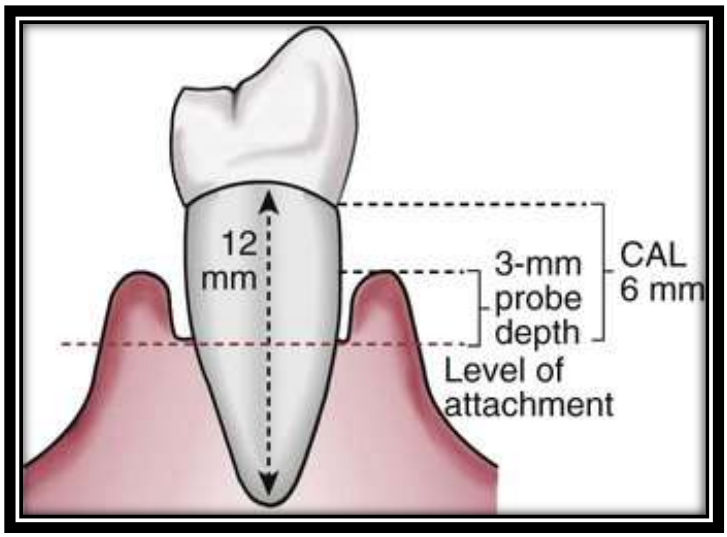


Fig 3.9 (a) : Clinical Attachment Loss (CAL)

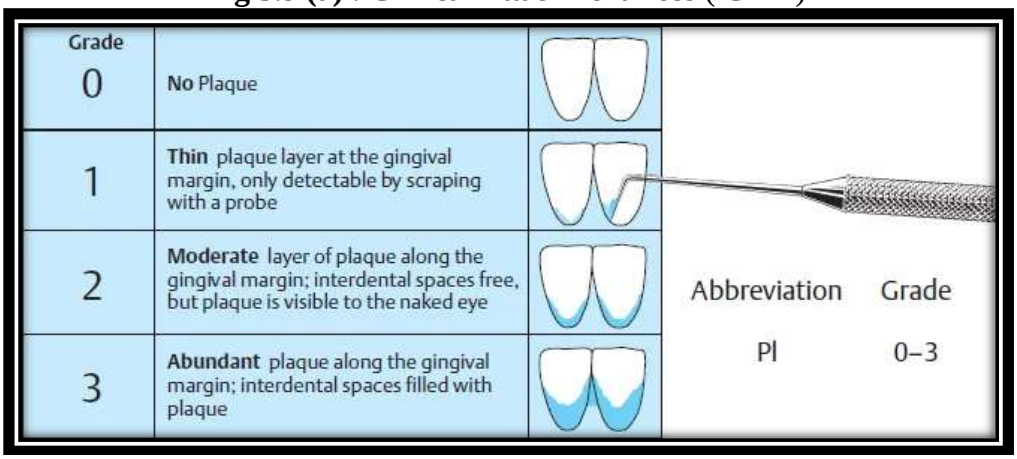


Fig 3.9 (b): Plaque Index (PI)

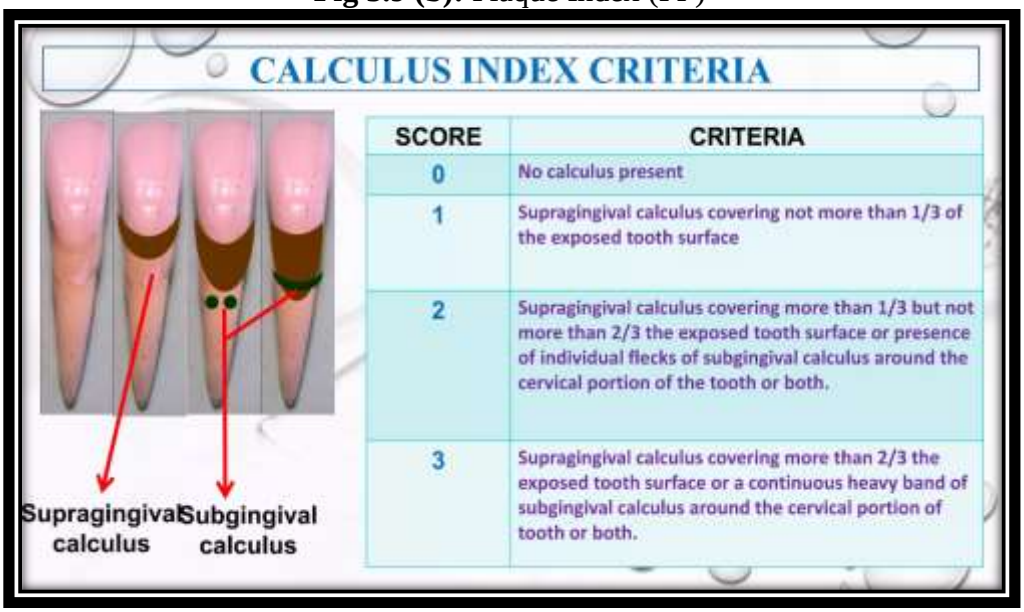


Fig 3.9 (c): Calculus Index (CI)

Grade	Abbreviation	Grade	
0–3	GI	0	Normal gingiva; no inflammation; no discoloration (erythema); no bleeding
		1	Mild inflammation; slight erythema; minimal superficial alterations. **No bleeding**
		2	Moderate inflammation; erythema; **bleeding on probing**
		3	Severe inflammation; severe erythema and swelling; **tendency to spontaneous bleeding;** possible ulceration.

Fig 3.9 (d): Bleeding on Probing (BOP)

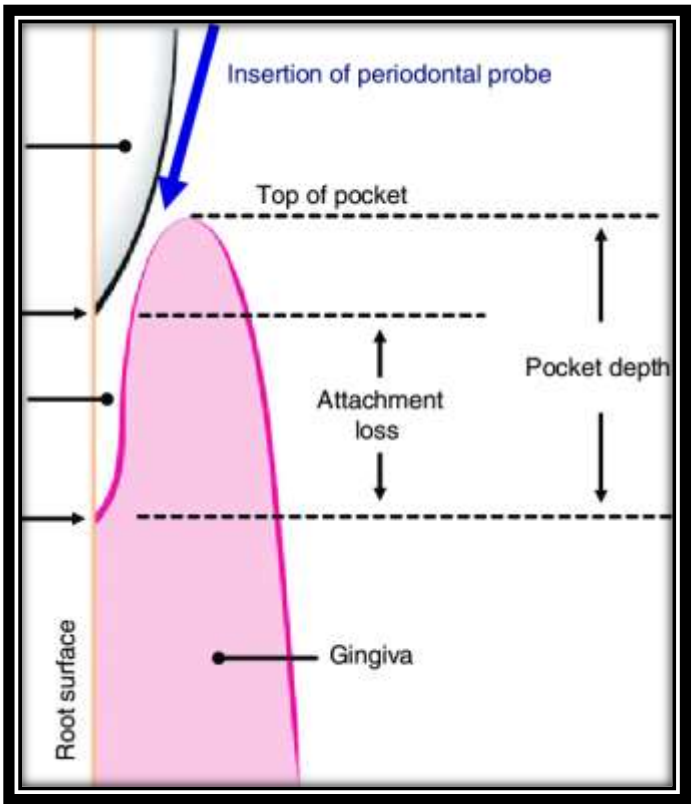


Fig 3.9 (e) : Periodontal Probing Depth (PPD)

3.11.3 BIOCHEMICAL PARAMETERS

3.11.3.1 Matrix Metalloproteinaeses-8: Unstimulated Saliva samples were collected of the participants, and sent to the BUHSC, MDRL laboratory and were measured by using the Human Matrix Metalloproteinase 8 ELISA Kit was procured from BT LAB.
CATALOGUE NO: E0903Hu.

3.11.3.2 Interleukin-1 beta: Unstimulated Saliva samples were collected of the participants, and sent to the BUHSC, MDRL laboratory and were measured by using the Human Interleukin 6 ELISA Kit was procured from BT LAB.
CATALOGUE NO: E0090Hu

3.11.3.2 Interleukin 6 (IL-6): Unstimulated Saliva samples were collected of the participants, and sent to the BUHSC, MDRL laboratory and were measured by using the Human Interleukin 1β ELISA Kit was procured from BT LAB.
CATALOGUE NO: E0143Hu

3.11.3 RADIOLOGICAL PARAMETERS

OPG and Periapical Radiographs are used to detect PDL attachment loss and alveolar bone destruction. (Figure 3.10)

3.12 PROTOCOL OF STUDY

- The study was designed as a case control study. It was approved by the Institutional Review Board (IRB) of Bahria University Health Sciences Campus.
- 76 subjects who participated in this trial were randomly selected from patients seeking periodontal treatment at the Department of Periodontology in Bahria University Medical Health Sciences.
- These participants were further divided into 2 groups. 38 participants in each group. According to their periodontal status and general health condition, eligible patients were invited to take part in the study.
- Patients with periodontitis referred for periodontal therapy were initially screened to assess their eligibility for recruitment. After applying inclusion/exclusion criteria, a total of (n=76) patients were included in the final analysis.
- Thirty-eight patients were assigned to the periodontitis group and thirty-eight periodontally healthy subjects were assigned to the non-periodontitis group.
- All the male and female patients of periodontitis as per inclusion criteria were enrolled in the study prior taking written informed consent(attached). All the demographic information of patients with their medical, oral and periodontal clinical parameters were recorded.
- **Clinical Evaluation:**
- Each participant received a full mouth periodontal examination and a medical and dental history examination. All permanent teeth were measured by CPITN probe. Plaque index (PI), Calculus index (CI),

Bleeding on probing (BOP), Probing depth (PD) and Clinical Attachment Loss (CAL) were measured at 6 sites on all teeth. Probing depths and attachment loss were measured at six sites (distobuccal, buccal, mesiobuccal, distolingual, lingual, and mesiolingual) for each tooth, excluding third molars, using a CPITN probe.

- Probing pocket depths were assessed from the edge of marginal gingiva to the bottom of the pocket, and attachment loss is assessed from the cementoenamel junction to the bottom of the pocket.

Saliva Sampling

- As we were collecting non-stimulatory saliva, participants were encouraged to refrain from eating, drinking, chewing gum, or cleaning their teeth. Or they may remain isolated for 30 minutes prior to the saliva sample collection.
- First, each participant took a seat and gargled with tap water. Then, they had to expectorate entire saliva in a small sterile cup for up to 5 minutes using the drooling technique. Following that, 2 to 5 ml of unstimulated saliva sample will be transferred to a polypropylene tube.
- Immediately, collected saliva samples were centrifuged for 15 minutes (6000 rpm, 4°C), then distributed in Eppendorf tubes and frozen at −80°C until analysis.

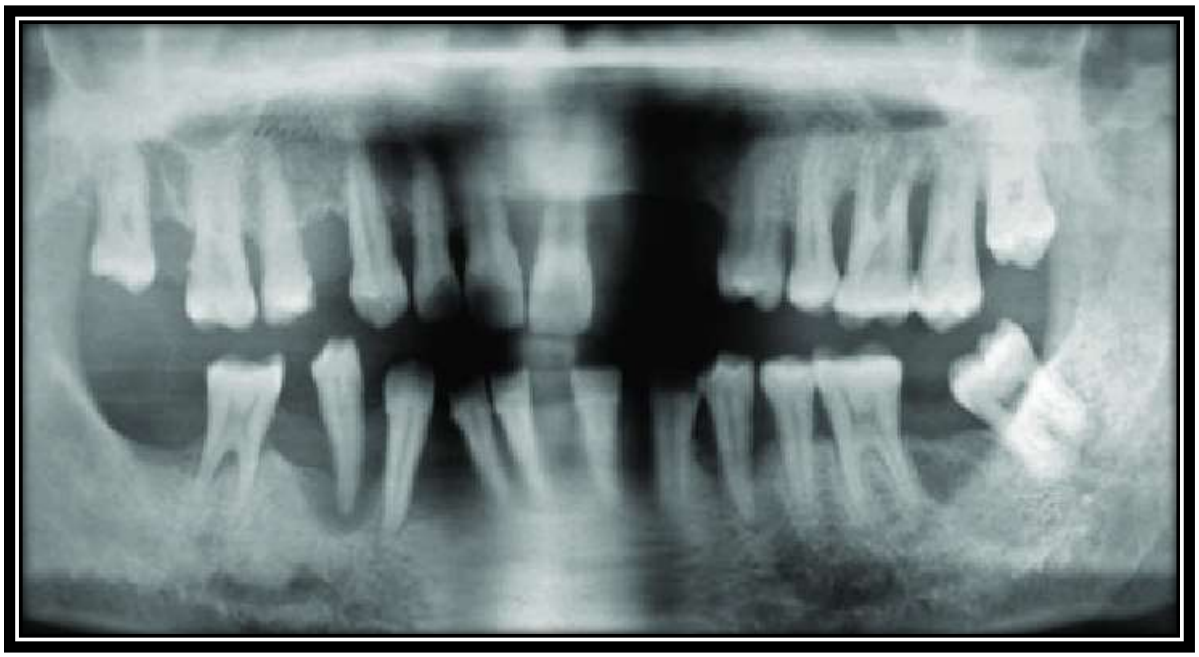
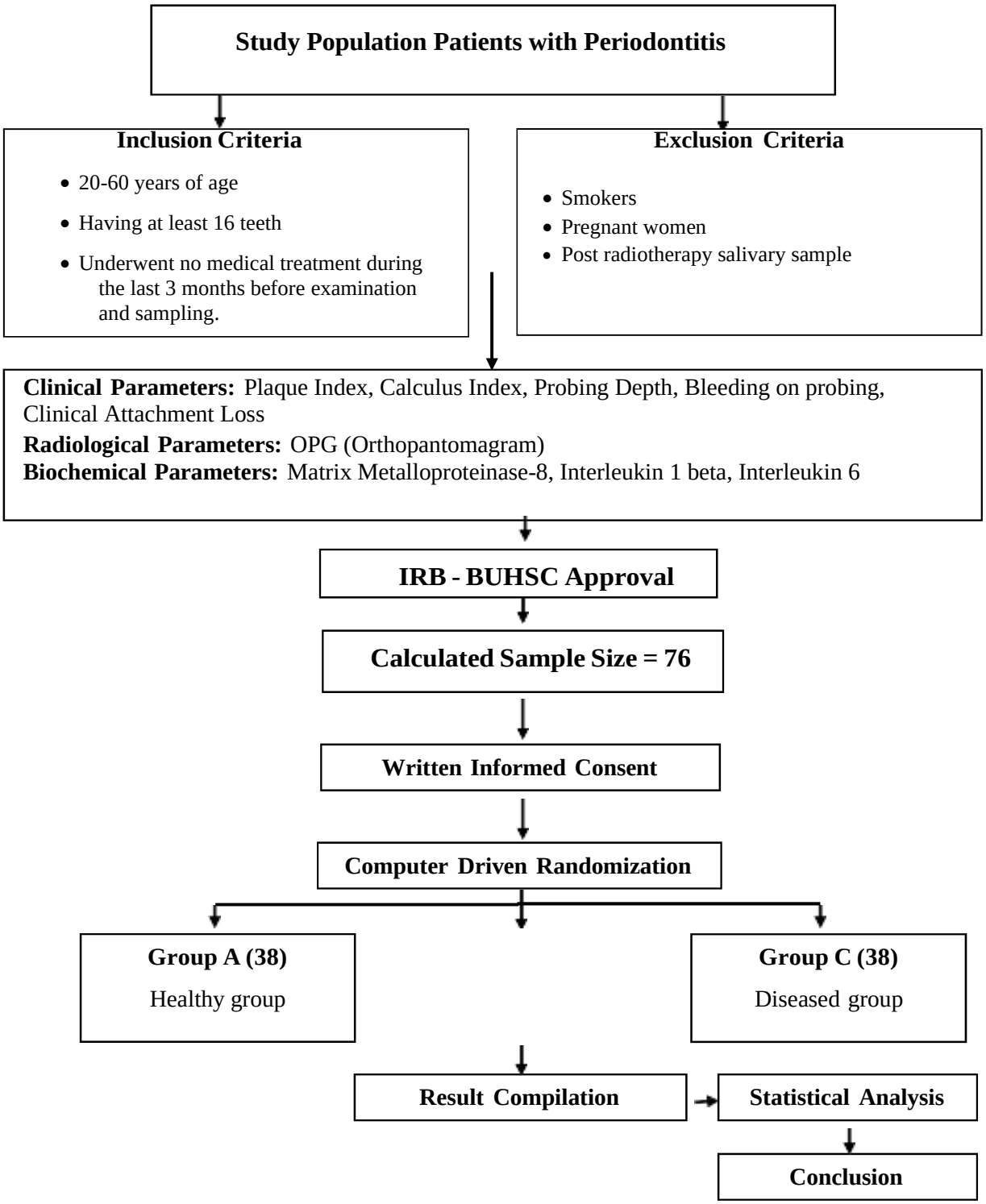


Fig 3.10: OPG (Orthopantomagram)

3.13 ALGORITHM OF STUDY



3.14 STATISTICAL ANALYSIS

- Data analysis was done by using IBM SPSS Statistics v27.
- Mean and standard deviation were calculated for quantitative variables whereas frequency and percentage were Reported for qualitative variables. Mean comparison of IL-1BETA, IL-6 and MMP-8 according to study groups were done by using independent t test.
- P- value less than 0.05 were considered as significant.

CHAPTER 4

RESULTS

The study was conducted at Bahria University Health Sciences Campus Karachi, in the Department of Periodontology, Dental section in February in the year of 2024. A total number of 76 samples were taken according to the selection criteria mentioned earlier and were split evenly into two groups: the cases and the controls. The related clinical data was documented in the subject evaluation form. After defining the standard clinical features in the patient's saliva, the data was analyzed and then compared with different clinical parameters.

4.1 Gender:

Tables 4.1 provide gender distribution in both case and control groups, and it shows that there were 60.5% male and 39.5% female patients in the cases, and 34.2% male and 65.8% female patients in the controls.

4.2 Age:

Tables 4.3 and 4.4 provide comprehensive age descriptive information comparing the cases and controls, respectively.
Figure 4.2 shows that 50% of patients and 94.7% of controls were above 45 years old. The average age was 28.52±8.63 years and 44.92±13.01 years in the cases.

4.3 Co-Morbidities:

Tables 4.3 provide comprehensive descriptive statistics on co-morbidities

between patients and controls.

In the case group, 26.3% of patients had any kind of comorbid condition, compared to 5.3% of patients in the control group. The most frequent co-morbid conditions in cases were diabetes mellitus (20%), hypertension (40%).

4.5 Plaque Grading:

Table 4.5 and 4.6 shows that among cases, 68.4% of patients had moderate plaque at the gingival margin and 31.6% had abundant plaque, while among controls, 28.3% of patients had thin plaque, 71.1% had moderated plaque, Mean plaque index was 1.31±0.47 and 0.76±0.48 respectively among cases and controls.

4.7 Calculus Grading:

Tables 4.7 and 4.8 provide a detailed frequency distribution of calculus between cases and controls. Regarding calculus, 44.7% of patients had supragingival calculus covering more than 2/3 of the exposed tooth surface, 52.6% had supragingival calculus covering more than 1/3 but not more than 2/3 of the exposed tooth surface, and 2.6% had supragingival calculus covering not more than 1/3 of the exposed tooth surface.

In contrast, 68.4% of patients in the control group had no calculus, and 31.6% had supragingival calculus covering no more than 1/3 of the exposed tooth surface. Mean calculus index was 2.57±0.55 among cases and 0.31±0.47 among controls.

4.9 Grading of Bleeding on Probing:

Mean bleeding index on probing was 1.26±0.68 among cases and 0.34±0.48 among controls shows in Tables 4.9 and 4.10, there were 13.2% of patients with several isolated bleeding spots, 47.4% participants showed spontaneous bleeding

and interdental triangle filled with blood, and 39.5% presented with profuse bleeding on probing. In contrast, among the controls, 65.8% of patients had normal gingival tissue and 34.2% had no bleeding and mild inflammation.

4.11 Probing Depth:

Tables 4.11 shows probing depth, the mean probing depth for patients and controls were 4.94±0.83 mm and 1.57±0.75 mm, respectively,

4.12 Clinical Attachment Loss:

The mean clinical attachment loss was 6.42±1.08 mm and 0.68±0.73 mm. As shown in Tables 4.12 and 4.13, respectively, all patients in the cases were found to have stage-III and IV clinical attachment loss, while 44.7% of patients in the controls had no clinical attachment loss, 52.6% had stage-I, and 2.6% had stage-II clinical loss of attachment.

4.14: Mean Levels of MMP-8, IL-1β and IL-6:

Normality was checked for IL-1β, IL-6, and MMP-8 by Shapiro wilk test as presented in Table 4.14. Among cases, the mean levels of IL-1β, IL-6, and MMP-8 were 4.95±1.26 pg/mL, 4.68±1.41 pg/mL, and 4.15±0.82 pg/mL, respectively, whereas among controls, the mean levels of these three factors were 3.90±1.01 pg/mL, 3.78±1.52 pg/mL, and 3.45±0.98 pg/mL. Tables 4.15 to 4.17, respectively, provide comprehensive descriptive statistics of IL-1β, IL-6, and MMP-8 in patients and controls.

4.18: Comprehensive Comparison of IL-1β:

A comprehensive comparison of IL-1β means is shown in Tables 4.18. The mean IL-1β was 4.95±1.26 among cases with significant mean difference

(p=0.000) and 3.90±1.01 among controls. Additionally, mean comparisons were conducted for male and female patients, as well as those with co-morbid conditions and those without, as well as patients who were 45 years of age or older. We discovered a significant mean IL-1β difference for patients who were male (p=0.007), female (p=0.010), older than 45 (p=0.030), co-morbid (p=0.021), and non-co-morbid (p=0.001).

4.19: Comprehensive Comparison of IL-6:

Tables 4.41 to 4.46 display comprehensive mean comparisons of IL-6.
Table 4.40 shows that the mean IL-6 was 4.68±1.41 among cases and 3.78±1.52 among controls with a significant mean difference (p=0.009). Additionally, mean comparisons were conducted for male and female patients, as well as those with co-morbid conditions and those without, as well as patients who were 45 years of age or older. We discovered a significant mean IL-6 difference for patients with no co-morbid conditions (p=0.014) and for individuals ≤45 years old (p=0.004).

4.20: Comprehensive Comparison of MMP-8:

The MMP-8 mean comparisons in detail are shown in Tables 4.20. The mean MMP-8 was 4.15±0.82 for controls and 3.45±0.98 for patients with a significant mean difference (p=0.000). Additionally, mean comparisons were conducted for male and female patients, as well as those with co-morbid conditions and those without, as well as patients who were 45 years of age or older. A noteworthy variation in mean MMP-8 was seen among female patients (p=0.013), patients aged 45 years or older (p=0.004), and patients without co-morbid conditions (p=0.001).

4.21: Association of demographic and clinical parameters:

According to study groups we found significant association for gender(p=0.022), age groups(p=0.000), any co-morbid (p=0.012), plaque (p=0.001), calculus(p=0.000), bleeding on probing (p=0.001) probing depth (p=0.001) and clinical attachment loss (p=0.001) according to study groups whereas no significant association were found with dietary habits, and medication use (p=0.108). Detailed results of associations are presented in Table 4.21.

4.22 Optimal cut-off values for IL-1β, IL-6 and MMP-8 in detection of periodontitis

The diagnostic performance of three biomarkers (IL-1β, IL-6, and MMP-8) were evaluated using the area under the receiver operating characteristic curve. IL-1β demonstrated the highest AUC (AUC=0.720), suggesting it is the most effective biomarker among the three for detection of periodontitis. IL-6(AUC=0.670) and MMP-8(AUC=0.684) also show significant discriminatory abilities, though slightly lower compared to IL-1β as presented in Table 4.22 (Figure 4.1). Optimal cut-off for IL-1β, IL-6, and MMP-8 was identified by doing a pilot study on 10 healthy controls. For IL-1β, the optimal cutoff was found to be 3.9 pg/mL with 51.75% sensitivity and 86.8% specificity. IL-6 had a cutoff value of 3.7 pg/mL, yielding a sensitivity of 63.2% and specificity of 68.4% MMP-8 had the cutoff value of 3.45 pg/mL, with a sensitivity of 71.1% and a specificity of 55.3%

Overall, IL-1β provided a more balanced diagnostic performance, while MMP-8 showed the highest sensitivity, and IL-6 had moderate performance with better sensitivity compared to IL-1β.

Table 4.1: Frequency distribution of gender among cases and controls (n=76)

	Frequency (Percent)	
	Cases	**Controls**
Male	**23 (60.5)**	**13 (34.2)**
Female	**15 (39.5)**	**25 (65.8)**
TOTAL	**38**	**38**

Table 4.2: Descriptive statistics of age (years) among cases and controls (n=76)

Age(years)	Mean	Std. Deviation	Min	Max	Range
Cases	**44.92**	**13.01**	**21**	**67**	**46**
Controls	**28.52**	**8.63**	**20**	**50**	**30**

Table 4.3: Frequency distribution of co-morbid among cases and controls (n=76)

	Frequency (Percent)	
	Cases	**Controls**
Yes	**10 (26.3)**	**2 (5.3)**
No	**28 (73.7)**	**36 (94.7)**
TOTAL	**38**	**38**

Table 4.4: Frequency distribution of co-morbid type among cases and controls (n=6)

	Frequency (Percent)	
	Cases	**Controls**
Diabetes Mellitus	**2 (20)**	**0**
Hypertension	**4 (40)**	**0**
TOTAL	**6**	**0**

Table 4.5: Descriptive statistics of plaque index among cases and controls (n=76)

Plaque index	Mean	Std. Deviation	Min	Max	Range
Cases	1.31	0.47	1	2	1
Controls	0.76	0.48	0	2	2

Table 4.6: Frequency distribution of plaque among cases and controls (n=76)

	Frequency (Percent)	
	Cases	Controls
No Plaque	0	0
Thin Plaque layer at the gingival margin	0	11 (28.3)
Moderate layer of plaque along the gingival margin, free interdental spaces.	26 (68.4)	27 (71.1)
Abundant layer of plaque, interdental spaces filled with plaque	12 (31.6)	0
TOTAL	38	38

Table 4.7: Descriptive statistics of calculus index among cases and controls (n=76)

Calculus index	Mean	Std. Deviation	Min	Max	Range
Cases	1.57	0.55	1	3	2
Controls	0.31	0.47	0	1	1

Table 4.8: Frequency distribution of calculus among cases and controls (n=76)

Calculus Index	Frequency (Percent)	
	Cases	Controls
No Calculus	0	26 (68.4)
Supragingival calculus covering not more than 1/3 of the exposed tooth surface	1 (2.6)	12 (31.6)
Supragingival calculus covering more than 1/3 but not more than 2/3 the exposed tooth surface and presence of flacks of subgingival calculus	20 (52.6)	0
Supragingival calculus covering more than 2/3 the exposed tooth surface or a continuous band of subgingival calculus around the cervical portion of the tooth	17 (44.7)	0
TOTAL	38	38

Table 4.9: Descriptive statistics of bleeding on probing index among cases and controls (n=76)

Bleeding on probing index	Mean	Std. Deviation	Min	Max	Range
Cases	1.26	0.68	0	2	2
Controls	0.34	0.48	0	1	1

Table 4.10: Frequency distribution of bleeding on probing among cases and controls (n=76)

	Frequency (Percent)	
	Cases	Controls
Normal gingival	0	25 (65.8)
Bleeding point appears on probing	0	13 (34.2)
Several isolated bleeding points or single fine line of blood appears	5 (13.2)	0
Interdental triangle filled with blood	18 (47.4)	0
Profuse bleeding	15 (39.5)	0
TOTAL	38	38

Table 4.11: Descriptive statistics of probing depth (mm) among cases and controls (n=76)

Probing depth (mm)	Mean	Std. Deviation	Min	Max	Range
Cases	4.94	0.83	4	7	3
Controls	1.57	0.75	0	3	3

Table 4.12: Descriptive statistics of clinical attachment loss (mm) among cases and controls (n=76)

Clinical attachment loss (mm)	Mean	Std. Deviation	Min	Max	Range
Cases	6.42	1.08	5	9	4
Controls	0.68	0.73	0	3	3

Table 4.13: Frequency distribution of clinical attachment loss among cases and controls (n=76)

	Frequency (Percent)	
	Cases	**Controls**
No clinical attachment loss	**0**	**17 (44.7)**
Stage-1 (1-2 mm)	**0**	**20 (52.6)**
Stage-II (3-4 mm)	**0**	**1 (2.6)**
Stage-III (>4 mm)	**38 (100)**	**0**
TOTAL	**38**	**38**

Table 4.14: Normality testing for IL-1β, IL-6 and MMP-8 (n=76)

	Study Group	Kolmogorov-Smirnov[a]		Shapiro-Wilk	
		Statistic	P-value	Statistic	P-value
IL-1β (pg/mL)	Control	.104	.200*	.968	.348
	Cases	.121	.179	.943	.051
IL-6 (pg/mL)	Control	.128	.120	.952	.108
	Cases	.115	.200*	.966	.301
MMP-8 (pg/mL)	Control	.144	.045	.920	.010
	Cases	.128	.116	.932	.023

Table 4.15: Descriptive statistics of IL-1β (pg/mL) among cases and controls (n=76)

IL-1β (pg/mL)	Mean	Std. Deviation	Min	Max	Range
Cases	4.95	1.26	2.47	6.74	4.27
Controls	3.90	1.01	1.71	5.64	3.93

Table 4.16: Descriptive statistics of IL-6 (pg/mL) among cases and controls (n=76)

IL6 (pg/mL)	Mean	Std. Deviation	Min	Max	Range
Cases	4.68	1.41	2.26	7.88	5.62
Controls	3.78	1.52	1.23	7.33	6.10

Table 4.17: Descriptive statistics of MMP-8 (pg/mL) among cases and controls (n=76)

MMP-8 (pg/mL)	Mean	Std. Deviation	Min	Max	Range
Cases	4.15	0.82	3.00	5.76	2.76
Controls	3.45	0.98	0.25	5.11	4.86

Table 4.18: Mean comparison of IL-1β according to study groups (n=76)

	IL-1β (pg/mL) Mean± Std. Dev		p-value
	Cases	Controls	
Overall (n=76)	4.95±1.26	3.90±1.01	0.000*
Male (n=36)	5.00±1.32	3.99±0.78	0.007*
Female (n=40)	4.89±1.21	3.86±1.13	0.010*
≤45 years (n=55)	4.63±1.36	3.89±1.04	0.030*
>45 years (n=21)	5.28±1.10	4.08±0.37	0.151**
With comorbid (n=12)	5.08±1.22	4.01±0.14	0.021
Without comorbid (n=64)	4.91±1.30	3.90±1.04	0.001*

Independent t-test was applied.

***Significant at 0.05 levels.**

****Not Significant at 0.05 levels.**

Table 4.19: Mean comparison of IL-6 according to study groups (n=76)

	IL-6 (pg/mL) Mean± Std. Dev		p-value
	Cases	Controls	
Overall (n=76)	4.68±1.41	3.78±1.52	0.009*
Male (n=36)	4.70±1.40	3.90±1.10	0.086**
Female (n=40)	4.65±1.47	3.72±1.71	0.090*
≤45 years (n=55)	5.12±1.50	3.81±1.55	0.004*
>45 years (n=21)	4.24±1.20	3.24±0.98	0.274**
With comorbid (n=12)	4.70±1.43	4.78±1.47	0.947**
Without comorbid (n=64)	4.67±1.43	3.72±1.52	0.014*

Independent t-test was applied.

***Significant at 0.05 levels.**

****Not Significant at 0.05 levels.**

Table 4.20: Mean comparison of MMP-8 according to study groups (n=76)

	MMP (pg/mL) Mean± Std. Dev		p-value
	Cases	Controls	
Overall (n=76)	4.15±0.82	3.45±0.98	0.001*
Male (n=36)	4.17±0.75	3.82±0.65	0.181**
Female (n=40)	4.13±0.94	3.25±1.08	0.013*
≤45 years (n=55)	4.30±1.01	3.43±1.00	0.004*
>45 years (n=21)	4.01±0.56	3.81±0.43	0.639**
With comorbid (n=12)	3.98±0.69	4.03±0.68	0.933**
Without comorbid (n=64)	4.21±0.86	3.41±0.99	0.001*

Independent t-test was applied.

***Significant at 0.05 levels.**

****Not Significant at 0.05 levels.**

Table 4.21: Association of demographic and clinical parameters according to study groups (n=64)

		Study Groups Frequency(percent)		p-value
		Controls	Cases	
Gender	Male	13(34.2)	23(60.5)	0.022*
	Female	25(65.8)	15(39.5)	
Age Groups	≤45 years	36(94.7)	19(50)	0.000*
	>45 years	2(5.3)	19(50)	
Any Co-morbid	Yes	2(5.3)	10(26.3)	0.012*
	No	36(94.7)	28(73.7)	
Dietary Habits	Regular	38(100)	35(92.1)	0.240
	Irregular	0(0)	3(7.9)	
Use of Medications	Yes	1(2.6)	6(15.8)	0.108
	No	37(97.4)	32(84.2)	
Plaque	No Plaque	10(26.3)	0(0)	0.001*
	Thin Plaque	27(71.1)	26(68.4)	
	Moderate Plaque	1(2.6)	12(31.6)	
Calculus	No Calculus Present	26(68.4)	0(0)	0.002*
	Supragingival calculus covering not more than 1/3 of the exposed tooth surface	12(31.6)	17(44.7)	

	Supragingival calculus covering more than 1/3 but not more than 2/3 the exposed tooth surface	**0(0)**	**20(52.6)**	
	Supragingival calculus covering more than 2/3 the exposed tooth surface	**0(0)**	**1(2.6)**	
Bleeding on Probing	**Normal gingival**	**25(65.8)**	**5(13.2)**	**0.001***
	No Bleeding/Mild Inflammation	**13(34.2)**	**18(47.4)**	
	Bleeding on probing/Moderate Inflammation	**0(0)**	**15(39.5)**	
Clinical attachment loss	**No Clinical attachment loss**	**17(44.7)**	**0(0)**	**0.001***
	Stage I	**20(52.6)**	**0(0)**	
	Stage II	**1(2.6)**	**0(0)**	
	Stage III	**0(0)**	**38(100)**	

Chi-square/fisher exact test was applied.

***Significant at 0.05 levels.**

****Not Significant at 0.05 levels.**

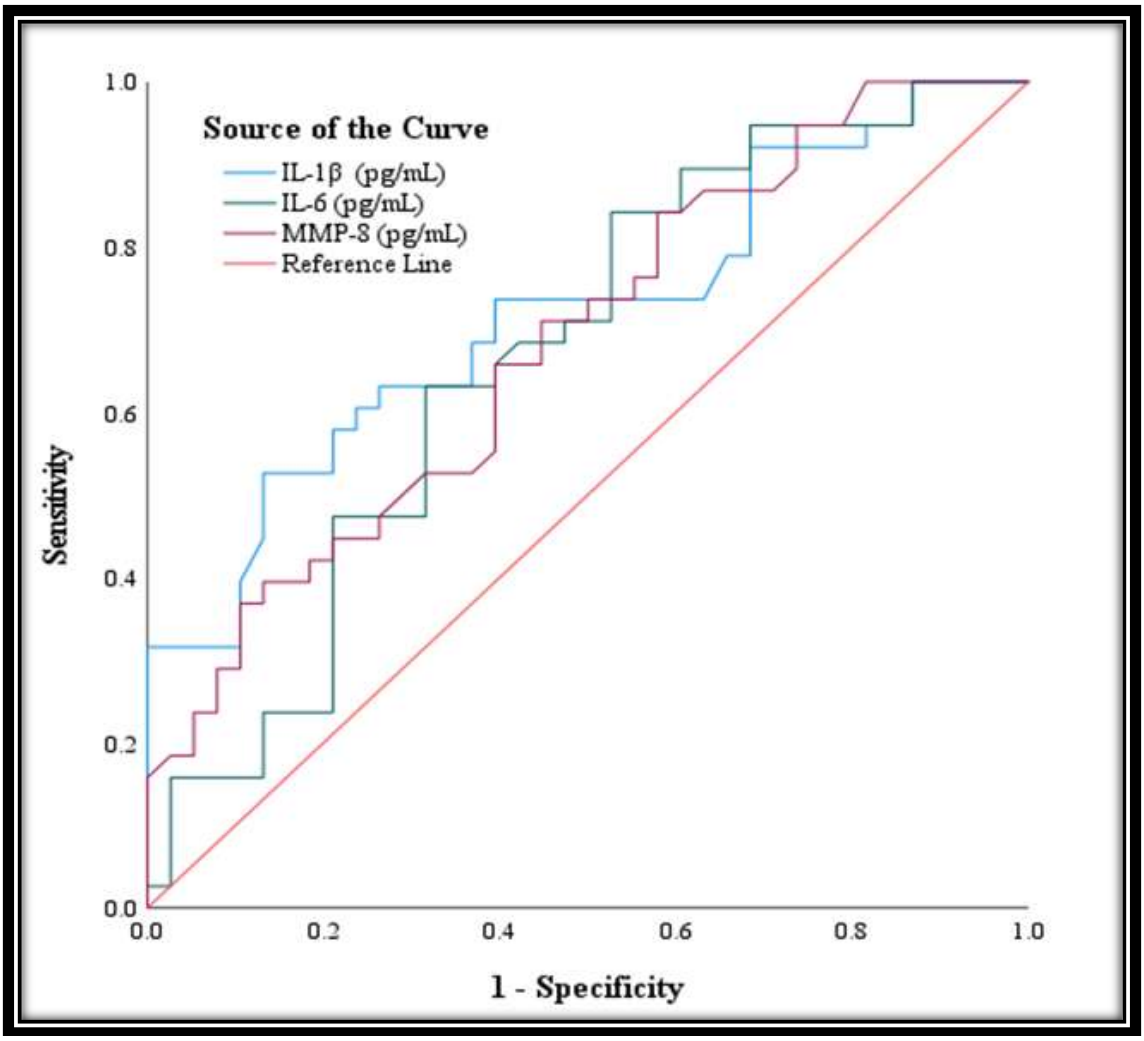


Figure 4.1: Receiver Operating Curve (ROC)

Table 4.22: Area under curve (AUC) for IL-1β, IL-6 and MMP-8

Test Result Variable(s)	AUC	Std. Error	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
IL-1β (pg/mL)	.720	.059	.001	.605	.834
IL-6 (pg/mL)	.670	.062	.011	.548	.792
MMP-8 (pg/mL)	.684	.060	.006	.565	.802

Table 4.23: Optimal cut-off for IL-1β, IL-6 and MMP-8 in detection of periodontitis

	Cutt Off	Sensitivity	Specificity
IL-1β (pg/mL)	3.9	52.6%	86.8%
IL-6 (pg/mL)	3.78	63.2%	68.4%
MMP-8 (pg/mL)	3.45	71.1%	55.3%

CHAPTER 5

DISCUSSION

5.1 SEQUENCE OF DISCUSSION EXPERIMENT

The discussion chapter has covered the elaborate analysis considering the results of the present study. It has analyzed the parameters like calculus index, plaque index, bleeding on probing, periodontal probing depth and clinical attachment loss. This study analyzed salivary levels of IL-1β, IL-6 and MMP-8 in periodontally healthy and diseased subjects as clinical parameters which are used for prediction of destructive periodontal disease. They are evaluated one by one in the context of their results for the present study. It has also discussed age, gender and co-morbidity in the results. Role of all parameters have been discussed in the discussion section one by one. The various reviews which are taken from the literature from previous records to compare the present study output.

In our study a total of 76 patients were included and split evenly into two groups: 38 cases and 38 controls. The study showed that there were 60.5% male and 39.5% female patients in the cases, and 34.2% male and 65.8% female patients in the controls. The average age was 28.52±8.63 years and 44.92±13.01 years in the cases. And it shows that 50% of cases were above 45 years old.

The demographic characteristics of the studied population showed significant differences in age and gender between periodontitis and healthy groups (p=0.000 and p=0.022, respectively)

A study conducted in Pakistan found that 82% of those 60 years of age and older had periodontitis. This is comparable to the national survey, which found that 93% of people in the same age group had the disease (Khawaja Khail, A. A et al 2015). Other studies carried out in Pakistan, like Chaudhry et al 2003, found that 98% of cases aged 18 to 52 had the disease, with 31% having advanced periodontitis.

Periodontitis is more prevalent in men than in women. Studies have shown that men

have a higher prevalence of this disease, with approximately 57% of men being affected compared to 39% of women. (Könönen, E., Gursoy, M.,2019). Data from NHANES show that men have higher rates of periodontal disease compared to women across various age groups. This survey provides comprehensive health and nutrition data for the U.S. population and includes periodontal assessments. Data from 2009-2014 shows that men have higher rates of periodontal disease compared to women. Specifically, the prevalence of total periodontitis was found to be 50.2% in men and 34.6% in women The American Academy of Periodontology reports that men are less likely to take proactive steps in maintaining their oral health, such as regular brushing, flossing, and dental check-ups, leading to a higher prevalence of periodontitis.

Our research highlighted that in the case group, 26.3% of patients had some kind of comorbid condition, compared to 5.3% of patients in the control group. The *p* value is significant (p=0.012). The most frequent co-morbid conditions in cases were diabetes mellitus (20%), hypertension (40%).

Patients with moderate to severe periodontitis had a 50% higher risk of hypertension than those without periodontal disease, according to a systematic review and meta-analysis research (Munoz Aguilera, E., et al 2020). In a comparable manner, a retrospective cross-sectional study found that people with periodontitis had a 20% greater chance of having blood pressure (Pietropaoli, D., et al 2018). The findings are corroborated by an observational research that examined the prevalence of both hypertension and periodontitis (Padmanabhan, P., et al 2015).

Hosadurga, R., 2020 conducted a study with 270 patients, SBP was higher among the participants who were edentulous than partially edentulous. However, there was no significant association between tooth loss and SBP and DBP after adjusting for confounding factors. In another study, a higher number of missing teeth in individuals with higher blood pressure rates was observed. However, the reasons for tooth loss in the included patients can be a consequence of periodontitis, as well as other reasons, such as periapical lesions, fractures, trauma, and caries. (Al-Ahmad, B. E. M., et al 2018)

The relationship between hypertension and periodontitis is associated with a chronic

immune–inflammatory disorder increased mainly by periodontal inflammation. First, hypertension can intensify the activation of innate and adaptive immune cells, such as monocytes, macrophages, and T and B lymphocytes (Hosadurga, R., 2020). Conversely, periodontopathogens can intensify the inflammatory cascade through the activation of Th1 and Th17 lymphocytes, which in turn triggers an inflammatory mechanism that leads to hypertension (Suvan, J. at al 2021). Furthermore, periodontitis raises levels of systemic and local inflammatory markers by encouraging modifications in neutrophil activity, which lead to endothelial and vascular dysfunction (Suvan, J et al 2021). Specifically, endothelial dysfunction upsets the balance of regulation by lowering nitric oxide and raising interleukin-6 (IL-6), tumor necrosis factor (TNF-α), and CRP levels in the blood (Leira, Y et al 2020).

Studies currently available indicate a directly proportional relationship between diabetes and periodontitis. Diabetes mellitus is a major risk factor for periodontitis (Li, Y et al 2021). Research has shown that individuals with insufficient control of their diabetes had an 86% higher prevalence of periodontitis development when compared to people without diabetes or with well-controlled diabetes (Nascimento, G. G et al 2018). However, individuals with periodontitis have worse control over their blood sugar, an increased chance of developing insulin resistance, and a greater frequency of problems associated with diabetes (George, A. K et al 2021) Numerous research investigations have confirmed that periodontal treatment can lower the amount of periodontal inflammation, which in turn improves blood levels of glycated hemoglobin (Di Domenico, G. L et al 2023). As a result, screening for periodontitis in people with diabetes is essential, and vice versa (Stöhr, J., Barbaresko, J. et al 2021)

Patients with diabetes are two to three times more likely than the general population to develop periodontitis (Mealey, B. L., et al 2007), glycemic management is a major factor in predicting risk (Trentin, M. S., 2018). Diabetes mellitus is one of the actual risk factor for periodontal disease (Ganesan, S. M., Joshi, V et al 2017)) and has been added to the "grading" system of the new European Federation of Periodontology (EFP) and American Academy of Periodontology (AAP)

classification of periodontal diseases (Tonetti, M. S., et al 2018).

Our study showed that shows that among cases, 68.4% of patients had moderate plaque at the gingival margin, they were graded as 2 and 31.6% had abundant plaque and they were graded as 3, while among controls, 28.3% of patients had thin plaque, 71.1% had moderated plaque, Mean plaque index was 1.31±0.47 and 0.76±0.48 respectively among cases and controls. Regarding calculus, 44.7% of patients had supragingival calculus covering more than 2/3 of the exposed tooth surface they were graded as 4, 52.6% had supragingival calculus covering more than 1/3 but not more than 2/3 of the exposed tooth surface and they were graded as 3, and 2.6% had supragingival calculus covering not more than 1/3 of the exposed tooth surface. In contrast, 68.4% of patients in the control group had no calculus, and 31.6% had supragingival calculus covering no more than 1/3 of the exposed tooth surface. Mean calculus index was 1.57±0.55 among cases and 0.31±0.47 among controls. P value is significant for both plaque and calculus index (p=0.000). Research indicates that individuals with periodontitis often exhibit moderate to severe calculus due to the chronic nature of the disease and its impact on oral hygiene and plaque control. This relationship is supported by findings from various studies and clinical observations that highlight the prevalence of significant calculus build-up in patients with periodontitis (Saleh, M. H., 2022).

Study by Hajishengallis and Lamont (2021) highlights how dysbiosis, a microbial imbalance in dental plaque, contributes to periodontitis. It shows that pathogenic bacteria disrupt the homeostasis of the oral microbiome, leading to a pro-inflammatory environment and tissue destruction.

Kolenbrander et al. (2022) discusses the resilience of dental plaque biofilms and their ability to resist host defenses and antimicrobial treatments. It emphasizes the importance of mechanical disruption of biofilms in periodontal therapy.

Graves et al. (2022) focuses on the interaction between plaque bacteria and the host immune system. It demonstrates how bacterial components like lipopolysaccharides (LPS) from Gram-negative bacteria in plaque can trigger a robust immune response, leading to chronic inflammation and tissue damage.

A review explores new therapeutic strategies targeting dental plaque biofilms, such as the use of biofilm-disrupting agents and targeted antimicrobial peptides. These

approaches aim to reduce the microbial load and mitigate the inflammatory response associated with periodontitis (Marsh and Devine et al 2023).

A review discusses the multifactorial nature of periodontitis, highlighting the role of both dental plaque and calculus in its pathogenesis and progression. (Kornman, K.S. et al 2022).

Another study highlights the microbial composition of periodontal biofilms, including the role of calculus in providing a niche for pathogenic bacteria. (Teles, R., et al 2013).

In our study, in the case group, the mean levels of IL-1β, IL-6, and MMP-8 were 4.95±1.26 pg/mL, 4.68±1.41 pg/mL, and 4.15±0.82 pg/mL, respectively. In the control group, the mean levels of these three factors were 3.90±1.01 pg/mL, 3.78±1.52 pg/mL, and 3.45±0.98 pg/mL. P value is significant for these markers.

Table 4.47 shows that the mean for MMP-8 was 4.15±0.82 for cases and 3.45±0.98 for controls with a significant mean difference (p=0.000). Mean comparisons were conducted for male and female patients, as well as those with co-morbid conditions and those without, as well as patients who were 45 years of age or older. A noteworthy variation in mean MMP-8 was seen among female patients (p=0.013), patients aged 45 years or older (p=0.004), and patients without co-morbid conditions (p=0.001)

The primary gingival collagenase, MMP-8, is the most researched biomarker for the detection of periodontitis in gingival crevicular fluid and is correlated with the state, severity, and development of periodontal disease (Hernández, M et al 2020) (Arias-Bujanda, N., et al 2020).

Metalloproteinase-8 (MMP-8), released by neutrophils, is a molecule having the unique ability to break down type I and III collagens, which are the major collagen species within the periodontium (Rathnayake, N. et al 2013). Probing depth, clinical attachment loss, BOP, and PI were all clinical periodontal indicators that were linked to high levels of MMP-8 in saliva. According to Fatemi, K., et al 2020, individuals with generalized moderate to severe periodontitis had higher concentrations of MMP-8 in their saliva.

In our study a noteworthy variation in mean MMP-8 was seen among female patients

(p=0.013) than males.

Studies indicate that MMP-8 (matrix metalloproteinase-8) levels are generally higher in females with periodontitis. Research has shown that women tend to have elevated levels of MMP-8 in their saliva and gingival crevicular fluid compared to men, which suggests a higher inflammatory response in females with periodontitis. (Nardi, G. M., et al 2020). Hormones such as estrogen and progesterone can influence the expression and activity of MMPs. During menstrual cycles, pregnancy, and menopause, fluctuations in these hormones can lead to changes in periodontal tissue response and inflammation. Estrogen, in particular, has been shown to affect collagen metabolism and the regulation of MMPs, including MMP-8. (Virtanen, E.,2017).

Our study highlighted p value significant (p=0.004) in patients aged 45 years or older.

Studies indicate that Matrix Metalloproteinase-8 (MMP-8) levels tend to be elevated in elderly patients with periodontitis. MMP-8, a collagenase, plays a crucial role in the degradation of extracellular matrix components, contributing to tissue destruction observed in periodontal disease. In comparison to individuals without periodontitis, people with the disease have considerably greater levels of matrix metalloproteinase-8 (MMP-8). This rise is observed regardless of any comorbid diseases, suggesting that MMP-8 is a valid biomarker for periodontitis diagnosis.

The higher levels of MMP-8 in elderly patients could be attributed to age-related changes in immune response and tissue turnover, which may exacerbate inflammatory processes in periodontal tissues. (Checchi, V.,2020).

Our study showed that p value (p=0.001) is significant in patients without co-morbid conditions.

A study highlighted that in comparison to individuals without periodontitis, people with the disease have considerably greater levels of matrix metalloproteinase-8 (MMP-8). This rise is observed regardless of other comorbid diseases, suggesting that MMP-8 is a valid biomarker for periodontitis diagnosis (Yuan, C., 2018).

Some studies reported that no statistical difference could be found between gingivitis and healthy periodontium regarding saliva levels of MMP-8 (Noack, B., et al 2017).

Another study showed that total MMP-8 may not be able to reflect effectively periodontal breakdown or progression of periodontitis, and they stated that instead of total MMP-8, the assessment of active MMP-8 (aMMP-8) levels may reflect a proinflammatory state of periodontal disease and may help in staging and grading periodontitis (Sorsa, T., et al 2020).

Salivary MMP-8 levels were altogether higher in patients with periodontal infection compared to healthy people, concurring to a precise survey and meta-analysis by Preshaw et al. (2016), which raised the possibility that salivary MMP-8 could be a helpful biomarker for the diagnosis of periodontal disease. Periodontal disease and blood MMP-8 levels did not correlate, according to (Passoja et al 2008). According to Özçaka et al.'s 2011 investigation, there was no significant difference seen in the blood levels of MMP-8 between patients with chronic periodontitis and periodontal healthy persons. Serum biomarker levels were shown by Kinney et al. to be insignificant in the diagnosis of periodontitis. (Kinney, J. S., 2014).

In the case of localized aggressive periodontitis, Gonçalves et al 2013 showed that SRP and systemic antibiotic usage successfully lowered local levels of particular MMPs. Leppilahti et al 2015, demonstrated that MMP-8 levels in GCF in particular are more stable and stay below a certain threshold level in patients who received azithromycin antimicrobial therapy. SRP improves all clinical periodontal markers evaluated, with the exception of CAL,

MMP-8 as a supplementary tool for periodontal disease screening may be limited due to variations in measuring techniques with varying degrees of agreement (Gursoy, U. K et al 2010). Here, we demonstrate that: (a) MMP-8 exhibited the highest accuracy in differentiating between periodontitis and healthy sites; and (t) MMP-8 showed the best diagnostic precision in differentiating between moderate and severe periodontitis sites (Sorsa, T., 2016).

Another study concluded that the concentration of MMP-8 was greater in the diseased group even though there was no statistically significant difference between the levels in the healthy and diseased groups ($p=0.057$). The little population size in the healthy group is the primary reason for the absence of meaningful correlation.

Early research revealed that individuals with periodontitis had significantly greater MMP-8 levels than those without the illness (Keles Yucel, Z. P et al 2020) (Syrjäläinen, S et al 2019).

Matrix metalloproteinase-8 (MMP-8) has been shown to have significant correlations with key clinical parameters of periodontal disease, including bleeding on probing (BOP), clinical attachment loss (CAL), and periodontal probing depth (PPD).

Studies have demonstrated that elevated levels of MMP-8 are associated with increased BOP. This correlation arises because MMP-8, an enzyme involved in the breakdown of extracellular matrix components, can lead to heightened inflammation and tissue degradation, which results in bleeding upon probing (Sorsa et al., 2006).

Another study concluded that elevated levels of MMP-8 have been associated with increased BOP. MMP-8, contributes to inflammation and tissue degradation, leading to bleeding upon probing. Studies have shown a significant correlation between MMP-8 levels in gingival crevicular fluid and BOP (Kinney et al., 2007). Some studies have found no significant correlation between MMP-8 levels and BOP. For example, a study by Eley and Cox (2003) did not find a consistent association between MMP-8 levels in gingival crevicular fluid and BOP, suggesting that other factors might influence bleeding responses in periodontal tissues.

MMP-8 levels are strongly correlated with CAL. Higher concentrations of MMP-8 indicate increased degradation of collagen fibers in the periodontal ligament and alveolar bone, contributing to the loss of attachment observed in periodontitis patients (Gursoy et al., 2010). Contradictory evidence regarding the correlation between MMP-8 levels and CAL has been reported. In a study by Kinney et al. (2007), the researchers found that MMP-8 levels did not consistently correlate with CAL across different patient populations, indicating variability in the enzyme's role in tissue destruction.

There is a significant correlation between elevated MMP-8 levels and deeper periodontal pockets (PPD). MMP-8 activity results in the destruction of periodontal tissues, which manifests as increased probing depths, a hallmark of advanced periodontal disease (Kraus et al., 2012). Discrepant findings have also been observed concerning PPD. A study by Marcaccini et al. (2010) reported that while there was

some association between MMP-8 levels and PPD, the correlation was not strong enough to be considered a reliable marker for all patients with periodontitis. This suggests that MMP-8 alone may not fully reflect the severity of periodontal pocketing in all cases.

These contradictory findings underscore the complexity of periodontal disease and suggest that MMP-8 levels may be influenced by multiple factors, including individual patient differences and the presence of other inflammatory mediators.

Studies have found a positive correlation between MMP-8 levels and plaque index. Higher MMP-8 levels are often associated with increased plaque accumulation, reflecting the enzyme's response to bacterial biofilm and its role in periodontal inflammation (Kornman et al., 1997). MMP-8 levels also show a correlation with calculus index. Calculus, which serves as a reservoir for bacterial plaque, can contribute to the inflammatory response and subsequent release of MMP-8, linking higher levels of this enzyme to increased calculus formation (Räisänen et al., 2019).

Some studies have reported no significant correlation between MMP-8 levels and PI. For example, a study by Mantyla et al. (2006) found that MMP-8 levels in gingival crevicular fluid did not consistently correlate with the amount of dental plaque, suggesting that MMP-8 might be more indicative of tissue inflammation and destruction rather than plaque accumulation alone.

Similarly, conflicting results have been observed regarding the relationship between MMP-8 levels and calculus index. In a study by Ramseier et al. (2009), researchers did not find a strong correlation between MMP-8 levels and calculus formation, indicating that MMP-8 levels might not be directly influenced by the presence of calculus but rather by the inflammatory response to bacterial biofilm.

In our study the mean probing depth for patients and controls were 4.94±0.83 mm and 1.57±0.75 mm, respectively, whereas the mean clinical attachment loss was 6.42±1.08 mm and 0.68±0.73 mm. All patients in the cases were found to have stage-III clinical attachment loss and according to the 2017 Classification of Periodontal and Peri-Implant Diseases they are classified as stage III periodontitis and rated as Grade C periodontitis, while 44.7% of patients in the controls had no clinical attachment loss, 52.6% had stage-I and classified as stage I periodontitis, and 2.6%

had stage-II clinical loss of attachment and classified as stage II periodontitis.

Our study also showed that among cases, there were 13.2% of patients with several isolated bleeding spots and they were graded as 2, 47.4% participants showed spontaneous bleeding and interdental triangle filled with blood and they were graded as 3, and 39.5% presented with profuse bleeding on probing and they were graded as 4. In contrast, among the controls, 65.8% of patients had normal gingival tissue and 34.2% had no bleeding and mild inflammation. Mean bleeding index on probing was 1.26±0.68 among cases and 0.34±0.48 among controls P value is significant for clinical attachment loss, periodontal probing depth and bleeding on probing (p=0.000)

In periodontitis, BOP is mostly seen in Grade 2 and Grade 3, reflecting a more widespread and severe inflammatory response. Studies show that these higher grades of BOP are strongly associated with active periodontal disease and are used to monitor the effectiveness of periodontal therapy (Gupta, N.,2015).

Our study highlighted that the mean IL-1β was 4.95±1.26 among cases and 3.90±1.01 among controls with a significant mean difference (p= 0.000) Additionally, mean comparisons were conducted for male and female patients, as well as those with co-morbid conditions and those without, as well as patients who were 45 years of age or older. We discovered a significant mean IL-1β difference for patients who were male (p=0.007), female (p=0.010), older than 45 (p=0.030), co-morbid (p=0.021), and non-co-morbid (p=0.001).

IL-1β is a proinflammatory cytokine recognized as an important mediator in the pathophysiology of periodontitis. Gursoy et al. suggested that IL-1β is a well-potent inflammatory stimulator that can help discriminating between inactive and active periodontal lesions (Gursoy, U. K., et al 2009). Its properties include promoting bone resorption and inducing the production of tissue-degrading proteinases (Oh, H., et al 2015).

Our study showed that we discovered a significant mean IL-1β difference for patients who were male (p=0.007), female (p=0.010). The similar levels of IL-1β in both male and female periodontitis patients can be attributed to the similar immune responses both genders exhibit towards bacterial infections causing periodontitis.

The immune response involves the release of cytokines like IL-1β to combat pathogens, which leads to comparable levels of this cytokines across genders. This is supported by studies that have found no significant gender-specific differences in the levels of IL-1β among periodontitis patients (Zhang, Y., 2021).

Another study concluded that the findings of higher IL-1*β* concentrations in saliva from patients with grade B periodontitis are in agreement with several earlier studies that showed higher salivary levels of this analyte in patients suffering from periodontitis in comparison with healthy subjects(Cheng, R., et al 2020) (Rangbulla, V et al 2017) (Syrjäläinen, S., 2019).It has been reported that patients with deeper pocket depths and more severe bleeding on probing (BOP) had increased value levels of salivary IL-1*β*.

A study evaluated salivary interleukin IL-1*β*, matrix metalloproteinase MMP-8, as potential diagnostic tools for periodontitis diagnosis. They concluded that as a single marker IL-1*β* showed the best diagnostic value with 90% sensitivity and 76% specificity for discriminating periodontitis subjects from healthy subjects. Thus, IL-1*β* is a strong promising biomarker for the early diagnosis of periodontal disease (Zhang, Y., et al 2021).

According to Garlet, G. P. 2010, proinflammatory cytokines including IL-1α, IL-1β, TNF-α, IL-6, and IL-17 can lead to severe chronic inflammation and tissue degradation.

In several investigations, Il-6 was also evaluated as a proinflammatory cytokine that contributes to bone loss when an infection is present (Rathnayake, N., et al 2013). In fact, it has been proposed that salivary IL-6 concentrations rose markedly in individuals with periodontitis relative to healthy controls, and that these increases coincided with the disease's advancement (Batool, H., et al 2018). According to a study, there is a substantial positive correlation between salivary IL-6 levels and increases in clinical attachment loss, periodontal probing depth, and bleeding on probing (Isola, G., et al 2021). In saliva samples from the Taiwanese population, the authors did not identify any statistically significant differences in the IL-6 level value (Wu, Y. C., et al 2018).

According to a study, the group with periodontitis had greater levels of IL-1B than

the groups with gingivitis and the healthy control. A study comparing the salivary IL-1 levels of healthy individuals and patients with periodontitis found a similar tendency. They were much greater than those found in healthy controls, even though there was no statistically significant difference between the periodontitis groups. In this study, it was discovered that the levels of IL-B in gingivitis were higher than in healthy controls. However, there was no discernible difference in IL-1B levels between the gingivitis and periodontitis groups and the healthy controls. Additionally, this data matched the clinical measurements. The study's conclusions confirmed the value of saliva as a sample technique in periodontal disease due to immunological factors. Increased amounts of IL-1β have been proposed as a potential host-response factor linked to periodontal disease clinical symptoms (Tobón-Arroyave, S. I., et al 2008).

Furthermore, individuals suffering from chronic periodontitis experience elevated blood levels of IL-1β, which might have a systemic effect (Kusuhara, M et al. 2006). This result suggests a potential causative relationship between systemic disorders including cardiovascular diseases and periodontitis through IL-1β.(Zhu, H et al. 2015). The inflammasome signaling pathway associated with nod-like receptor protein-3 (NLRP3) is implicated in periodontal disease by activating IL-1β. In both gingivitis and periodontitis, the NLRP3 inflammasome complex's mRNA expression was increased. (Bostanci, N et al. 2009).

Elevated levels of IL-1β are significantly associated with increased BOP. IL-1β is a pro-inflammatory cytokine that plays a crucial role in the inflammatory response and tissue degradation seen in periodontitis, contributing to increased bleeding upon probing (Yucel-Lindberg & Båge, 2013). Some studies have reported conflicting results regarding the association between IL-1β levels and BOP. For instance, a study by Loe et al. (1998) found that while IL-1β levels correlated with other markers of inflammation, the correlation with BOP was not consistently strong across all subjects, suggesting variability in individual inflammatory responses.

There is a strong correlation between higher IL-1β levels and greater CAL. IL-1β promotes the breakdown of collagen and other extracellular matrix components in the periodontal ligament, leading to the loss of attachment between the tooth and the

surrounding supportive tissues (Engebretson et al., 2002). Increased IL-1β levels are associated with deeper periodontal pockets. IL-1β contributes to the inflammatory process that results in the destruction of periodontal tissues, reflected in increased probing depths, a clinical indicator of the severity of periodontal disease (Tonetti et al., 2007). Contradictory evidence exists regarding the correlation between IL-1β levels and CAL/PPD. While IL-1β is known to contribute to tissue destruction, not all studies have found a robust correlation with CAL and PPD in all patient populations (Engebretson et al., 2002).

IL-1β levels have also been correlated with plaque and calculus indices. Higher IL-1β levels are associated with increased plaque accumulation and calculus formation, reflecting the cytokine's role in the inflammatory response to bacterial biofilm and its contribution to periodontal tissue destruction (Bickel et al., 2009). The relationship between IL-1β and CI/PI also shows variability. Some studies have reported significant correlations, indicating that IL-1β levels may reflect the extent of plaque accumulation and calculus formation. However, conflicting results suggest that IL-1β alone may not consistently predict these indices across different patient groups (Bickel et al., 2009).

Our study also shows that the mean IL-6 was 4.68±1.41 among cases and 3.78±1.52 among controls with a significant mean difference (p=0.009) Additionally, mean comparisons were conducted for male and female patients, as well as those with co-morbid conditions and those without, as well as patients who were 45 years of age or older. We discovered a significant mean IL-6 difference between patients with no co-morbid conditions (p=0.014) and for individuals ≤45 years old (p=0.004). Another most promising salivary biomarkers for the early detection of periodontitis is interleukin-6 (IL-6). Ahn et al. (2011) carried out the first study with the goal of assessing the diagnostic capacity of salivary IL-6 for the identification of periodontitis. 40 patients with periodontitis and 40 healthy controls were enrolled in the study. In comparison to healthy controls, periodontitis patients had considerably greater levels of salivary IL-6. Salivary IL-6, according to the study, may be used as a possible biomarker for the early detection of periodontitis. The findings showed that the periodontitis group had considerably greater salivary IL-6 levels than the

control group. Salivary IL-6 may be employed as an easy-to-use, non-invasive diagnostic tool for the early diagnosis of periodontitis, according to the study.

Salivary cytokines, particularly IL-1β, IL-6, can serve as reliable markers for periodontal disease severity and may aid in monitoring disease progression. The growing interest in salivary biomarkers for periodontal disease marks a significant shift in dental diagnostics and treatment approaches.

Elevated levels of IL-6 are significantly associated with increased BOP. IL-6 plays a role in the acute-phase response to inflammation, contributing to increased vascular permeability and tissue damage, which can lead to bleeding upon probing (Stashenko et al., 1991). Some studies have reported contradictory findings regarding the association between IL-6 levels and BOP. For instance, a study by Hienz et al. (2000) found that while IL-6 levels were elevated in patients with periodontitis, the correlation with BOP was not consistently significant across all subjects, suggesting variability in individual inflammatory responses. There is evidence supporting a correlation between higher IL-6 levels and greater CAL. IL-6 promotes the production of other inflammatory mediators and tissue-destructive enzymes, contributing to the breakdown of periodontal tissues and subsequent attachment loss (Loos et al., 2000). Increased IL-6 levels have been associated with deeper periodontal pockets. IL-6 is involved in the regulation of bone metabolism and the inflammatory response within the periodontal tissues, contributing to the progression of periodontitis and deeper probing depths (Loos et al., 2000).

Contradictory evidence exists regarding the correlation between IL-6 levels and CAL/PPD. While IL-6 is involved in the inflammatory cascade leading to tissue destruction, not all studies have found a strong and consistent correlation with CAL and PPD in all patient populations (Lamster et al., 2003).

IL-6 levels have also been correlated with plaque and calculus indices. Higher IL-6 levels are associated with increased plaque accumulation and calculus formation, reflecting the cytokine's role in the inflammatory response to bacterial biofilm and its contribution to periodontal tissue destruction (Kobayashi et al., 2007).

The relationship between IL-6 and PI/CI also shows variability. Some studies have reported significant correlations, indicating that IL-6 levels may reflect the extent of

plaque accumulation and calculus formation. However, conflicting results suggest that IL-6 alone may not consistently predict these indices across different patient groups (Ebersole et al., 1997).

Some salivary analytes can be utilized as a technique to "intercept" the early stages of periodontal disorders since they are biological reflections of the inflammatory and tissue-destructive processes that occur during periodontitis. Numerous studies have suggested that evaluating a panel of biomarkers may be a more effective approach to diagnosing periodontitis than focusing on a single salivary biomarker (Meisner, A. et al 2019). A panel comprising IL-1β, MMP-8, and IL-6 has special diagnostic relevance for periodontitis (Ebersole, J. L et al 2013).

In our study, we have identified several asymptomatic cases among controls, who were otherwise healthy. Out of 38 controls, 5 participants had elevated levels of interleukin 6 and 6 participants had elevated levels of interleukin 1β. Despite the absence of clinical symptoms, these individuals exhibited raised levels of interleukin 1 beta, interleukin 6. This finding suggests that these subjects are at a high risk for developing periodontitis. The raised levels of these biomarkers indicate underlying inflammatory processes, highlighting their potential utility in monitoring the early stages and progression of periodontal disease, even before clinical symptoms manifest.

The value of 8 pg/mL of IL-1β in the context of periodontitis is considered indicative of mild to moderate disease. According to a recent study published in the *Journal of Clinical Medicine* in 2023, IL-1β levels are significantly higher in patients with periodontitis compared to healthy controls. The study reports that higher IL-1β levels correlate with more severe forms of the disease, with levels above 61 pg/mL associated with severe periodontitis. Therefore, a level of 8 pg/mL suggests a less severe, early-stage periodontitis condition. (Relvas, M.,2023). This classification implies that while the patient may have some level of periodontal inflammation, the overall risk for rapid progression and severe tissue destruction is low. (O'Neill, C. M., 2013).

According to a meta-analysis two-biomarker combinations in oral fluids show high diagnostic accuracy for periodontitis, which is not substantially improved by

incorporating more biomarkers. In saliva, the dual combinations of IL-1β, IL-6 and in asymptomatic periodontitis, IL-6 levels reflect the ongoing inflammatory process within periodontal tissues. Monitoring IL-6 levels aids in detecting and monitoring disease activity, providing insights into disease progression and response to treatment (Loos et al., 2000).

Elevated IL-1β levels in asymptomatic periodontitis indicate ongoing localized inflammation and tissue breakdown, despite the absence of clinical symptoms. Monitoring IL-1β levels helps in assessing disease severity and guiding treatment strategies (Yucel-Lindberg & Båge, 2013).

Our study also highlighted the diagnostic performance of these three biomarkers (IL-1β, IL-6, and MMP-8) and they were evaluated using the area under the receiver operating characteristic curve. IL-1β demonstrated the highest AUC (AUC=0.720), suggesting it is the most effective biomarker among the three for detection of periodontitis. IL-6(AUC=0.670) and MMP-8(AUC=0.684) also show significant discriminatory abilities, though slightly lower compared to IL-1β.

Optimal cut-off for IL-1β, IL-6, and MMP-8 was identified by doing a pilot study on 10 healthy controls. For IL-1β, the optimal cutoff was found to be 3.9 pg/mL with 51.75% sensitivity and 86.8% specificity. IL-6 had a cutoff value of 3.7 pg/mL, yielding a sensitivity of 63.2% and specificity of 68.4% MMP-8 had the cutoff value of 3.45 pg/mL, with a sensitivity of 71.1% and a specificity of 55.3%

Overall, IL-1β provided a more balanced diagnostic performance, while MMP-8 showed the highest sensitivity, and IL-6 had moderate performance with better sensitivity compared to IL-1β.

Recent studies have highlighted the diagnostic performance of biomarkers IL-1β, IL-6, and MMP-8 in the detection of periodontitis, focusing on their sensitivity and specificity. IL-1β has been consistently shown to be one of the most effective biomarkers. In a study, IL-1β exhibited a sensitivity of 88.24% and specificity of 62.5% for diagnosing periodontitis, indicating its strong discriminatory ability. Comparatively, IL-6 and MMP-8 also demonstrated significant diagnostic value when used in combination with IL-1β. The combination of these three biomarkers yielded a sensitivity range of 78-94% and specificity between 77-97%, which

suggests that using them together may enhance diagnostic accuracy.(Cafiero, C at al.,2021).Clinical evidence has shown that periodontal disease lead to excessive release of IL-1β, with a sensitivity ranging from 54% to 88% and a specificity ranging from 52% to 100% across five studies (Wu, Y. C. et al 2018;Ebersole, J. L et al 2013; Ebersole, J. L et al 2015;Ramseier, C. A. et al 2009;Sánchez, G. A., et al 2013)

With sensitivity ranging from 65% to 87% and specificity ranging from 48% to 87%, MMP-8 has been described by several authors as one of the most potent indicators of tissue degradation and as a promising biomarker for early periodontal diagnosis (de Lima, C. L et al 2016; Arias-Bujanda, N et al 2020; Ebersole, J. L., et al 2015).

Recent studies have reported that IL-6 showed a sensitivity of approximately 52% to 80% and specificity ranged from 48% to 87% for diagnosing periodontitis. This reflects its moderate effectiveness in identifying periodontitis cases. (Wu, Y. C. et al 2018;Ebersole, J. L et al 2013; Ebersole, J. L et al 2015;Ramseier, C. A. et al 2009)

A study discovered that IL-1β, MMP-8, demonstrated a strong potential for identifying individuals with periodontitis among the ten salivary biomarkers they investigated (Wu, Y. C., et al 2018). Furthermore, it is possible to distinguish between people with gingivitis and healthy participants using the combination of IL-1β and MMP-8 (Zhang, Y., et al 2021).

According to some systematic reviews, our studied salivary molecules, IL-1β, IL-6, and MMP-8 are recognized as key salivary biomarkers with acceptable diagnostic reliability for periodontal disease (Kc, S., et al 2020). When developing the new framework for staging and grading in the case definition system of periodontitis, the teamwork suggested that specific biomarkers and their thresholds may be incorporated in diagnostic criteria. Indeed, validated biomarkers may help improving a periodontitis diagnosis notably in the early stages, and probably also to assess disease development and clinical response to treatment in an otherwise healthy individual with high risk to develop periodontitis (Tonetti, M. S., et al 2018).

To our knowledge, this study is the first one looking for three different salivary biomarkers in patients with periodontitis in a Pakistani population. There may be some variation in the findings between research regarding the presence of cytokines

in periodontitis. This could be due to studies protocols, sample size of the studied groups, and because periodontitis are chronic diseases with dynamic states of activity and remission.

Furthermore, disparities in geography and ethnicity can be a factor in the variations in the statistics. Indeed, in addition to dysbiosis and host response, ageing, poor socioeconomic position, smoking, heredity, and regional variables may all have a role in the onset and progression of periodontal disorders (Chikte, U et al 2019). Therefore, further research is required to determine the potential of a single biomarker or a panel of biomarkers to be linked to the development of periodontitis, to be related with the diagnosis of periodontitis, or even to be used as a tool to measure the periodontal response to treatments

This study has several drawbacks. Initially, even if we succeeded in identifying statistically significant variations in the levels between the sick and well groups, the sample size was probably too small to identify the real variations in MMP-8, iL-1beta, and IL-6 levels within the population under investigation. Patients with gingivitis were excluded from these short-term results. These results complement earlier research and may be used with other studies focusing on biomarkers and thresholds in the future to help with the clinical diagnosis of periodontitis

5.2 IMPLICATIONS OF THE STUDY

5.2.1 THEORETICAL IMPLICATION

Present study has established a relationship between increased levels of biomarkers in Pakistani population. Through careful analysis of patient's data collected from BUHSC, Dental Section, it has been proved that periodontitis patients have increased levels of MMP-8, IL-1β and IL-6. Salivary biomarkers could enable early detection of periodontitis, may help to identify individuals at high risk of developing periodontitis, allowing for timely interventions and potentially preventing disease progression. These biomarkers could lead to the creation of chair-side tests for periodontitis diagnosis and monitoring, making dental care more efficient, enable point-of-care testing, allowing dental professionals to make informed decisions during patient visits.

Current studies suggest that periodontal disease can be asymptomatic or in many periodontal patients may be at risk of systemic conditions and remain asymptomatic and undiagnosed. Thus, implicating these levels of biomarkers can also be used for successful screening of periodontitis in healthy individuals.

5.2.2 PRACTICAL IMPLICATIONS

It has a strong practical implication. Dental practitioners may advice levels of these markers to be evaluated in the laboratory to diagnose periodontitis as the current study has proved that its levels are increased in asymptomatic and symptomatic patients. The management of periodontal disease may undergo a revolution if salivary periodontal indicators were included into standard dental procedures. Salivary biomarkers provide personalized therapy, early diagnosis, and a deeper comprehension of the relationship between oral and systemic health by offering a non-invasive, economical, and all-encompassing diagnostic tool. This method improves public health outcomes in addition to improving patient care.

5.2.3 POLICY IMPLICATIONS

It is possible to greatly enhance public health outcomes by incorporating salivary periodontal indicators into healthcare programs. These policies can improve dental health and general well-being, lower healthcare costs, and encourage a more comprehensive approach to health management by promoting early identification, individualized treatment, and preventative care. It is imperative that policymakers, healthcare providers, and researchers collaborate to devise and execute plans that optimize the advantages of salivary diagnostics for the advancement of society. Further studies are required to use the results of this research work in policy formulation and implementation.

5.3 LIMITATIONS & STRENGTHS OF STUDY

5.3.1 LIMITATIONS

- Single centered study so the results cannot be applied to the whole population
- Short duration of study of six months
- Small sample size.
- Limited Resources

5.3.2 STRENGTHS

- To the best of our knowledge and extensive literature review the levels of these three biomarkers are being detected first time in Pakistani population

- Comparison of cases with controls will give better idea about the periodontal alteration in high-risk patients

5.4 FUTURE RESEARCH DIRECTIONS / RECOMMENDATIONS

- Ongoing research aims to further elucidate the role of MMP-8, IL-1β and IL-6 in periodontal disease and explore its potential as a therapeutic target. Studies

are investigating the molecular mechanisms regulating MMP-8, IL-1β and IL-6 expression and activity, as well as the development of novel inhibitors to modulate its activity in periodontal tissues.

- Recommendations for **MMP-8**
- **Longitudinal Studies:** Conduct long-term studies to understand the changes in MMP-8 levels over the course of periodontal disease and treatment.
- **Mechanistic Studies:** Investigate the molecular pathways regulating MMP-8 expression and activity to identify new therapeutic targets.
- **Point-of-Care Diagnostics:** Develop and validate new, more sensitive, and specific point-of-care tests for MMP-8 to enable early diagnosis and real-time monitoring of periodontal disease.
- **Therapeutic Trials:** Conduct clinical trials to evaluate the efficacy of MMP-8 inhibitors in preventing or reducing periodontal tissue destruction.

Recommendations for **IL-1β**

- **Biomarker Validation:** Further validate IL-1β as a reliable biomarker for early detection and monitoring of periodontal disease through large-scale clinical studies.
- **Genetic Research:** Expand genetic studies to identify additional polymorphisms and their impact on IL-1β expression and periodontal disease risk.
- **Anti-inflammatory Therapies:** Explore the development of therapies targeting IL-1β signaling pathways to reduce inflammation and bone loss in periodontal disease.
- **Combination Biomarkers:** Study the combined use of IL-1β with other biomarkers (e.g., MMP-8, IL-6) to improve diagnostic accuracy and predictive value.

Recommendations for **IL-6**

- **Therapeutic Approaches:** Investigate IL-6 inhibitors and other anti-cytokine therapies as potential treatments for periodontal disease, especially in patients

with comorbid conditions.

- **Systemic Connections:** Explore the bidirectional relationship between periodontal disease and systemic diseases mediated by IL-6 to develop integrated treatment strategies.

Integrated Approach:

- **Multifactorial Analysis:** Promote research that simultaneously investigates MMP-8, IL-1β, and IL-6 to understand their combined effects on periodontal disease pathogenesis.

- **Preventive Strategies:** Focus on preventive measures by identifying at-risk individuals through genetic screening and biomarker analysis, enabling early intervention and potentially preventing disease onset.

  By advancing our understanding of these biomarkers and developing targeted diagnostic and therapeutic strategies, we can improve the management and outcomes of periodontal disease

5.5 CONCLUSION:

In conclusion, MMP-8, IL-1β, and IL-6 are consistently expressed in the saliva of periodontitis patients. However, their elevated levels in individuals with periodontitis suggest these biomarkers play a significant role in the inflammatory processes associated with periodontal disease.

Current study found significant statistical difference of means between IL-1β, IL-6 and MMP-8 with age gender and co-morbid. The use of salivary biomarkers IL-1β, IL-6, and MMP-8 represents a significant advancement in the field of periodontal diagnostics. Their ability to provide a non-invasive, accurate, and cost-effective means of assessing periodontal health holds great potential for improving patient outcomes and advancing periodontal care. However, further research and standardization are necessary to fully integrate these biomarkers into routine clinical practice.

REFERENCES

Aaron, S. L., & DeBlois, K. W. (2020). Acute necrotizing ulcerative gingivitis.

Abdulkareem, A. A., Al-Taweel, F. B., Al-Sharqi, A. J., Gul, S. S., Sha, A., & Chapple, I. L. (2023). Current concepts in the pathogenesis of periodontitis: from symbiosis to dysbiosis. Journal of Oral Microbiology, 15(1), 2197779.

Albandar, J. M. (2014). Aggressive periodontitis: case definition and diagnostic criteria. Periodontology 2000, 65(1), 13-26.

Alfant, B., Shaddox, L. M., Tobler, J., Magnusson, I., Aukhil, I., & Walker, C. (2008). Matrix metalloproteinase levels in children with aggressive periodontitis. Journal of periodontology, 79(5), 819-826.

Al-Rawi, N. H., Al-Marzooq, F., Al-Nuaimi, A. S., Hachim, M. Y., & Hamoudi, R. (2020). Salivary microRNA 155, 146a/b and 203: A pilot study for potentially non-invasive diagnostic biomarkers of periodontitis and diabetes mellitus. PLoS One, 15(8), e0237004.

Altıngöz, S. M., Kurgan, Ş., Önder, C., Serdar, M. A., Ünlütürk, U., Uyanık, M., ... & Günhan, M. (2021). Salivary and serum oxidative stress biomarkers and advanced glycation end products in periodontitis patients with or without diabetes: A cross-sectional study. Journal of periodontology, 92(9), 1274-1285.

Apolinario Vieira, G. H., Aparecida Rivas, A. C., Figueiredo Costa, K., Ferreira Oliveira, L. F., Tanaka Suzuki, K., Reis Messora, M., ... & Taba Jr, M. (2021). Specific inhibition of IL-6 receptor attenuates inflammatory bone loss in experimental periodontitis. Journal of periodontology, 92(10), 1460-1469.

Arias-Bujanda, N., Regueira-Iglesias, A., Balsa-Castro, C., Nibali, L., Donos, N., &

Tomás, I. (2020). Accuracy of single molecular biomarkers in saliva for the diagnosis of periodontitis: A systematic review and meta-analysis. *Journal of clinical periodontology*, *47*(1), 2-18.

Armitage, G. C. (2010). Comparison of the microbiological features of chronic and aggressive periodontitis. Periodontology 2000, 53(1).

Babel, N., Cherepnev, G., Babel, D., Tropmann, A., Hammer, M., Volk, H. D., & Reinke, P. (2006). Analysis of tumor necrosis factor-α, transforming growth factor-β, interleukin-10, IL-6, and interferon-γ gene polymorphisms in patients with chronic periodontitis. Journal of periodontology, 77(12), 1978-1983.

Baeza, M., Garrido, M., Hernández-Ríos, P., Dezerega, A., García-Sesnich, J., Strauss, F., ... & Hernández, M. (2016). Diagnostic accuracy for apical and chronic periodontitis biomarkers in gingival crevicular fluid: an exploratory study. Journal of clinical periodontology, 43(1), 34-45.

Bahammam, M. A., & Attia, M. S. (2018). Effects of systemic simvastatin on the concentrations of visfatin, tumor necrosis factor-α, and interleukin-6 in gingival crevicular fluid in patients with type 2 diabetes and chronic periodontitis. Journal of immunology research, 2018.

Balli, U. M. U. T., Ongoz Dede, F., Bozkurt Dogan, S., Gulsoy, Z., & Sertoglu, E. (2016). Chemerin and interleukin-6 levels in obese individuals following periodontal treatment. Oral Diseases, 22(7), 673-680.

Balogun, A. O., Taiwo, J. O., Opeodu, O. I., Adeyemi, B. F., & Kolude, B. M. (2020). Diagnostic Utility of Salivary Matrix Metalloproteinase-8 (MMP-8) in Chronic Periodontitis: A Novel Approach. Open Journal of Stomatology, 10(4), 41-49.

Balta, M. G., Papathanasiou, E., Blix, I. J., & Van Dyke, T. E. (2021). Host modulation and treatment of periodontal disease. Journal of Dental

Research, 100(8), 798-809.

Bartold, P. M. (2018). Lifestyle and periodontitis: The emergence of personalized periodontics. Periodontology 2000, 78(1), 7-11.

Bartold, P. M., & Van Dyke, T. E. (2013). Periodontitis: a host-mediated disruption of microbial homeostasis. Unlearning learned concepts. Periodontology 2000, 62(1), 203-217.

Barutta, F., Bellini, S., Durazzo, M., & Gruden, G. (2022). Novel insight into the mechanisms of the bidirectional relationship between diabetes and periodontitis. Biomedicines, 10(1), 178.

Batool, H., Nadeem, A., Kashif, M., Shahzad, F., Tahir, R., & Afzal, N. (2018). Salivary levels of IL-6 and IL-17 could be an indicator of disease severity in patients with calculus associated chronic periodontitis. *BioMed research international*, *2018*(1), 8531961.

Belstrøm, D., Sembler-Møller, M. L., Grande, M. A., Kirkby, N., Cotton, S. L., Paster, B. J., ... & Holmstrup, P. (2018). Impact of oral hygiene discontinuation on supragingival and salivary microbiomes. JDR Clinical & Translational Research, 3(1), 57-64.

Bibi, T., Arshad Hassan, D. R. A., Kumar, C., Naz, A., Tanwir, F., Qadir, F., & Mazhar, S. (2023) PREVALENCE OF PERIODONTITIS BASED ON ETHNIC DISPARITY IN KARACHI, PAKISTAN.

Billings, M., Holtfreter, B., Papapanou, P. N., Mitnik, G. L., Kocher, T., & Dye, B. A. (2018). Age-dependent distribution of periodontitis in two countries: findings from NHANES 2009 to 2014 and SHIP-TREND 2008 to 2012. *Journal of clinical periodontology*, *45*, S130-S148.

Borgnakke, W. S. (2015). Does treatment of periodontal disease influence systemic disease?. Dental Clinics, 59(4), 885-917.

Bostanci, N., Emingil, G., Saygan, B., Turkoglu, O., Atilla, G., Curtis, M. A., & Belibasakis, G. N. (2009). Expression and regulation of the NALP3 inflammasome complex in periodontal diseases. Clinical & Experimental Immunology, 157(3), 415-422.

Cafiero, C., & Matarasso, S. (2013). Predictive, preventive, personalised and participatory periodontology:'the 5Ps age'has already started. EPMA Journal, 4, 1-29.

Cafiero, C., Spagnuolo, G., Marenzi, G., Martuscelli, R., Colamaio, M., & Leuci, S. (2021). Predictive periodontitis: the most promising salivary biomarkers for early diagnosis of periodontitis. Journal of clinical medicine, 10(7), 1488.

Caton, J. G., Armitage, G., Berglundh, T., Chapple, I. L., Jepsen, S., Kornman, K. S., ... & Tonetti, M. S. (2018). A new classification scheme for periodontal and peri-implant diseases and conditions–Introduction and key changes from the 1999 classification. Journal of periodontology, 89, S1-S8.

Chapple, I. L., Mealey, B. L., Van Dyke, T. E., Bartold, P. M., Dommisch, H., Eickholz, P., ... & Yoshie, H. (2018). Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of periodontology, 89, S74-S84.

Chen, M. X., Zhong, Y. J., Dong, Q. Q., Wong, H. M., & Wen, Y. F. (2021). Global, regional, and national burden of severe periodontitis, 1990–2019: An analysis of the Global Burden of Disease Study 2019. Journal of clinical periodontology, 48(9), 1165-1188.

Chen, M., Cai, W., Zhao, S., Shi, L., Chen, Y., Li, X., ... & Huang, S. (2019). Oxidative stress-related biomarkers in saliva and gingival crevicular fluid associated with chronic periodontitis: A systematic review and meta-analysis. Journal of clinical periodontology, 46(6), 608-622.

Cheng R, Wu Z, Li M, Shao M, Hu T. Interleukin-1β is a potential therapeutic target for periodontitis: a narrative review. International journal of oral science. 2020 Dec;12(1):2.

Daalderop, L. A., Wieland, B. V., Tomsin, K., Reyes, L., Kramer, B. W., Vanterpool, S. F., & Been, J. V. (2018). Periodontal disease and pregnancy outcomes: overview of systematic reviews. JDR Clinical & Translational Research, 3(1), 10-27.

Derruau, S., Robinet, J., Untereiner, V., Piot, O., Sockalingum, G. D., & Lorimier, S. (2020). Vibrational spectroscopy saliva profiling as biometric tool for disease diagnostics: A systematic literature review. Molecules, 25(18), 4142.

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W. H., ... & Wade, W. G. (2010). The human oral microbiome. Journal of bacteriology, 192(19), 5002-5017.

Dietrich, T., Ower, P., Tank, M., West, N. X., Walter, C., Needleman, I., ... & Chapple, I. L. C. (2019). Periodontal diagnosis in the context of the 2017 classification system of periodontal diseases and conditions–implementation in clinical practice. British dental journal, 226(1), 16-22.

Du, M., Wang, Y., Liu, Z., Wang, L., Cao, Z., Zhang, C., ... & He, H. (2019). Effects of IL-1β on MMP-9 expression in cementoblast-derived cell line and MMP-mediated degradation of type I collagen. Inflammation, 42, 413-425.

Earle, K. A., Billings, G., Sigal, M., Lichtman, J. S., Hansson, G. C., Elias, J. E., ... & Sonnenburg, J. L. (2015). Quantitative imaging of gut microbiota spatial organization. Cell host & microbe, 18(4), 478-488.

Ebersole, J. L., Nagarajan, R., Akers, D., & Miller, C. S. (2015). Targeted salivary biomarkers for discrimination of periodontal health and disease (s). Frontiers in cellular and infection microbiology, 5, 62.

Ebersole, J. L., Schuster, J. L., Stevens, J., Dawson, D., Kryscio, R. J., Lin, Y., ... &

Miller, C. S. (2013). Patterns of salivary analytes provide diagnostic capacity for distinguishing chronic adult periodontitis from health. Journal of clinical immunology, 33, 271-279.

Elías-Boneta, A. R., Toro, M. J., Rivas-Tumanyan, S., Rajendra-Santosh, A. B., & Brache, M. (2018). Prevalence, severity, and risk factors of gingival inflammation in Caribbean adults: a multi-city, cross-sectional study. Puerto Rico health sciences journal, 37(2), 115-123.

Esteves, E. J. M. (2023). Saliva as a non-invasive diagnostic tool: COVID-19 and T2DM as case-study.

Fahim, A., Shakeel, S., Shahid, T. N., Anwar, H. M., Raja, A. A., & Khan, A. (2022). Prevalence of periodontitis in Pakistan: A systematic review. Journal of University College of Medicine and Dentistry, 1(1), 30-34.

Fatemi, K., Rezaee, S. A., Banihashemrad, S. A., Keyvanfar, S., & Eslami, M. (2020). Importance of MMP-8 in salivary and gingival crevicular fluids of periodontitis patients. *Iranian journal of immunology*, *17*(3), 236-243.

Franco, C., Patricia, H. R., Timo, S., Claudia, B., & Marcela, H. (2017). Matrix metalloproteinases as regulators of periodontal inflammation. International journal of molecular sciences, 18(2), 440.

Frodge, B. D., Ebersole, J. L., Kryscio, R. J., Thomas, M. V., & Miller, C. S. (2008). Bone remodeling biomarkers of periodontal disease in saliva. Journal of periodontology, 79(10), 1913-1919.

Gaphor, S. M., Ali, S. H., & Abdullah, M. J. (2014). Evaluation of salivary interleukin-1beta (IL-1β) level in relation to the periodontal status in smoker and non-smoker individuals. J Interdiscipl Med Dent Sci, 2(2376-032X).

Genco, R. J., & Borgnakke, W. S. (2013). Risk factors for periodontal disease. Periodontology 2000, 62(1), 59-94.

Ghallab, N. A. (2018). Diagnostic potential and future directions of biomarkers in gingival crevicular fluid and saliva of periodontal diseases: Review of the current evidence. Archives of oral biology, 87, 115-124.

Go, H., Park, T., Shin, A. R., Jung, Y. S., Amano, A., Song, K. B., & Choi, Y. H. (2022). Validity of a combination of periodontal pathogens and salivary biomarkers as predictors of periodontitis. Journal of Periodontal Research, 57(5), 1083-1092.

Gonçalves, P. F., Huang, H., McAninley, S., Alfant, B., Harrison, P., Aukhil, I., ... & Shaddox, L. M. (2013). Periodontal treatment reduces matrix metalloproteinase levels in localized aggressive periodontitis. Journal of periodontology, 84(12), 1801-1808.

Graetz, C., Mann, L., Krois, J., Sälzer, S., Kahl, M., Springer, C., & Schwendicke, F. (2019). Comparison of periodontitis patients' classification in the 2018 versus 1999 classification. Journal of clinical periodontology, 46(9), 908-917.

Gursoy, U. K., Könönen, E., Huumonen, S., Tervahartiala, T., Pussinen, P. J., Suominen, A. L., & Sorsa, T. (2013). Salivary type I collagen degradation end-products and related matrix metalloproteinases in periodontitis. Journal of clinical periodontology, 40(1), 18-25.

Haririan, H., Andrukhov, O., Böttcher, M., Pablik, E., Wimmer, G., Moritz, A., & Rausch-Fan, X. (2018). Salivary neuropeptides, stress, and periodontitis. Journal of periodontology, 89(1), 9-18.

He, W., You, M., Wan, W., Xu, F., Li, F., & Li, A. (2018). Point-of-care periodontitis testing: biomarkers, current technologies, and perspectives. Trends in biotechnology, 36(11), 1127-1144.

Heikkinen, A. M., Raivisto, T., Kettunen, K., Kovanen, L., Haukka, J., Pakbaznejad Esmaeili, E., ... & Sorsa, T. (2017). Pilot study on the genetic background of

an active matrix metalloproteinase-8 test in finnish adolescents. Journal of Periodontology, 88(5), 464-472.

Hernández, M., Baeza, M., Räisänen, I. T., Contreras, J., Tervahartiala, T., Chaparro, A., ... & Hernández-Ríos, P. (2021). Active MMP-8 quantitative test as an adjunctive tool for early diagnosis of periodontitis. Diagnostics, 11(8), 1503.

Hong, I., Pae, H. C., Song, Y. W., Cha, J. K., Lee, J. S., Paik, J. W., & Choi, S. H. (2020). Oral fluid biomarkers for diagnosing gingivitis in human: A cross-sectional study. Journal of clinical medicine, 9(6), 1720.

Isaza-Guzmán, D. M., Medina-Piedrahíta, V. M., Gutiérrez-Henao, C., & Tobón-Arroyave, S. I. (2017). Salivary levels of NLRP3 inflammasome-related proteins as potential biomarkers of periodontal clinical status. Journal of periodontology, 88(12), 1329-1338.

Isola, G., Giudice, A. L., Polizzi, A., Alibrandi, A., Murabito, P., & Indelicato, F. (2021). Identification of the different salivary Interleukin-6 profiles in patients with periodontitis: a cross-sectional study. Archives of Oral Biology, 122, 104997.

Iwashita, M. (2023). Association between periodontal disease and arteriosclerosis-related diseases. Journal of Atherosclerosis and Thrombosis, 30(11), 1517-1524.

Izadi Borujeni, S., Mayer, M., & Eickholz, P. (2015). Activated matrix metalloproteinase-8 in saliva as diagnostic test for periodontal disease? A case–control study. Medical microbiology and immunology, 204, 665-672.

Jacob, P. S., Nath, S., & Patel, R. P. (2014). Evaluation of interleukin-1β and 8 in gutka chewers with periodontitis among a rural Indian population. Journal of periodontal & implant science, 44(3), 126.

Jaedicke, K. M., Preshaw, P. M., & Taylor, J. J. (2016). Salivary cytokines as

biomarkers of periodontal diseases. Periodontology 2000, 70(1), 164-183.

Jakubovics, N. S., Goodman, S. D., Mashburn-Warren, L., Stafford, G. P., & Cieplik, F. (2021). The dental plaque biofilm matrix. Periodontology 2000, 86(1), 32-56.

Johannsen, B., Müller, L., Baumgartner, D., Karkossa, L., Früh, S. M., Bostanci, N., ... & Mitsakakis, K. (2019). Automated pre-analytic processing of whole saliva using magnet-beating for point-of-care protein biomarker analysis. Micromachines, 10(12), 833.

Johnson, N., Ebersole, J. L., Kryscio, R. J., Danaher, R. J., Dawson III, D., Al-Sabbagh, M., & Miller, C. S. (2016). Rapid assessment of salivary MMP-8 and periodontal disease using lateral flow immunoassay. Oral diseases, 22(7), 681-687.

Kang, S., Narazaki, M., Metwally, H., & Kishimoto, T. (2020). Historical overview of the interleukin-6 family cytokine. Journal of Experimental Medicine, 217(5).

Kassebaum, N. J., Bernabé, E., Dahiya, M., Bhandari, B., Murray, C. J. L., & Marcenes, W. (2014). Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. Journal of dental research, 93(11), 1045-1053.

Kato, T., Fujiwara, N., Kuraji, R., & Numabe, Y. (2020). Relationship between periodontal parameters and non-vital pulp in dental clinic patients: a cross-sectional study. BMC Oral Health, 20, 1-6.

Kaur, S., Bansal, Y., Kumar, R., & Bansal, G. (2020). A panoramic review of IL-6: Structure, pathophysiological roles and inhibitors. Bioorganic & medicinal chemistry, 28(5), 115327.

Kc, S., Wang, X. Z., & Gallagher, J. E. (2020). Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease

amongst adults: systematic review. Journal of clinical periodontology, 47(3), 289-308.

Kc, S., Wang, X. Z., & Gallagher, J. E. (2020). Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: systematic review. *Journal of clinical periodontology*, *47*(3), 289-308.

Keles Yucel, Z. P., Afacan, B., Emingil, G., Tervahartiala, T., Kose, T., & Sorsa, T. (2020). Local and systemic levels of aMMP-8 in gingivitis and stage 3 grade C periodontitis. Journal of Periodontal Research, 55(6), 887-894

Khurshid, Z. (2018). Salivary point-of-care technology. European Journal of Dentistry, 12(01), 001-002.

Kiarashi, M., Mahamed, P., Ghotbi, N., Tadayonfard, A., Nasiri, K., Kazemi, P., ... & Joudaki, A. (2024). Spotlight on therapeutic efficiency of green synthesis metals and their oxide nanoparticles in periodontitis. Journal of Nanobiotechnology, 22(1), 21.

Kim, J., & Amar, S. (2006). Periodontal disease and systemic conditions: a bidirectional relationship. Odontology, 94, 10-21.

Kinane, D. F. (2000). Regulators of tissue destruction and homeostasis as diagnostic aids in periodontology. Periodontology 2000, 24(1), 215-225.

Kinane, D. F., Stathopoulou, P. G., & Papapanou, P. N. (2017). Periodontal diseases. Nature reviews Disease primers, 3(1), 1-14.

Kinney, J. S., Morelli, T., Oh, M., Braun, T. M., Ramseier, C. A., Sugai, J. V., & Giannobile, W. V. (2014). Crevicular fluid biomarkers and periodontal disease progression. Journal of clinical periodontology, 41(2), 113-120.

Konopka, Ł., Pietrzak, A., & Brzezińska-Błaszczyk, E. (2012). Effect of scaling and root planing on interleukin-1β, interleukin-8 and MMP-8 levels in gingival

crevicular fluid from chronic periodontitis patients. Journal of periodontal research, 47(6), 681-688.

Kusuhara, M., Isoda, K., & Ohsuzu, F. (2006). Interleukin-1 and occlusive arterial diseases. Cardiovascular & Hematological Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Cardiovascular & Hematological Agents), 4(3), 229-235.

Kwon, T., Lamster, I. B., & Levin, L. (2021). Current concepts in the management of periodontitis. International dental journal, 71(6), 462-476.

Larsen, T., & Fiehn, N. E. (2017). Dental biofilm infections–an update. Apmis, 125(4), 376-384.

Leppilahti, J. M., Hernández-Ríos, P. A., Gamonal, J. A., Tervahartiala, T., Brignardello-Petersen, R., Mantyla, P., ... & Hernández, M. (2014). Matrix metalloproteinases and myeloperoxidase in gingival crevicular fluid provide site-specific diagnostic value for chronic periodontitis. Journal of clinical periodontology, 41(4), 348-356.

Leppilahti, J. M., Kallio, M. A., Tervahartiala, T., Sorsa, T., & Mäntylä, P. (2014). Gingival crevicular fluid matrix metalloproteinase-8 levels predict treatment outcome among smokers with chronic periodontitis. Journal of Periodontology, 85(2), 250-260.

Leppilahti, J. M., Sorsa, T., Kallio, M. A., Tervahartiala, T., Emingil, G., Han, B., & Mäntylä, P. (2015). The utility of gingival crevicular fluid matrix metalloproteinase-8 response patterns in prediction of site-level clinical treatment outcome. Journal of Periodontology, 86(6), 777-787.

Lertpimonchai, A., Rattanasiri, S., Vallibhakara, S. A. O., Attia, J., & Thakkinstian, A. (2017). The association between oral hygiene and periodontitis: a systematic review and meta-analysis. International dental journal, 67(6), 332-343.

Liu, Y., Duan, D., Ma, R., Ding, Y., Xu, Y., Zhou, X., ... & Xu, X. (2020). The combined use of salivary biomarkers and clinical parameters to predict the outcome of scaling and root planing: A cohort study. Journal of clinical periodontology, 47(11), 1379-1390.

MAHMOOD, D. H., NAEEM, D. S., SAEED, D. U., & ZARA, D. B. (2021). A case-control study on risk predictors associated with periodontitis in systemically healthy patients; a Regression Analysis.

Mauramo, M., Ramseier, A. M., Mauramo, E., Buser, A., Tervahartiala, T., Sorsa, T., & Waltimo, T. (2018). Associations of oral fluid MMP-8 with periodontitis in Swiss adult subjects. Oral diseases, 24(3), 449-455.

Meisner, A., Parikh, C. R., & Kerr, K. F. (2019). Biomarker combinations for diagnosis and prognosis in multicenter studies: Principles and methods. Statistical methods in medical research, 28(4), 969-985.

Miranda, T. S., de Freitas Figueiredo, N., Figueiredo, L. C., da Silva, H. D. P., Rocha, F. R. G., & Duarte, P. M. (2020). Cytokine profiles of healthy and diseased sites in individuals with periodontitis. Archives of Oral Biology, 120, 104957.

Mohammed, H. A., Abdulkareem, A. A., Zardawi, F. M., & Gul, S. S. (2022). Determination of the accuracy of salivary biomarkers for periodontal diagnosis. Diagnostics, 12(10), 2485.

Monje, A., Amerio, E., Farina, R., Nart, J., Ramanauskaite, A., Renvert, S., ... & Wang, H. L. (2021). Significance of probing for monitoring peri-implant diseases. Int. J. Oral Implantol, 14, 385-399.

Nazir, M. A. (2017). Prevalence of periodontal disease, its association with systemic diseases and prevention. International journal of health sciences, 11(2), 72.

Nazir, M. A. (2017). Prevalence of periodontal disease, its association with systemic diseases and prevention. International journal of health sciences, 11(2), 72.

Nikiforov, N. G., Kirichenko, T. V., Kubekina, M. V., Chegodaev, Y. S., Zhuravlev, A. D., Ilchuk, L. A., ... & Orekhov, A. N. (2023). Macrophages derived from LPS-stimulated monocytes from individuals with subclinical atherosclerosis were characterized by increased pro-inflammatory activity. Cytokine, 172, 156411.

Noack, B., Kipping, T., Tervahartiala, T., Sorsa, T., Hoffmann, T., & Lorenz, K. (2017). Association between serum and oral matrix metalloproteinase-8 levels and periodontal health status. *Journal of periodontal research*, *52*(5), 824-831.

Nualart Grollmus, Z. C., Morales Chávez, M. C., & Silvestre Donat, F. J. (2007). Periodontal disease associated to systemic genetic disorders. Medicina Oral, Patología Oral y Cirugía Bucal (Internet), 12(3), 211-215.

Nwhator, S. O., Ayanbadejo, P. O., Umeizudike, K. A., Opeodu, O. I., Agbelusi, G. A., Olamijulo, J. A., ... & Opedun, D. O. (2014). Clinical correlates of a lateral-flow immunoassay oral risk indicator. Journal of Periodontology, 85(1), 188-194.

Odanaka, H., Obama, T., Sawada, N., Sugano, M., Itabe, H., & Yamamoto, M. (2020). Comparison of protein profiles of the pellicle, gingival crevicular fluid, and saliva: possible origin of pellicle proteins. Biological research, 53.

Ombao, H., Lindquist, M., Thompson, W., & Aston, J. (2016). Handbook of neuroimaging data analysis. Chapman and Hall/CRC.

Özçaka, Ö., Bıçakcı, N., Pussinen, P., Sorsa, T., Köse, T., & Buduneli, N. (2011). Smoking and matrix metalloproteinases, neutrophil elastase and myeloperoxidase in chronic periodontitis. Oral diseases, 17(1), 68-76.

Özçaka, Ö., Bıçakcı, N., Pussinen, P., Sorsa, T., Köse, T., & Buduneli, N. (2011). Smoking and matrix metalloproteinases, neutrophil elastase and myeloperoxidase in chronic periodontitis. Oral diseases, 17(1), 68-76.

Park, S., Kim, I., Han, S. J., Kwon, S., Min, E. J., Cho, W., ... & Park, Y. M. (2023). Oral Porphyromonas gingivalis infection affects intestinal microbiota and promotes atherosclerosis. Journal of Clinical Periodontology, 50(11), 1553-1567.

Persson, G. R. (2018). Periodontal complications with age. Periodontology 2000, 78(1), 185-194.

Pihlstrom, B. L., Michalowicz, B. S., & Johnson, N. W. (2005). Periodontal diseases. The lancet, 366(9499), 1809-1820.

Plachokova, A. S., Andreu-Sánchez, S., Noz, M. P., Fu, J., & Riksen, N. P. (2021). Oral microbiome in relation to periodontitis severity and systemic inflammation. International journal of molecular sciences, 22(11), 5876.

Preshaw, P. M., & Bissett, S. M. (2013). Periodontitis: oral complication of diabetes. Endocrinology and Metabolism Clinics, 42(4), 849-867.

Prieto, D., Maurer, G., Sáez, M., Cáceres, F., Pino-Lagos, K., & Chaparro, A. (2021). Soluble Neuropilin-1 in gingival crevicular fluid from periodontitis patients: An exploratory cross-sectional study. Journal of Oral Biology and Craniofacial Research, 11(1), 84-87.

Ptasiewicz, M., Grywalska, E., Mertowska, P., Korona-Głowniak, I., Poniewierska-Baran, A., Niedźwiedzka-Rystwej, P., & Chałas, R. (2022). Armed to the teeth—the oral mucosa immunity system and microbiota. International Journal of Molecular Sciences, 23(2), 882.

Räisänen, I. (2023). Risk factors and active matrix metalloproteinase-8 (aMMP-8) diagnostics for initial periodontitis in adolescents.

Ramseier, C. A., Kinney, J. S., Herr, A. E., Braun, T., Sugai, J. V., Shelburne, C. A., ... & Giannobile, W. V. (2009). Identification of pathogen and host-response markers correlated with periodontal disease. Journal of periodontology, 80(3), 436-446.

Rangbulla, V., Nirola, A., Gupta, M., Batra, P., & Gupta, M. (2017). Salivary IgA, interleukin-1β and MMP-8 as salivary biomarkers in chronic periodontitis patients. Chin J Dent Res, 20(1), 43-51.

Rathee, M., & Jain, P. (2022). Gingivitis. In StatPearls [Internet]. StatPearls Publishing.

Rathnayake, N., Gustafsson, A., Norhammar, A., Kjellström, B., Klinge, B., Ryden, L., ... & PAROKRANK Steering Group. (2015). Salivary matrix metalloproteinase-8 and-9 and myeloperoxidase in relation to coronary heart and periodontal diseases: a subgroup report from the PAROKRANK study (periodontitis and its relation to coronary artery disease). PloS one, 10(7), e0126370.

Ray, P.; Le Manach, Y.; Riou, B.; Houle, T.T. Statistical evaluation of a biomarker. Anesthesiology 2010, 112, 1023–1040.

Rheu, G. B., Ji, S., Ryu, J. J., Lee, J. B., Shin, C., Lee, J. Y., ... & Shin, S. W. (2011). Risk assessment for clinical attachment loss of periodontal tissue in Korean adults. The journal of advanced prosthodontics, 3(1), 25.

Richards, D. (2013). Oral diseases affect some 3.9 billion people. Evidence-based dentistry, 14(2), 35-35

Ridgeway, E. E. (2000). Periodontal disease: diagnosis and management. Journal of the American Academy of Nurse Practitioners, 12(3), 79-84.

Ross, J. H., Hardy, D. C., Schuyler, C. A., Slate, E. H., Mize, T. W., & Huang, Y. (2010). Expression of periodontal interleukin-6 protein is increased across patients with neither periodontal disease nor diabetes, patients with periodontal disease alone and patients with both diseases. Journal of periodontal research, 45(5), 688-694.

Salminen, A., Gursoy, U. K., Paju, S., Hyvärinen, K., Mäntylä, P., Buhlin, K., ... & Pussinen, P. J. (2014). Salivary biomarkers of bacterial burden,

inflammatory response, and tissue destruction in periodontitis. Journal of clinical periodontology, 41(5), 442-450.

Sánchez, G. A., Miozza, V. A., Delgado, A., & Busch, L. (2013). Salivary IL-1β and PGE 2 as biomarkers of periodontal status, before and after periodontal treatment. Journal of clinical periodontology, 40(12), 1112-1117.

Sanz, M., Papapanou, P. N., Tonetti, M. S., Greenwell, H., & Kornman, K. (2020). Guest editorial: Clarifications on the use of the new classification of periodontitis. Journal of Clinical Periodontology, 47(6), 658-659.

Schett, G., Dayer, J. M., & Manger, B. (2016). Interleukin-1 function and role in rheumatic disease. Nature Reviews Rheumatology, 12(1), 14-24.

Sever, E., Božac, E., Saltović, E., Simonić-Kocijan, S., Brumini, M., & Glažar, I. (2023). Impact of the tobacco heating system and cigarette smoking on the oral cavity: a pilot study. Dentistry journal, 11(11), 251.

Sexton, W. M., Lin, Y., Kryscio, R. J., Dawson III, D. R., Ebersole, J. L., & Miller, C. S. (2011). Salivary biomarkers of periodontal disease in response to treatment. Journal of clinical periodontology, 38(5), 434-441.

Sitompul, S. I., Pikir, B. S., Aryati, Kencono Wungu, C. D., Supandi, S. K., & Sinta, M. E. (2023). Analysis of the Effects of IL-6-572 C/G, CRP-757 A/G, and CRP-717 T/C Gene Polymorphisms; IL-6 Levels; and CRP Levels on Chronic Periodontitis in Coronary Artery Disease in Indonesia. Genes, 14(5), 1073.

Slots, J. (2017). Periodontitis: facts, fallacies and the future. Periodontology 2000, 75(1), 7-23.

Song, Y., Huang, Y. Y., Liu, X., Zhang, X., Ferrari, M., & Qin, L. (2014). Point-of-care technologies for molecular diagnostics using a drop of blood. Trends in biotechnology, 32(3), 132-139.

Sorsa, T., Alassiri, S., Grigoriadis, A., Räisänen, I. T., Pärnänen, P., Nwhator, S. O., ... & Sakellari, D. (2020). Active MMP-8 (aMMP-8) as a grading and staging biomarker in the periodontitis classification. Diagnostics, 10(2), 61.

Sorsa, T., Hernández, M., Leppilahti, J., Munjal, S., Netuschil, L., & Mäntylä, P. (2010). Detection of gingival crevicular fluid MMP-8 levels with different laboratory and chair-side methods. Oral diseases, 16(1), 39-45.

Sorsa, T., Tjäderhane, L., Konttinen, Y. T., Lauhio, A., Salo, T., Lee, H. M., ... & Mäntylä, P. (2006). Matrix metalloproteinases: contribution to pathogenesis, diagnosis and treatment of periodontal inflammation. Annals of medicine, 38(5), 306-321.

Stöhr, J., Barbaresko, J., Neuenschwander, M., & Schlesinger, S. (2021). Bidirectional association between periodontal disease and diabetes mellitus: a systematic review and meta-analysis of cohort studies. *Scientific Reports*, *11*(1), 13686.

Struppek, J., Schnabel, R. B., Walther, C., Heydecke, G., Seedorf, U., Lamprecht, R., ... & Aarabi, G. (2021). Periodontitis, dental plaque, and atrial fibrillation in the Hamburg City Health Study. PLoS One, 16(11), e0259652.

Susin, C., Haas, A. N., & Albandar, J. M. (2014). Epidemiology and demographics of aggressive periodontitis. Periodontology 2000, 65(1), 27-45.

Tobón-Arroyave, S. I., Jaramillo-González, P. E., & Isaza-Guzman, D. M. (2008). Correlation between salivary IL-1β levels and periodontal clinical status. Archives of oral biology, 53(4), 346-352.

Tonetti, M. S., & Sanz, M. (2019). Implementation of the new classification of periodontal diseases: Decision-making algorithms for clinical practice and education. *Journal of clinical periodontology*, *46*(4), 398-405.

Toraman, A., Arabaci, T., Aytekin, Z., Albayrak, M., & Bayir, Y. (2020). Effects of vitamin C local application on ligature-induced periodontitis in diabetic rats.

Journal of applied oral science, 28, e20200444.

Uwitonze, A. M., Uwambaye, P., Isyagi, M., Mumena, C. H., Hudder, A., Haq, A., ... & Razzaque, M. S. (2018). Periodontal diseases and adverse pregnancy outcomes: Is there a role for vitamin D?. The Journal of steroid biochemistry and molecular biology, 180, 65-72.

Van Dyke, T. E., Bartold, P. M., & Reynolds, E. C. (2020). The nexus between periodontal inflammation and dysbiosis. Frontiers in immunology, 11, 530286.

Wu, M., Chen, S. W., & Jiang, S. Y. (2015). Relationship between gingival inflammation and pregnancy. Mediators of inflammation, 2015.

Wu, Y. C., Ning, L., Tu, Y. K., Huang, C. P., Huang, N. T., Chen, Y. F., & Chang, P. C. (2018). Salivary biomarker combination prediction model for the diagnosis of periodontitis in a Taiwanese population. *Journal of the Formosan Medical Association*, *117*(9), 841-848.

Yasuda, H. (2021). Discovery of the RANKL/RANK/OPG system. Journal of bone and mineral metabolism, 39(1), 2-11.

Zhang, Y., Kang, N., Xue, F., Qiao, J., Duan, J., Chen, F., & Cai, Y. (2021). Evaluation of salivary biomarkers for the diagnosis of periodontitis. BMC Oral Health, 21(1), 266.

Zhang, Y., Sun, J., Lin, C. C., Abemayor, E., Wang, M. B., & Wong, D. T. (2016). The emerging landscape of salivary diagnostics. Periodontology 2000, 70(1), 38-52.

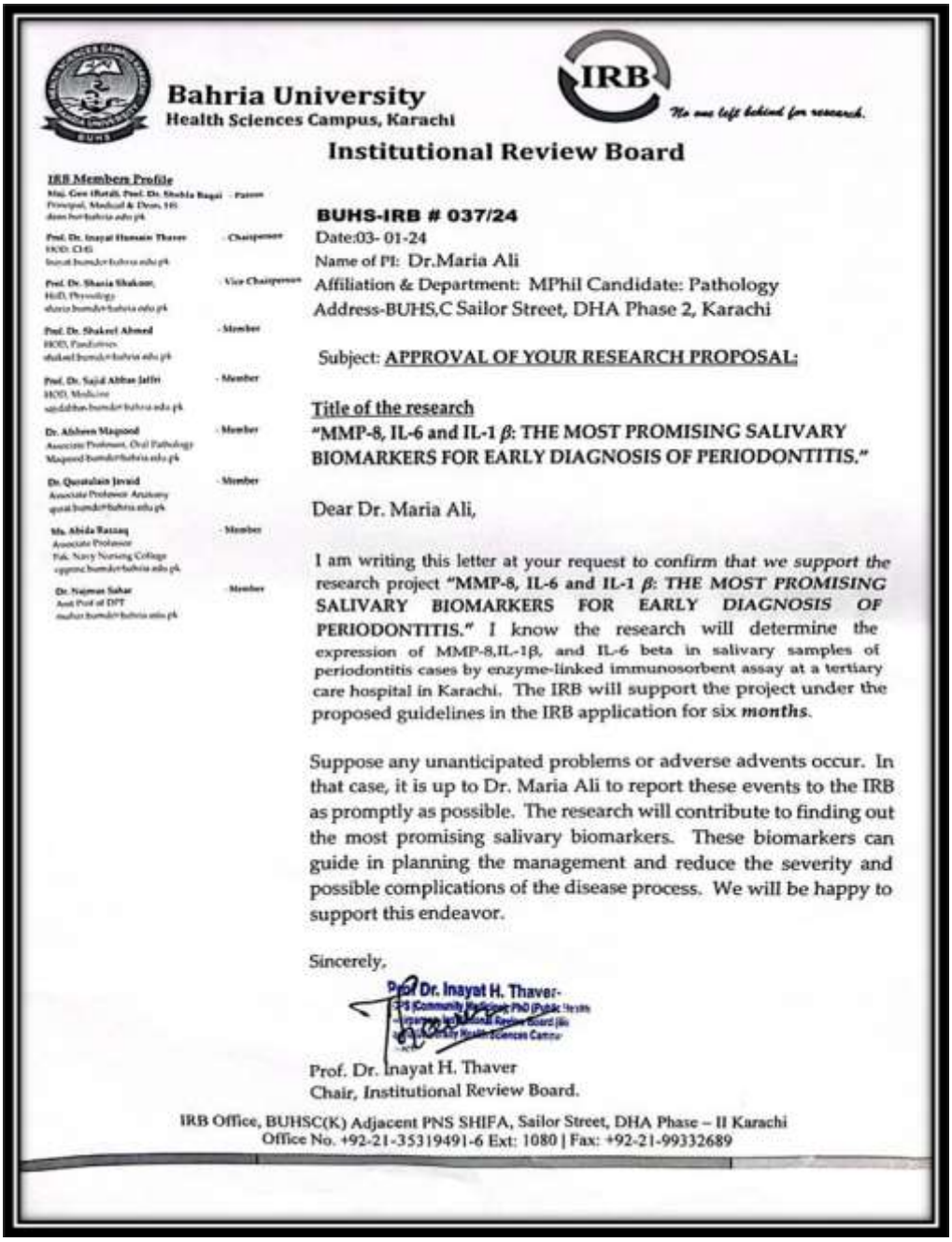
Zhao, M., Xie, Y., Gao, W., Li, C., Ye, Q., & Li, Y. (2023). Diabetes mellitus promotes susceptibility to periodontitis—novel insight into the molecular mechanisms. Frontiers in Endocrinology, 14, 1192625.

Zhou, S. Y., Duan, X. Q., Hu, R., & Ouyang, X. Y. (2013). Effect of non-surgical

periodontal therapy on serum levels of TNF-α, IL-6 and C-reactive protein in periodontitis subjects with stable coronary heart disease. Chin J Dent Res, 16(2), 145-51.

Zhu, H., Lin, X., Zheng, P., & Chen, H. (2015). Inflammatory cytokine levels in patients with periodontitis and/or coronary heart disease. International Journal of Clinical and Experimental Pathology, 8(2), 2214.

(A) BUHSC IRB Approval Letter



Bahria University
Health Sciences Campus, Karachi

IRB — No one left behind for research.

Institutional Review Board

IRB Members Profile

Maj. Gen (Retd). Prof. Dr. Shehla Baqai - Patron
Principal, Medical & Dean, HS
dean.hsc@bahria.edu.pk

Prof. Dr. Inayat Hussain Thaver - Chairperson
HOD, CHS
Inayat.hsmdc@bahria.edu.pk

Prof. Dr. Shazia Shakoor, - Vice Chairperson
HoD, Physiology
shazia.hsmdc@bahria.edu.pk

Prof. Dr. Shakeel Ahmed - Member
HOD, Paediatrics
shakeel.hsmdc@bahria.edu.pk

Prof. Dr. Sajid Abbas Jaffri - Member
HOD, Medicine
sajidabbas.hsmdc@bahria.edu.pk

Dr. Afsheen Maqsood - Member
Associate Professor, Oral Pathology
Maqsood.hsmdc@bahria.edu.pk

Dr. Quratulain Javaid - Member
Associate Professor Anatomy
qurat.hsmdc@bahria.edu.pk

Ms. Abida Razzaq - Member
Associate Professor
Pak Navy Nursing College
cpgsnc.hsmdc@bahria.edu.pk

Dr. Najmus Sahar - Member
Asst Prof of DPT
nsahar.hsmdc@bahria.edu.pk

BUHS-IRB # 037/24
Date:03- 01-24
Name of PI: Dr.Maria Ali
Affiliation & Department: MPhil Candidate: Pathology
Address-BUHS,C Sailor Street, DHA Phase 2, Karachi

Subject: **APPROVAL OF YOUR RESEARCH PROPOSAL:**

Title of the research
"MMP-8, IL-6 and IL-1 β: THE MOST PROMISING SALIVARY BIOMARKERS FOR EARLY DIAGNOSIS OF PERIODONTITIS."

Dear Dr. Maria Ali,

I am writing this letter at your request to confirm that we support the research project **"MMP-8, IL-6 and IL-1 β: THE MOST PROMISING SALIVARY BIOMARKERS FOR EARLY DIAGNOSIS OF PERIODONTITIS."** I know the research will determine the expression of MMP-8,IL-1β, and IL-6 beta in salivary samples of periodontitis cases by enzyme-linked immunosorbent assay at a tertiary care hospital in Karachi. The IRB will support the project under the proposed guidelines in the IRB application for six ***months***.

Suppose any unanticipated problems or adverse advents occur. In that case, it is up to Dr. Maria Ali to report these events to the IRB as promptly as possible. The research will contribute to finding out the most promising salivary biomarkers. These biomarkers can guide in planning the management and reduce the severity and possible complications of the disease process. We will be happy to support this endeavor.

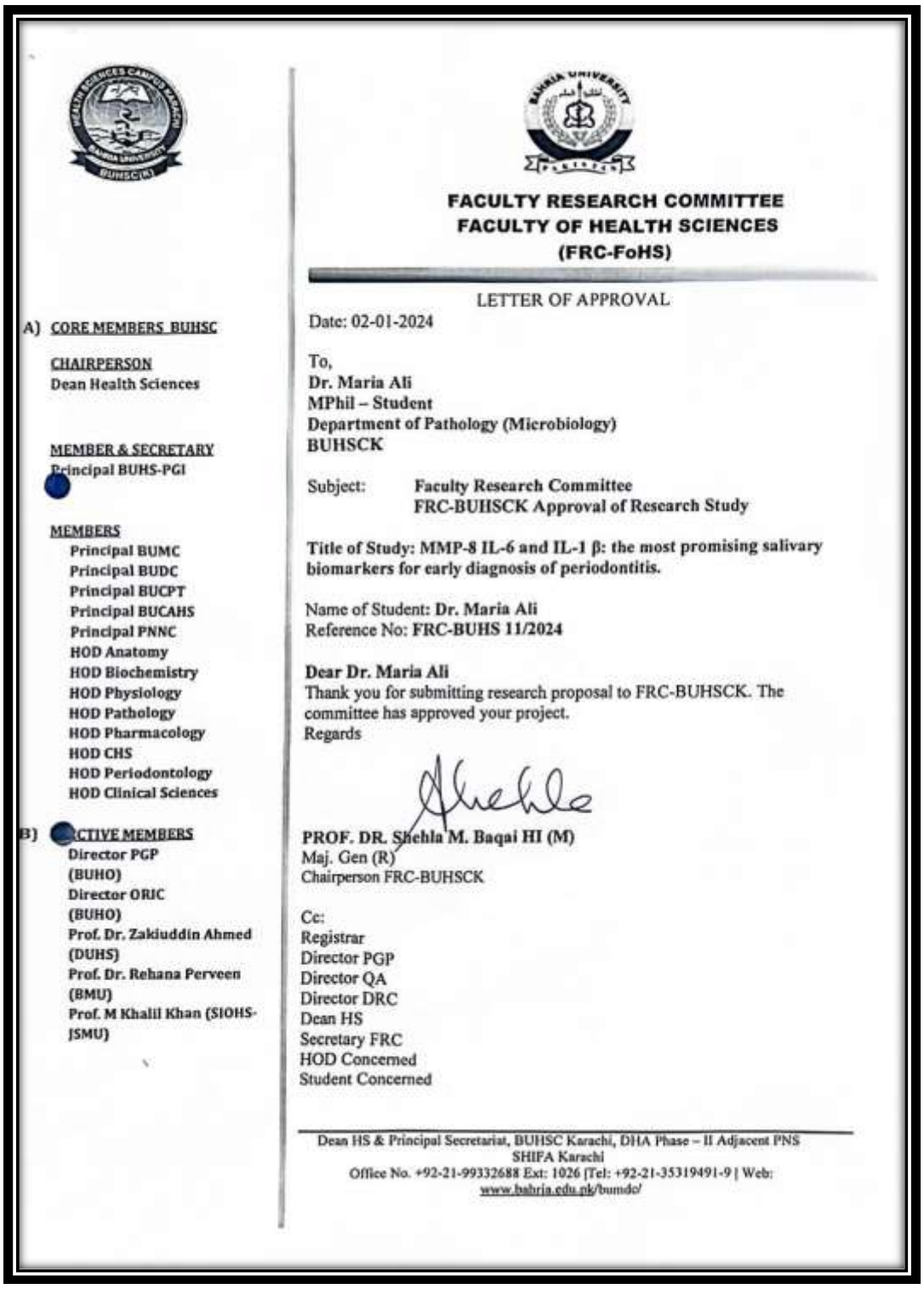
Sincerely,

Prof. Dr. Inayat H. Thaver-
[illegible]

Prof. Dr. Inayat H. Thaver
Chair, Institutional Review Board.

IRB Office, BUHSC(K) Adjacent PNS SHIFA, Sailor Street, DHA Phase – II Karachi
Office No. +92-21-35319491-6 Ext: 1080 | Fax: +92-21-99332689

(B)BUHSC FRC Approval Letter



FACULTY RESEARCH COMMITTEE
FACULTY OF HEALTH SCIENCES
(FRC-FoHS)

LETTER OF APPROVAL

Date: 02-01-2024

To,
Dr. Maria Ali
MPhil – Student
Department of Pathology (Microbiology)
BUHSCK

Subject: **Faculty Research Committee**
FRC-BUHSCK Approval of Research Study

Title of Study: MMP-8 IL-6 and IL-1 β: the most promising salivary biomarkers for early diagnosis of periodontitis.

Name of Student: **Dr. Maria Ali**
Reference No: **FRC-BUHS 11/2024**

Dear Dr. Maria Ali
Thank you for submitting research proposal to FRC-BUHSCK. The committee has approved your project.
Regards

PROF. DR. Shehla M. Baqai HI (M)
Maj. Gen (R)
Chairperson FRC-BUHSCK

Cc:
Registrar
Director PGP
Director QA
Director DRC
Dean HS
Secretary FRC
HOD Concerned
Student Concerned

A) CORE MEMBERS BUHSC

CHAIRPERSON
Dean Health Sciences

MEMBER & SECRETARY
Principal BUHS-PGI

MEMBERS
Principal BUMC
Principal BUDC
Principal BUCPT
Principal BUCAHS
Principal PNNC
HOD Anatomy
HOD Biochemistry
HOD Physiology
HOD Pathology
HOD Pharmacology
HOD CHS
HOD Periodontology
HOD Clinical Sciences

B) CTIVE MEMBERS
Director PGP (BUHO)
Director ORIC (BUHO)
Prof. Dr. Zakiuddin Ahmed (DUHS)
Prof. Dr. Rehana Perveen (BMU)
Prof. M Khalil Khan (SIOHS-JSMU)

Dean HS & Principal Secretariat, BUHSC Karachi, DHA Phase – II Adjacent PNS SHIFA Karachi
Office No. +92-21-99332688 Ext: 1026 |Tel: +92-21-35319491-9 | Web: www.bahria.edu.pk/bumdc/

(C)Consent Form (English Version)

WRITTEN INFORMED CONSENT FORM OF PATIENT

INFORMED CONSENT

You are giving your consent to participate voluntarily and at your own will in this research project that aims to detect early diagnostic salivary bio marker for periodontitis patients.

You have been explained in detail the nature and significance of participating in the project and you understand the provided explanation.

You have been told that findings of your disease and your data will be kept strictly confidential and will be used only for the benefit of community, publications and paper presentations.

You have been explained that laboratory investigations will be conducted for the diagnosis of disease. For this purpose, you fully agree to give your salivary samples at the beginning of study.

You also agree to give all relevant information needed, in full and to the best of your knowledge to the researcher. It is clarified to you that no incentive will be provided to you for participating in the study except the cost of lab investigations and drugs, whereas you do have the right to withdraw from the study at any time.

You are advised to contact Dr. Maria Ali on mobile number: 0333-7245926 or visit BUHS Dental section OPD/ Bahria University Dental College, Karachi in case of any query/ emergency related to your disease.

I have been explained that laboratory investigations will be conducted to evaluate my health status and to diagnose and monitor my disease process. For this purpose, I fully agree to give my salivary sample to the researcher.

I also agree to give all relevant information needed, in full and to the best of my knowledge to the researcher. It is clarified to me that no incentive, financial assistance or reimbursement will be provided to me for participating in the study whereas I do have the right to withdraw from the study at any time.

(D) Consent Form (Urdu Version)

INFORMED CONSENT (URDU)

آپ اس تحقیقی پروجیکٹ میں رضاکارانہ طور پر اور اپنی مرضی سے حصہ لینے کے لیے اپنی رضامندی دے رہے ہیں جس کا مقصد پیریڈونٹائٹس کے مریضوں میں ابتدائی تشخیصی نشان کا پتہ لگانا ہے۔ یہ پروجیکٹ پیریڈونٹائٹس کے مریضوں میں تشخیص کے لیے مارکر کا جائزہ لے گا مریضوں میں مارکر کی موجودگی کی تصدیق کرے گا۔

آپ کو پروجیکٹ میں حصہ لینے کی نوعیت اور اہمیت کے بارے میں تفصیل سے بتایا گیا ہے اور آپ فراہم کردہ وضاحت کو سمجھتے ہیں۔

آپ کو بتایا گیا ہے کہ آپ کی بیماری کے نتائج اور آپ کے ڈیٹا کو سختی سے خفیہ رکھا جائے گا اور صرف کمیونٹی کے فائدے ، اشاعتوں اورآرٹیکل اشاعت کے لیے استعمال کیا جائے گا۔

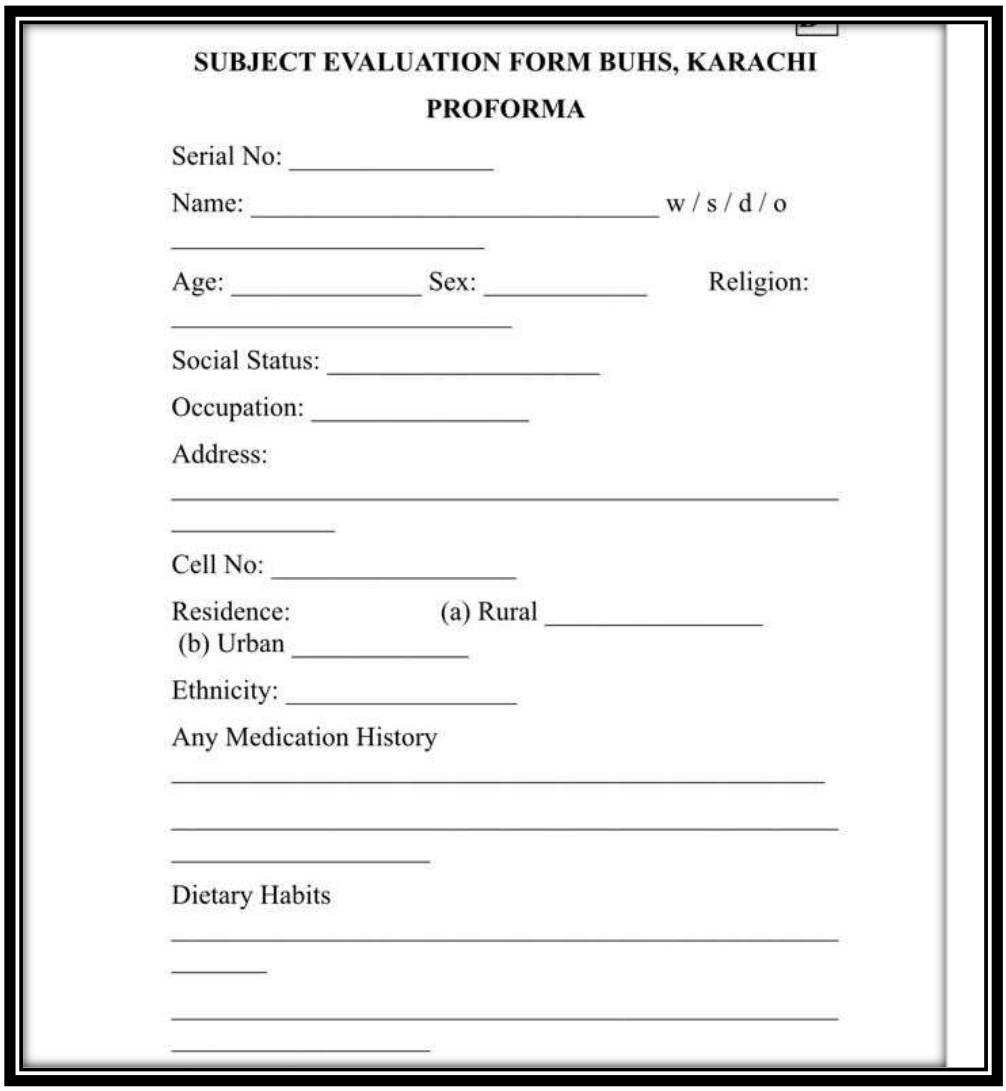
آپ پوری طرح سے اور بہتر علم کے ساتھ محقق کوتمام متعلقہ معلومات دینے پر اتفاق کرتے ہیں۔ یہ آپ کے لیے واضح کیا گیا ہے کہ سوائے لیب کی تحقیقات اور ادویات کی قیمت کے علاوہ مطالعہ میں حصہ لینے کے لئے آپ کو کوئی ادائیگی نہیں کی جائے گی ، جبکہ آپ کو کسی بھی وقت تحقیقاتی عمل کو چوڑنے کا حق حاصل ہے ۔

آپ کو مشورہ دیا جاتا ہے کہ ڈاکٹرماریہ علی سے موبائل نمبر:7245926-0333 پر رابطہ کریں یا اپنی بیماری سے متعلق کسی بھی سوال/ایمرجنسی کی صورت میں بحریہ یونیورسٹی ڈینٹل کالج، کراچی تشریف لائیں۔

مجھے سمجھایا گیا ہے کہ میری صحت کی حالت کا جائزہ لینے اور میری بیماری کے عمل کی تشخیص اور نگرانی کے لیے لیبارٹری تحقیقات کی جائیں گی۔ اس مقصد کے لیے ، میں محقق کو اپنےتھوک کا نمونہ دینے پر پوری طرح متفق ہوں۔

میں تمام متعلقہ معلومات کو مکمل طور پر اور میری بہترین معلومات کے مطابق محقق کو دینے سے بھی اتفاق کرتا ہوں۔ میرے لیے واضح کیا جاتا ہے کہ مجھے مطالعہ میں حصہ لینے کے لیے کوئی ترغیب، مالی امداد یا معاوضہ فراہم نہیں کیا جائے گا جبکہ مجھے کسی بھی وقت مطالعہ سے دستبردار ہونے کا حق حاصل ہے۔

(E) Subject Evaluation Form



SUBJECT EVALUATION FORM BUHS, KARACHI

PROFORMA

Serial No: _____

Name: _____ w / s / d / o

_____

Age: _____ Sex: _____ Religion:

_____

Social Status: _____

Occupation: _____

Address:

_____

_____

Cell No: _____

Residence: (a) Rural _____
(b) Urban _____

Ethnicity: _____

Any Medication History

_____

_____

_____

Dietary Habits

_____

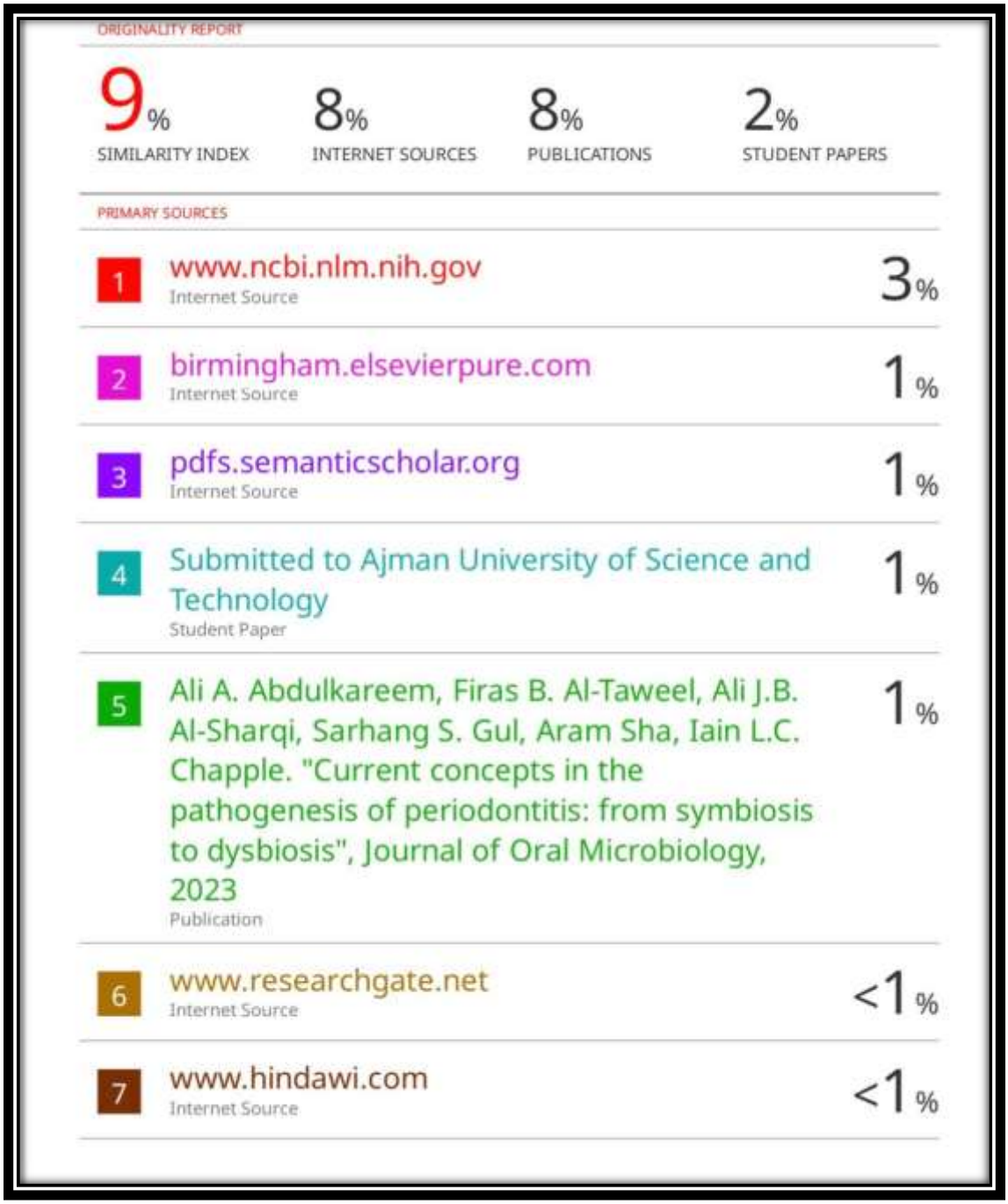
_____

_____

_____

(F) Hospital / Institute Card

(G) Turnitin Plagiarism Check report



ORIGINALITY REPORT

9%	8%	8%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet Source	3%
2	birmingham.elsevierpure.com Internet Source	1%
3	pdfs.semanticscholar.org Internet Source	1%
4	Submitted to Ajman University of Science and Technology Student Paper	1%
5	Ali A. Abdulkareem, Firas B. Al-Taweel, Ali J.B. Al-Sharqi, Sarhang S. Gul, Aram Sha, Iain L.C. Chapple. "Current concepts in the pathogenesis of periodontitis: from symbiosis to dysbiosis", Journal of Oral Microbiology, 2023 Publication	1%
6	www.researchgate.net Internet Source	<1%
7	www.hindawi.com Internet Source	<1%