EVALUATION OF SERUM ASYMMETRIC DIMETHYLARGININE (ADMA) AS A MARKER OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH PERIODONTAL DISEASE AND CORONARY HEART DISEASE

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APPROVAL FOR EXAMINATION

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TO MY BELOVED MOTHER

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ABSTRACT

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of Nitric oxide (NO) production. It is synthesized by post translational modification of L-arginine which involves the set of enzymes called protein arginine methyl transferases (PRMTs). It has been identified as a hallmark of vascular endothelium destruction since it inhibits NO-mediated vasodilation in the endothelial cells. Periodontal disease has been linked to cardiovascular diseases (coronary artery disease) because of the shared risk factors and etiology. However, the relevance of ADMA as a reliable measure of endothelial dysfunction in individuals with cardiovascular disorders and periodontal disease, where it has been discovered at elevated levels, remains unclear. ADMA might provide origin for the interaction of cardiovascular diseases and periodontitis. Thus, this research investigates serum ADMA as a measure of endothelial dysfunction in periodontal and coronary heart disease patients. The objectives of this study was to evaluate the levels of ADMA, inflammatory mediators, lipid profile, and periodontal parameters in individuals with cardiovascular diseases and periodontitis, which may have significant implications for understanding the interplay between these conditions. In this case control study, after the attainment of FRC and ERC approval; 22 subjects with chronic periodontitis (CP), 22 subjects with coronary heart disease (CHD), 22 subjects with both periodontitis and coronary heart disease (CHD) and 22 systemically healthy controls aged between 30-60 years were recruited. All the male and female individuals were selected on the basis of inclusion criteria prior taking written informed consent. The clinical and periodontal parameters (plaque index, probing pocket depth, clinical attachment level and bleeding on probing) were assessed in the participants of the study. The overnight fasting blood samples of patients were obtained for analysis of ADMA, C-reactive protein and lipid profile. The results were analyzed with SPSS version 23.0. ADMA was significant in all the groups (CP, CHD, CP+CHD and Controls). Subsequently the parameters of lipid profile TC, HDL and LDL were significant among the groups. Inflammatory mediator hs-CRP was also significant among all the groups. The periodontal clinical parameters which includes number of teeth, PD, CAL, BoP% and PI were also significant among all

the groups ($p<0.05$). The significant correlations were found for parameters which includes studied groups, gender, hsCRP, HDL, no. of teeth, BoP and PI. Multivariate regression analysis showed significant positive association for the case groups (CP, CHD and CP+CHD) for ADMA. Our results specified that serum ADMA levels may be a valuable measure of endothelial dysfunction in individuals with periodontal disease and coronary heart disease. The different threshold levels suggested by the receiver operator characteristics (ROC) curve (CP=4.7μmol/L) (CHD=7.30 μmol/L) (CP+CHD=10.3 μmol/L) depicted that ADMA levels can be assessed in these conditions based on those cut-off levels.

Keywords: ADMA, Coronary Heart Disease, C Reactive Protein, Lipid Profile, Periodontal Disease, Probing Depth, Clinical Attachment Level, Bleeding on Probing, Plaque Index.

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LIST OF ABBREVIATIONS

ACVD	Attherosclerotic cardiovascular disease
ADMA	N^G,N^G-dimethyl-L-arginine, asymmetric dimethylarginine
AMP3	Vesicle Associated Membrane Protein 3
ANRIL	Antisense Non Coding RNA in the *INK4* Locus
BMI	Body Mass Index
BoP	Bleeding on Probing
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
CAL	Clinical attachment level
CAMTA1	Calmodulin Binding Transcription Activator 1
CDKN2B-AS1	Cyclin Dependent Kinase Inhibitor 2B Antisense RNA 1
CEJ	Cementoenamel Junction
cGMP	Cyclic Guanosine Monophosphate
CHD	Coronary Heart Disease
CP	Chronic Periodontitis
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DDAH	Dimethylarginine Dimethylaminohydrolase
ELISA	Enzyme Linked Immunosorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
GCF	Gingival Crevicular Fluid
HDL	High Density Lipoprotein
Hs-CRP/CRP	High-sensitivity C-reactive protein
LDL	Low Density Lipoprotein
MMA	Monomethyl Arginine
NO	Nitric Oxide
NOS	Nitric Oxide Synthase

oxLDL	Oxidized Low Density Lipoprotein
PCI	Percutaneous Coronary Intervention
PD/PPD	Probing Depth
PI	Plaque Index
PLG	Plasminogen
PRMT	Protein Arginine Methyltransferases
SAM	S-adenosyl Methionine
SBP	Systolic Blood Pressure
SDMA	Symmetric Dimethylarginine
TC	Total Cholesterol
TG	Triglycerides

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CHAPTER 1

INTRODUCTION

BACKGROUND

Periodontal disease, also known as Periodontitis is a condition that affects the supporting structures of the tooth (gingiva, cementum, and alveolar bone). This disorder is caused by a combination of factors that are local and those that are systemic. Risk factors include gender, stress, genetic constitution, lifestyle such as drinking and smoking, dietary calcium, vitamin D intake, obesity, diabetes, metabolic syndrome and osteoporosis (Genco & Borgnakke, 2013). The mechanism of the disease is reflected in its most significant molecular pathways, that eventually leads to the induction of host-derived mechanisms particularly activation of proteinases. This activation of proteinases made by the host contributes to the loss of fibers of marginal periodontal ligament, movement of the junctional epithelium towards apex, and spread of the bacterial growth from the root tip to the root surface (Tonetti et al., 2018). Inflammation of the gingiva (gums), periodontal pockets measuring between 3 and 12 millimeters, gingival bleeding, tooth movement, and recession are all symptoms of this condition (Cardoso et al., 2018).

Periodontal disorders are one of the most widespread forms of disorders, affecting between 20% and 50% of the world's population (M. A. Nazir, 2017). According to the outcomes of the Global Burden of Disease Study carried out in 2016, periodontal disease of the most severe forms was the eleventh most frequent ailment in the globe (Vos et al., 2017). The prevalence of severe periodontitis is higher in nations with lower levels of economic

development. Between the years 1990 and 2019, the growth of the world's population was responsible for 67.9% of the rise in the number of individuals having severe periodontitis (Chen et al., 2021). It is one of the most common reasons for tooth loss, which can have negative effects on chewing, appearance, one's sense of self-worth, and overall quality of life (Jepsen et al., 2018; Tonetti et al., 2017). The prevalence of periodontitis in Asia varies, largely dependent on the investigated population and the diagnostic criteria used. A meta-analysis of 69 researches from 16 Asian nations conducted in 2021 discovered that the total prevalence of periodontitis in Asia was 45.3%. The highest prevalence was found in Southeast Asia (60.3%), followed by East Asia (44.9%), Central Asia (38.5%), and South Asia (34.7%). The total world population of about 20-50% is affected by this condition but a higher prevalence is observed in the Asian region, including Pakistan (Bokhari et al., 2014). Nationally, periodontal disease is estimated to affect roughly 58.4% of Pakistan's population. Periodontitis was estimated to be 37% prevalent in Punjab, 40% prevalent in Sindh, 20% prevalent in Khyber Pakhtunkhwa, and 3% prevalent in Baluchistan (Fahim et al., 2022).

Periodontitis is caused by a complicated interplay between a microbial assault and the host's immune system. This interaction changes the metabolism of connective tissue and bone, which damages the gums and causes clinical attachment loss (Yucel-Lindberg & Båge, 2013). It is a progression of a milder, reversible form of the disease known as gingivitis. It is the inflammation of the gum tissues of the tooth which progresses to periodontitis resulting in alveolar bone destruction and consequently tooth loss if left untreated. The disease initiates after the biofilm of dental plaque starts to form on the surface of the tooth. The subgingival biofilm infiltrated with bacteria which is associated with gingivitis and periodontitis is the result of dynamic interactions with its environment. In general, the constitution of microorganisms consists of species that cohabit in relative armistice. Nevertheless, if the surroundings alter as a consequence of gingival tissue inflammation or other, as-yet-unidentified biofilm processes, dysbiosis may occur in the proliferation of more infectious biofilm components and an exacerbation of periodontal inflammation (Darveau et al., 2012). As a result, gingivitis can be thought of as a rather generic inflammatory response to nonspecific (indigenous) subgingival bacteria. There is a transition in microbial composition as a result of the resulting inflammation and periodontitis development, and various potential

pathogens appear, leading to increased host-driven tissue damage (Teles et al., 2012). Bacterial components that cause inflammation, such as lipopolysaccharides (LPS), lipoteichoic acids, peptidoglycans, toxins and proteases, can be present in the biofilm on tooth surfaces (Yucel-Lindberg & Båge, 2013). The response of the host to the microbial assault includes various inflammatory cell types as well as tissue resident cells are activated and stimulated (Van Dyke & Van Winkelhoff, 2013). The "red complex" pathogens Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia, have been found in biofilms at areas expressing progressive periodontitis (Suzuki et al., 2013). In innate immunity, pathogens in the dental plaque, like LPS, cause mast cells to release vasoactive amines, TNF that has already been made, and inflammatory mediators. With the assistance of mediators, inflammatory cells are attracted to the tissue. Polymorphonuclear leucocytes make lysosomal enzymes, and when inflammatory factors are present, the levels of MMP rise. MMPs and lysosomal enzymes are both involved in the breakdown of gingival tissue. Cells called macrophages and lymphocytes move into the tissue. CD4 T helper cells (Th0) cells are turned on by the antigen-presenting cells. T-cell cytokines can either make more inflammatory molecules or stop them from being made (Yucel-Lindberg & Båge, 2013). Inflammatory mediators are responsible for turning on many of the processes that lead to bone loss. The nuclear factor-kappa B (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG) affiliation is one of the molecules that controls formation of bone. When RANK binds to its receptor on the surface of pre-osteoclasts, it causes them to grow into osteoclasts. On the other hand, when OPG binds to RANKL, it stops it from interacting with RANK and, as a result, stops all of the molecular steps that lead to bone resorption. In normal circumstances, the rebuilding process involves both osteoclasts, which break down the bone matrix, and osteoblasts, which fix it. When the balance changes towards alveolar bone resorption, the activity of osteoclasts goes up. This causes bone modelling to be messed up, which is what happens in periodontitis. In periodontitis, LPS (lipopolysaccharides) from Gram-negative bacteria are a major cause of changes in inflammation and bone loss. The imbalance of pro-inflammatory and anti-inflammatory cytokines is another molecular process that leads to the loss of periodontal tissue. Cytokines are known to be important for organ homeostasis, regulating immune responses, and cell signaling. During an inflammatory response, LPS from Gram-negative bacteria leads to the production of pro-inflammatory

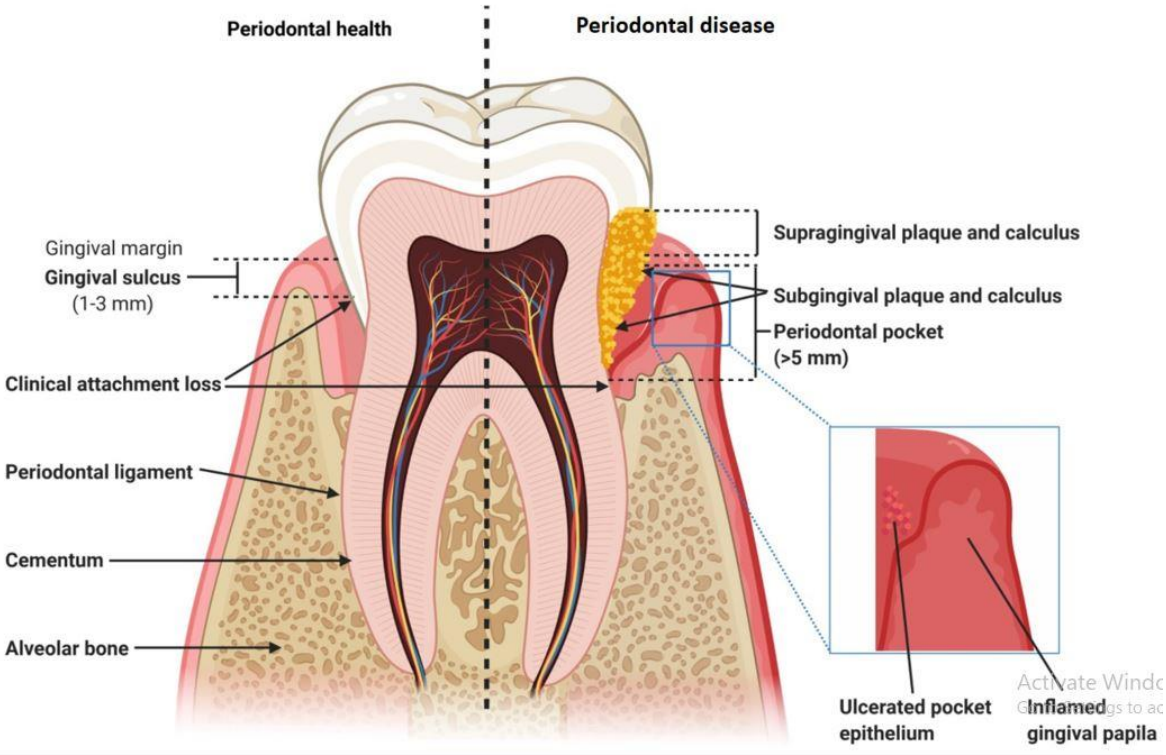


Figure 1.1 The progression from periodontal health to periodontitis disease (González-ramírez et al., 2022)

proteins (Ben-Sasson et al., 2009). PGE2 and cytokines affect the development of RANKL and OPG, which causes osteoclasts that can break down alveolar bone to be made and activated (Yucel-Lindberg & Båge, 2013).

Periodontitis is a multifactorial infectious disease with multiple causative and contributing factors. Periodontal disease assessment and classification have been revised on a regular basis. The recently developed periodontal disease classification (Jepsen et al., 2018) intends to identify clinical entities with clear boundaries by means of precise criteria capable of linking diagnosis with prevention and therapy, hence progressing on the road to precision and personalized dental practice. It establishes unambiguous diagnostic criteria for the following conditions: periodontal health (Lang & Bartold, 2018); gingivitis (Trombelli et al., 2018); reduced but healthy periodontium (periodontitis which is treated); gingival inflammation in periodontitis (treated periodontitis with persistent inflammation) (Chapple et al., 2018); v) periodontitis (Papapanou et al., 2018; Tonetti et al., 2018); This concept represents a significant departure from the previous system of classification (Armitage, 1999), which documented diverse categories of periodontitis (chronic periodontitis, aggressive periodontitis, necrotizing ulcerative periodontitis, and periodontitis as a manifestation of systemic diseases) and caused challenges with implementation and ambiguity in diagnosis because of conflicts that arise from the overlapping definitions of various individuals. Forms of periodontitis formerly categorised as "chronic" or "aggressive" were merged into a single group called "periodontitis," and further characterised using a multi-dimensional grading and staging system, as per the new periodontal disease categorization scheme (Papapanou et al., 2018).

For many years, oncologists have used staging system. A standard staging system was developed to provide a method of identifying periodontitis at many different points in time, which can be easily conveyed to others to facilitate the treatment and may be an influential variable in determining prognosis. Standardized measures of periodontitis severity and extent upon presentation are used in staging. However, the complexity of patient management is expanded to include this new factor. The information gained from periodontitis staging should be accompanied with evidence on the disease's underlying

biological grade. This is based on a combination of parameters such as: 1) the rate of progression of periodontitis; 2) known predisposing factors for periodontitis advancement; and 3) the hazard of an individual's case influencing the subject's systemic well-being. Stage I periodontitis is the stage of attachment loss that occurs between gingivitis and periodontitis. As a result, patients with stage I periodontitis have periodontitis as a consequence of persistent inflammation of the gums and plaque's microbial imbalances The interdental CAL measurements is 1-2mm at this stage, and radiographic bone loss in the coronal third area is less than 15%. With horizontal bone loss, the maximum PD is 4mm. At stages I and II, there is no tooth loss. Periodontitis at stage II is known as established periodontitis. It has interdental CAL of 3-4mm and radiographic bone loss of 15-33% in the coronal third of the root surface. With horizontal bone loss, the maximum PD is 5mm. The presence of severe periodontal infections that spread out to the central portion of the root obscures management, such as extensive intrabony defects, a history of tooth exfoliation due to periodontitis, furcation involvement and the presence of localized ridge defects that interfere with implant tooth replacement. The interdental CAL is 5 mm, and radiographic bone loss continues to the root's middle third region as well as beyond. The maximal PD is 6mm, with a 3mm vertical bone loss. In this condition, tooth loss is associated with 4 teeth. In addition, there is involvement of the furcation area classified as II or III with moderate ridge defects. Stage IV is marked by deep periodontal infections that go all the way to the tip of the root and/or a history of losing multiple teeth.; it can often be exacerbated by excessive movement of the tooth due to minor occlusal trauma and secondary effects of loss of teeth: posterior bite collapse and drifting. The masticatory function has been lost. The interdental CAL is 5 mm, and radiographic bone loss continues to the root's middle third and beyond. In this condition, tooth loss is associated with 5 teeth. In addition to Stage III complexity, significant therapy is required owing to masticatory impairment. Secondary occlusal damage (2nd degree tooth mobility), significant ridge defects, drifting, flaring, and bite collapse are also visible. At this stage of the disease, there are 20 surviving teeth (10 opposing pairs).

The grading system adds a new dimension by allowing progress over time to be evaluated. The fundamental criteria can be either direct or indirect indications of periodontitis advancement, and their existence is the fundamental determinant of the severity of

periodontitis. Longitudinal observations, like those that might be obtained from earlier radiographs of diagnostic quality, provide the basis of the direct evidence. The foundation of the inferences formed from the circumstantial evidence is the measurement of the loss of bone at the most severely damaged tooth in the dentition as as a result of age (estimated by radiographic bone loss in proportion to the length of the root divided by the individual's age). Therefore, the presence of risk factors can cause a change in periodontitis stage. There are three groups, from slowest to fastest, based on the rate at which the disease spreads from patient to patient. Grade A is regarded to be the slowest, Grade B to be intermediate, and Grade C to be the quickest. Grade A indicates no radiographic bone loss over a five-year period. Loss of 2 mm in radiographic bone over 5 years corresponds to grade B, and loss of 2 mm in radiographic bone over 5 years corresponds to grade C. Bone loss as a percentage of age is 0.25% for grade A, 0.25%-1.00 for grade B, and 1.00% for grade C (Tonetti et al., 2018). The purpose of this staging and grading system is to aid clinicians in developing effective treatment plans for patients with periodontitis. Also, the system was made to help doctors identify patients who are more likely to deteriorate from their condition or who would not respond as expected to standard periodontal care (Sanz et al., 2020).

Chronic periodontitis is the evolution of the illness over a period of time without therapy, whereas aggressive periodontitis displays prompt attachment loss and bone deterioration, as well as the possibility of familial aggregation of disease. Both forms of periodontitis are referred to as periodontitis. According to that unanimity, both can be broken down into generalized or local forms, conditional to the proportion of tooth sites that are affected (above or below 30%) and the extent of loss of attachment (slight: 1 or 2 mm, moderate: 3 or 4 mm, and severe: 5 mm). The percentage of tooth affected sites determines the local form, while the severity of attachment loss determines the general form. The dichotomy between aggressive and chronic periodontitis can also be made based on a number of clinical characteristics, including the age at which symptoms first appeared, the rates of advancement of the disease, the patterns of obliteration, the signals of swelling, and the comparative amounts of calculus and plaque that are present (Cardoso et al., 2018). The clinical presentation of periodontitis is distinguished by the presence of periodontal pockets, clinical attachment loss, and bleeding on probing. These are the hallmarks of periodontitis.

The degree and extent to which these clinical symptoms manifest varies greatly according to the stage at which the disease is being exhibited. In the early stages of periodontitis, the clinical presentation may be asymptomatic or exhibit moderate signs, such as bleeding from the gingiva and slight periodontal pocketing. Gingival bleeding and modest periodontal pocketing are two examples. In the later stages of periodontal disease, the clinical presentation is distinguished by the presence of deep periodontal pockets, substantial clinical attachment loss, and mobile teeth. In addition to these, other clinical manifestations of CP include receding gums, bone loss, and involvement of the furcation. Conditions that affect the body as a whole, such as diabetes and smoking, can also have an effect on the clinical manifestations of CP (Heitz-Mayfield & Lang, 2010).

There is not a universal consensus on which method is the most accurate, even though they all have their advantages; for example, probing depth can be used to measure the profundity of the periodontal pocket, which is the sign of active disease, clinical attachment loss (CAL) and radiographs can measure the disease built up over a period of time, and bleeding on probing (BOP) is useful to indicate the presence of active inflammation. Some clinicians rely on a single technique, while others use a hybrid approach (Natto et al., 2018). Different diagnostic classifications for accessing periodontitis have been used in numerous studies. The CPI/CPITN (Community Periodontal Index/Community Periodontal Index of Treatment Needs) categorization system employed PD 3.5mm as a cut point (in 13.4% of cases), making it the most popular single-criterion system. Time is saved by merely recording the upper and lower first molars, the central incisor on the upper right side and the lower left side. This facilitates its rapid implementation in large human populations (Listgarten et al., 1985). The biggest benefit is that it's consistent everywhere, but it doesn't keep track of permanent alterations like recession or periodontal attachment loss. In the final analysis the choice of classification could affect how well periodontitis can be predicted. Regardless of the classification, the ability to predict whether a young adult will get periodontitis could be improved by combining knowledge about their periodontal health, their social and demographic background, and their general health history (Leite et al., 2017).

Cardiovascular disease is a collection of disorders which affect both blood arteries and the heart, comprising coronary heart disease, coronary artery disease, and acute coronary syndrome, amid others (Sanchis-Gomar et al., 2016). Coronary heart disease is a disorder in which there is a constriction or obstruction of the blood vessels (coronary arteries) that supply oxygen and blood to the muscles of the heart. This can lead to reduced flow of blood and supply of oxygen to the myocardium, which can cause chest pain or discomfort (angina), shortness of breath, heart attack (myocardial infarction), or even death (sudden cardiac death). The supply of blood to the heart can be obstructed by atheromatous plaque, thrombosis (occlusion) or spasm (constriction) of coronary arteries. It is a progressive condition that can develop over many years and is often caused by accumulation of fatty deposits (plaque) in the coronary arteries, a process known as atherosclerosis (Vinay et al., 2020).

According to the Global Burden of Diseases study, there were 154 million cases of CAD around the world in 2016. This was 32.7% of all cases of cardiovascular disease and 2.2% of all cases of illness around the world (James et al., 2018). The American Heart Association (AHA) found that have coronary artery disease is prevalent in around 15.5 million of Americans. This indicates that in the United States, 7.6% of males and 5.0% of women were affected with CAD during this time frame (Henrique Wolff Gowdak, 2016). The total of deaths from coronary artery disease were predicted to rise from 9 million to 19 million in the duration from 1990 to 2010 among those living in developing countries (including China, Latin America, India, Sub-Saharan Africa, and the Far East). Although conventional factors of risk are associated with the frequent development of CAD in China, conventional risk factors do not explain why it is prevalent in India (Ferreira-González, 2014). Statistics from the Global Burden of Disease (GBD) in 2001 show that 43% of all deaths from CVD may be attributed to CAD. Different death rates and death patterns are seen in countries with high and low per capita incomes. (Gaziano et al., 2010). The high prevalence of CAD in Latin America is explained by an increase in obesity, sedentary lifestyle and smoking (Ralapanawa & Sivakanesan, 2021). According to a Malaysian study completed in 2000, CAD is responsible for 22,158 fatalities, or around one-fifth of all the mortalities (Seong & John, 2016). In 2015, the average death rate from CAD in Eastern

Europe and Central Asia was twice as much as in Central Europe (Murphy et al., 2018). According to the WHO GBD report, CAD is the main reason of mortality in South America, Central America, and the majority of Caribbean islands, accounting for 35% of all fatalities. CAD accounted for 13.6% of overall mortality in South Asia, or 1.8 million fatalities in the year 2001, (Gaziano et al., 2010). It appears at a younger age with more severe illness and have a three to five times greater chance of myocardial infarction when compared to Caucasians. Additionally, such individuals have a higher chance of mortality from the following condition (M. Gupta et al., 2006). Pakistan has higher prevalence rate of coronary artery disease (CAD) which represents more than 30% of the national population (above 45 years old) being affected by the disease (Shahid et al., 2017). A study carried out in Pakistan showed that CVDs occurred in 17.5% of the population with 16.6% individuals being male and 18.3% females. CVDs are the most prevalent cardiac condition in Punjab, Pakistan, affecting 17.5% of the population studied. From October 2014 to September 2015, the annual rate of coronary artery disease was 0.4%, according to a study conducted in various cities in Punjab, Pakistan (Zubair et al., 2018).

There are two categories of risk factors that influence Coronary Artery Disease: non-modifiable risk factors and modifiable risk variables. Unlike modifiable hazards, such as gender and age and the risk factors which are non-modifiable cannot be avoided, altered, or managed in any way. As a result, non-modifiable risk variables have less of an impact on risk factor management. Risk factors that can be modified, prevented, or managed are referred to as modifiable risk factors. A sedentary lifestyle, smoking, an imbalance in nutritional intake, reduced glucose tolerance elevated blood pressure, abnormal lipids in the blood, diabetes mellitus, and obesity are few of the harmful factors (Mageed, 2018).

The coronary artery disease (CAD) is a complicated prolonged inflammatory illness which involves remodeling and constriction of the coronary arteries that deliver oxygen to the heart. It may be observed in a number of clinical forms, including, acute coronary syndrome, stable angina and sudden death due to cardiac arrest. It has a complicated etiology and mechanism and a multifaceted origin connected to environmental variables such as nutrition, smoking, and physical activity, as well as genetic factors that influence disease risk

both individually and in combination. Inherent to the pathogenesis of CAD is the formation of atherosclerotic plaque (Soleimany et al., 2022). Atherosclerosis is a multifaceted, long-term inflammatory condition of the large and medium-sized arteries caused by increased absorption, trapping, and accretion of lipids, especially low-density lipoprotein (LDL), in the arterial wall's sub endothelium; interactions between white blood cells and components of the arterial wall. (Björkegren & Lusis, 2022). Plaque is an accumulation of fatty substances that blocks blood flow by narrowing the lumen of the artery. The first step is to produce what is called a "fatty streak." Macrophages, also known as foam cells, deposit their lipid cargo subendothelially, leading to the development of fatty streaks. The intima layer disintegrates after an injury to the vascular tissues, allowing monocytes to migrate into the subendothelial space and develop into macrophages. When macrophages engulf oxidised low-density lipoprotein (LDL) particles, it leads to the formation of foam cells. When T cells are stimulated, they produce cytokines that further the pathogenic process. The increased amount of foam cells is a consequence of the release of growth factors, which stimulate smooth muscles, leading to the intake of oxidised LDL particles and also collagen and unload them besides active macrophages. As a consequence of this process, a plaque develops in the sub endothelium. If the endothelium is not harmed any further, this plaque may grow or stay the same over time. A fibrous cap will form and calcification will commence if the lesion stabilizes. The symptoms of angina begin when the heart is unable to meet the increased demand associated with physical activity because of insufficient blood flow to the myocardial tissue. However, when resting, symptoms would lessen because of the reduced need for oxygen. Angina pectoris at rest requires a lesion to be stenosed by at least 90%. Plaques may break apart, exposing tissue factor and leading to thrombosis in certain cases. Acute coronary syndrome (ACS), which includes unstable angina, non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI), may arise from thrombosis depending on the severity of the damage (Nakahara et al., 2017) .

The cases of coronary artery disorders fall into one of two categories: stable ischemic heart disease (SIHD) or acute coronary syndrome (ACS). Unstable angina, ST-elevation myocardial infarction (STEMI), and NSTEMI are the three most common forms of ACS (Kovell & Aurigemma, 2023). Individuals with established or supposed ischemic heart

disease are considered to have stable ischemic heart disease (SIHD) if they do not experience any recent or acute changes in their clinical condition, which suggests that there is no active thrombotic process taking place right now. These patients include those who have one or more of the following conditions: recent-onset or stable angina or symptoms corresponding to ischemia such as shortness of breath or pain in the arm with exercise; stabilization after the ACS as a result of revascularization or medicinal treatment; and asymptomatic SIHD detected by abnormal stress assessments or other imaging investigations (Dai et al., 2016). Acute coronary syndrome also referred as Unstable angina, is a fatal form of coronary artery disease caused by abrupt coronary insufficiency. The involvement of unstable plaques in the pathogenesis of ACS has been confirmed by experimental and clinical studies. These individuals had plaque rupture with thrombosis, elevated levels of systemic inflammatory biomarkers and thrombosis, in addition to coagulation system activation. Acute coronary syndrome is caused by partial or full thrombotic vascular blockage. The common symptoms include shortness of breath, chest discomfort, inflammation, and disorientation. The majority of patients show chronic ECG changes, as well as persistent myocardial infarction with ST segment elevation (STEMI). Some individuals with acute chest pain, however, may not have ST-segment elevation but instead have inversion of T-waves or faux-normalization of T waves, or perhaps no ECG alterations (non-STEMI) (Casagrande et al., 2013). Angina develops when a plaque or blood clot narrows a blood artery and may occur as a prelude to a heart attack or remain stable for lengthy periods of time (TP & AV, 2019).

Conferring to the National Heart, Lung, and Blood Institute (2022), patients with CHD may exhibit a variety of symptoms. However, some people are asymptomatic and are unaware that they are having CHD up until they experience chest pain, cardiovascular blood flow is blocked, resulting in a fatal heart attack, otherwise the heart abruptly ceases to function, also known as cardiac arrest. In 2021, the Centers for Disease Control and Prevention say that angina, or pain and soreness in the chest, is the most common sign of CAD. Angina occurs when excessive plaque builds up within the arteries, causing them to narrow. Blood flow to the muscle of the heart and the remaining parts of the body may be impeded by constricted arteries, causing chest pain. A cardiac attack frequently represents the initial sign of coronary artery disease. Heart attack symptoms include chest pain or

discomfort (angina), lethargy, dizziness, vertigo, or a cold perspiration, pain or discomfort in the arms or shoulders, and shortness of breath. CAD may eventually weaken the cardiac muscle. This may result in cardiac failure, a potentially fatal state in which the heart cannot circulate blood as effectively as it should (Fang et al., 2019).

A number of scoring systems, including the SYNTAX score, Jenkins' score, Sullivan Extent scores, and Gensini scoring systems can be used to determine the severity of CAD (Neeland et al., 2012; Tolunay & Kurmus, 2016). The Gensini score is an effective tool for classifying coronary artery disease (Chiha et al., 2016). Lately, this score is found to accurately predict the severity of CAD. The Gensini score is frequently utilized in numerous investigations in a consistent and reliable manner. (Hashemi et al., 2018). It is calculated by assigning a severity score to each coronary artery which has stenosis in this manner: 1 point is designated to a narrowing of 25%, 2 number of points are assigned to 26-50% of narrowing, 4 number of points for a narrowing of 51-75%, 8 number of points for a narrowing of 76-90%, 16 number of points for a narrowing of 91-99%, and 32 points for total occlusion. Following that, each artery lesion score is multiplied by a factor which considers the significance of the injury's location in the coronary circulatory system (5 for the left main coronary artery, 2.5 for the proximal segment of the left anterior descending coronary artery, 2.5 for the proximal segment of the circumflex artery, 1.5 for the mid-segment of the left anterior descending coronary artery, 1.0 for the right coronary artery, the distal segment of the left anterior descending coronary artery, the posterolateral artery, and the obtuse marginal artery, and 0.5 for other segments). In the end, the score is determined by adding the scores from each segment of the coronary artery (Avci et al., 2016).

The oral cavity is home to a huge variety of microorganisms, the species of which vary depending on whether they are located in the teeth, gums, cheeks, gingival sulcus, or palate. These bacteria interact with their human host, both when they are healthy and when they are sick. The mouth cavity of an adult can contain upwards of roughly one billion germs in its whole. It is well knowledge that patients who suffer from some level of periodontal disease (PD) are at an increased risk of experiencing bouts of bacteremia following routine activities such as brushing their teeth or using chewing gum, and that these risks can be

multiplied over the 24 hours. It is a well-established fact that the mouth is a source of systemic infections. This is because the mouth is a portal through which bacteria can enter the bloodstream as a result of disruptions in the integrity of the tissues that are caused by inflammation in illnesses such as periodontitis (Carrizales-Sepúlveda et al., 2018). Several observational studies and epidemiologic have repeatedly demonstrated that Periodontal disease is connected to subclinical and clinical cardiovascular consitions in a variety of populations. Both of these diseases are multifactorial in nature and have many risk factors in common, with inflammation playing a substantial part in the course of disease. Large cohort studies have recently found that periodontitis is related with an elevated risk of coronary artery disease (CAD) and overall mortality; moreover, this connection extended to preclinical CVD as well as stable CAD. Furthermore, genetic studies have indicated a common susceptibility gene that is involved in the development of periodontitis as well as cardiovascular disease. Two theories have been postulated to explain how Periodontal disease affects atherosclerotic cardiovascular disease (ASCVD). First, there is a direct process through which periodontal bacteria infect endothelium cells (Priyamvara et al., 2020). Porphyromonas gingivalis, Campylobacter rectus Tannerella forsythia, Prevotella intermedia, Aggregatibacter actinomycetemcomitans, and Fusobacterium nucleatum are all gram-negative anaerobic bacteria that can cause periodontitis. In the mouth, these organisms live in a biofilm called "dental plaque" that is made up of many different kinds of cells (Gunaratnam et al., 1992). It's worth noticing that the bacterial load of the aforementioned species in subgingival plaque biofilm is demonstrated to be linked to silent thickening of the carotid intima media, even when standard heart disease risk factors are not present (Desvarieux et al., 2005). One of the things that makes it easier for the bacteria to move is that the mouth cavity has a lot of blood vessels and the sulcular epithelium is thin and easily broken. Also, because periodontitis is an inflammatory disease, the enlarged blood vessels in the gums can help bacteria get into the bloodstream. It is thought that this short-term bacteremia makes it possible for bacteria to get into vascular cells. Also, bacteria that are usually found in the mouth have been found at places where arterial lesions are common, and polymerase chain reaction has been used to find bacteria in atherosclerotic plaques (Leishman et al., 2010). When anaerobic bacteria make a biofilm, it causes inflammation, which then moves into the inner connective tissues. Osteoclasts, which are mostly activated

by the pro-inflammatory chemical PGE2 and can be found in a periodontal pocket, are in charge of this inflammatory process. This results in the death of the gum supporting tissues and teeth and the loss of alveolar bone. Polymorphonuclear leucocytes, macrophages, and lymphocytes, which are defense cells, move into the connective tissue next to the periodontal gap. So, this inflamed and sick periodontium serves as a storage place for gram-negative bacteria and their products, such as lipopolysaccharide (LPS; activates B-lymphocytes) and proinflammatory cytokines. Porphyromonas gingivalis is the organism that is most often found in people with periodontitis. This microbe is known to make protease enzymes like gingipain, which is known to cause the CD14 molecule to break down. CD14 usually works as a leukocyte receptor for LPS, which stops the immune system from reacting to antigens from bacteria. Gingipain also helps bacteria live in plaque by breaking down proteins into peptides and amino acids, which helps the bacteria stay alive. LPS links to Toll-like receptors, mostly receptors 2 and 4. This causes the epithelial cells of the host to release cytokines that cause inflammation, such as TNF, IL-1, IFN-, and PGE2 (Schenkein & Loos, 2013). Biomarkers of inflammation, like CRP and IL-6, have been found to be higher in people with periodontitis, which has been linked to an increased chance of CVD (Hernández-Ríos et al., 2017) .

The second proposed mechanism is an indirect channel, and it involves an upsurge of inflammatory cytokines in the systemic circulation as a result of PD. PD causes a systemic inflammatory response, which results in persistently high concentrations of numerous cytokines, which includes IL-1, IL-8, IL-6, TNF- and monocyte chemoattractant protein-1. These cytokines are also connected to atherosclerotic vascular disease. Some of them are able to speed up the formation and the release of intravascular plasma proteins by the liver, including CRP protein and fibrinogen (Zeigler et al., 2015). In addition, bacterial byproducts such as lipopolysaccharide (LPS) have the potential to enter the bloodstream and activate a robust immune response. Because of their effect on endothelial cells, ability to modulate lipid metabolism, and ability to increase oxidative stress, the variables indicated above have the potential to induce atherosclerosis (Stoll et al., 2004). The findings of a prior study that demonstrated endothelial dysfunction in individuals with periodontitis provided support for this theory (Amar et al., 2003).

It's plausible that an increase in overall systemic oxidative stress is perhaps at the root of the connection between poor oral health and cardiovascular disease. By modifying the activity of immune-inflammatory cells in two different ways, this may have an effect on the risk of cardiovascular events as well as the course of oral disease. To begin, intracellular oxidative stress could affect the pattern of cytokines generated by immune-inflammatory cells, so diminishing their ability to combat the effects of bacterial aggression to the oral cavity (Slocum et al., 2016). This is in addition to the possibility that it might limit the availability of nitric oxide, which would lead to an increase in endothelial dysfunction. Recent research has shown that periodontal treatment influences the generation of mitochondrial reactive oxygen species in immune-inflammatory cells. This is the strongest predictor of changes in endothelial function, and it is accompanied by variations in the production of cytokines have a crucial role in the regulation of both innate and adaptive immunity (Masi et al., 2018). Second, cellular ageing may be caused by the persistent activation of immune-inflammatory cells, as well as introduction to elevated amounts of oxidative stress. This may cause the cells to become exhausted, which eventually leads to cellular ageing. It has been demonstrated that individuals who have chronic periodontitis have a shorter telomere length of leukocytes and that the telomere size is closely connected to both the circulating levels of oxidative stress and the severity of the oral illnesses (Masi et al., 2011; Gkranias, et al., 2014) Consequently, shortening of telomeres has been linked to cardiovascular disease , and cells with dangerously short telomeres could contribute to the course of the oral illness because they generate larger quantities of proinflammatory cytokines and lose their ability to react efficiently to bacterial aggressions (Masi, D'Aiuto, et al., 2014).

The two diseases share a number of common risk elements for example age, diabetes mellitus physiological stress, smoking, and poor socioeconomic conditions. There have been few investigations on the influence of stress on periodontal disease progression in people; nonetheless, it is well-documented that the systemic level of inflammatory cytokines increases dramatically in response to chronic stress (Zardawi et al., 2021). These cytokines are frequently seen in PD-related damaging events. Animal investigations in vitro and in vivo

have shown potential pathways through which stress might contribute to periodontal tissue degradation. Chronic stress has been shown to accelerate the progression of periodontitis by stimulating the sympathetic nervous system, which leads to the secretion of the catecholamines such as epinephrine, which is capable of binding to the 1-adrenergic receptor (1-AR) on the surface of the periodontal cells diminishing cellular processes and causing a massive release of inflammation-related factors (H. Lu et al., 2014). Concurrently, oxidative stress, psychological stress, hemodynamic stress, and social stress all play roles in the progression of cardiovascular disorders. The relationship between stress and ACVD has been widely researched, and the findings imply that people with ACVD who are also under psychological stress are more likely to experience transitory myocardial ischemia, increased risk of recurrent ACVD, and higher mortality (Zardawi et al., 2021).

Smoking is a widely recognized risk factor for periodontal disease and cardiovascular disease. Nicotine is one of the most dangerous toxic chemicals produced by smoking. Tobacco use is thought to increase the risk, pathogenesis, and exacerbation of periodontal disease through a number of mechanisms, including: diminished gingival perfusion, which limits nutrient and oxygen delivery as well as waste product removal; suppression of immune response, particularly inflammation; suppression of the periodontium's structural and functional recovery; and imbalance and increased infiltration of oral microbiota. Together, they slow wound healing and increase the progression of gum disease. (Silva, 2021). Correspondingly, toxic byproducts of tobacco combustion activate the atherogenic mechanism by increasing LDL oxidation, resulting in persistent inflammation in the intima layer and following endothelial dysfunction (Li et al., 2019; Salahuddin et al., 2012). Tobacco use increases platelet aggregation, blood viscosity, and alters the prothrombotic/antithrombotic balance toward enhanced coagulation. The role of smoking in the pathophysiology of periodontal disease and ACVD has been proven by a considerable reduction in the intensity of the correlation between the two diseases when smoking was adjusted (Rydén et al., 2016).

Diabetes mellitus (DM) is a metabolic disorder that damages the body in a number of ways, including the health of the gums and the heart. High glucose levels caused by

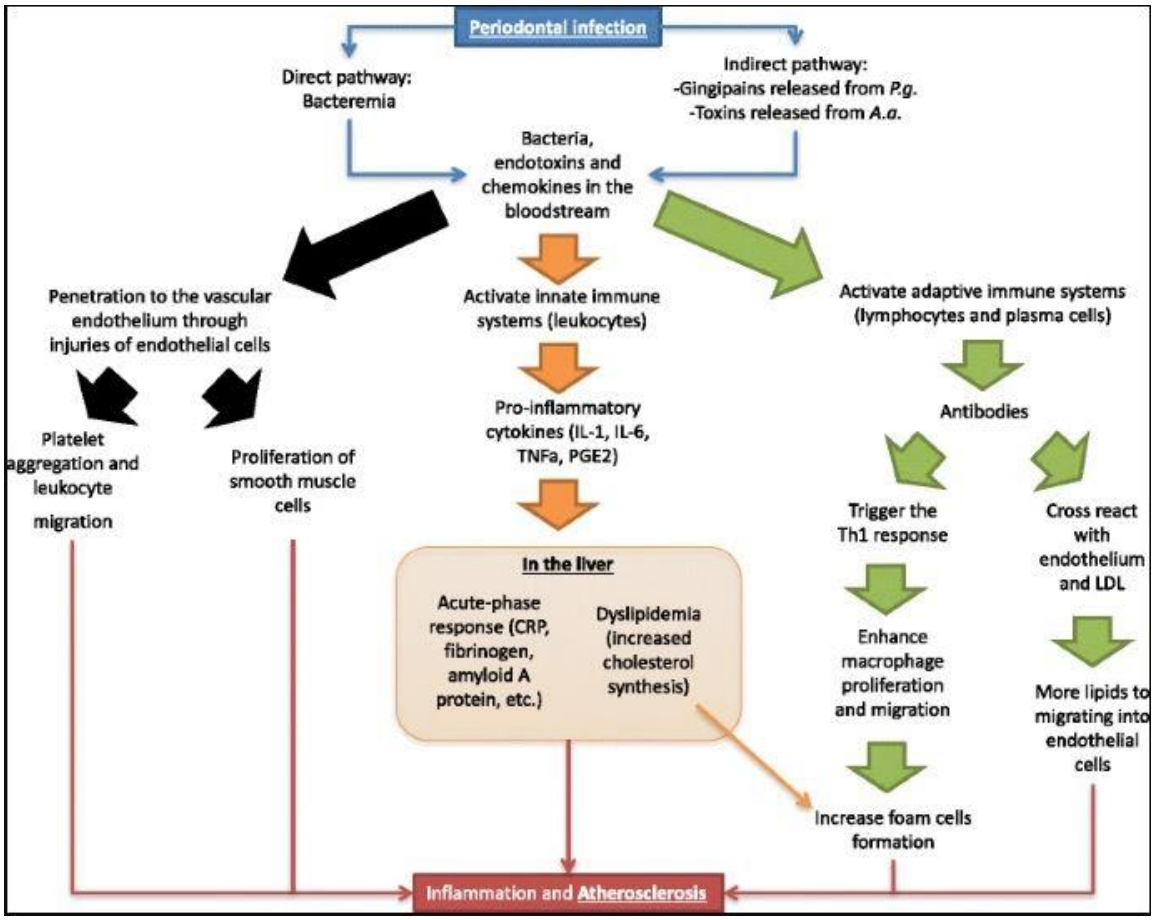


Figure 1.2 Mechanism coupling periodontal disease to inflammation of blood vessel tissues and atherosclerosis (Nguyen et al., 2018)

diabetes can make endothelial cells weaken and die, a process called necroptosis. Endothelial cells make a lot more receptor-interacting protein kinases 3 (RIPK3) when they have high blood sugar (Lin et al., 2018). When ECs die, their number and health go down and their covering becomes more permeable. This makes it easier for lipids to build up and for monocytes and vascular smooth muscle cells to move to the intima, which further damages the vascular system (Xu et al., 2018) Complications caused by diabetes also affect the bones' ability to heal, mineral mass, and rate of turnover (Brown et al., 2014). These systemic effects of DM make it much more likely that PD will get worse. Compared to healthy people, diabetics are almost three to four times more likely to get periodontitis (Apoorva et al., 2013).

There was a substantial link found between periodontal disease and four genes. Concomitantly, ANRIL/CDKN2B-AS1, PLG, and CAMTA1/VAMP3 were the three genes that were shown to be associated with atherosclerotic cardiovascular disease (Aarabi et al., 2017). It has been demonstrated that a particular long non-coding RNA that goes by the name ANRIL is concomitant with an augmented possibility of CAD as well as periodontitis (PD). According to the findings of the study, having both of the primary risk genes for coronary artery disease and Parkinson's disease was associated with having lower levels of particular ANRIL transcripts down order to zero down on certain ANRIL transcripts, the researchers utilized a "knock-down" technique. After further investigation, they discovered that ANRIL exerted its influence over three genes namely, ADIPOR1, VAMP3, and C11ORF10 in a manner that was dynamic over time. These genes contribute to the process by which glucose and fatty acids are used, as well as the inflammatory response. The research also discovered that a portion of CAMTA1 located upstream of VAMP3 was concomitant with an augmented possibility of both severe PD and CAD. In general, the findings of the study indicate that particular variants of ANRIL exert control over important genes, hence influencing the manner in which glucose and fatty acids are metabolized (Bochenek et al., 2013). PLG is the third gene that is involved in the development of both cholesterol and periodontitis as a risk factor. The transforming growth factor beta signaling pathway includes all of the genes that contribute to an individual's chance of developing coronary artery disease as well as periodontitis (Schaefer et al., 2015)

Asymmetric Dimethylarginine (ADMA) is synthesized as a result of proteolysis of methylated amino acid residues, namely L-arginine. It is an intrinsic inhibitor of the nitric oxide synthase enzyme, resulting in reduced nitric oxide synthesis, which protects the integrity of the basal vascular epithelium, limits platelet activation, prevents leukocyte adherence to the endothelium wall, and controls heart contractility (Tashie et al., 2020). It was isolated from human urine for the first time in 1970. ADMA can be distinguished by addition of two methyl groups on a single nitrogen atom, which are brought about by enzymes from the protein known as arginine N-methyltransferase (PRMT) family (Morales et al., 2016). S-adenosylmethionine (SAM), serves as a methyl group donor in the above reaction. Recent investigations on cardiovascular and renal systems, such as coronary artery disease, chronic kidney disease, Becker muscular disease, Duchene muscular disease (DMD), peripheral arterial occlusive disease, type I diabetes, rheumatoid arthritis and attention deficit hyperactivity disorder (ADHD) have found elevated ADMA levels (Tsikas, 2020). ADMA has been shown to be a biochemical indicator of endothelial dysfunction in humans, and greater ADMA levels have been related with an increased risk of CVD in several investigations. ADMA has been found to uncouple electron transport between NOS and L-Arginine, subsequently leading to the formation of reactive oxygen species (T. M. Lu et al., 2003).

There are three distinct states that may be achieved by methylating arginine residues on the guanidinium nitrogen atoms: NG-monomethylarginine (Rme1), symmetric NG, N'G-dimethylarginine (Rme2s) and asymmetric NG,NG-dimethylarginine (Rme2a), (Fulton et al., 2018). There are nine PRMTs in mammalian cells, and they all feature a highly conserved catalytic central part that consists of a Rossmann-like motif that interacts with SAM and an alpha barrel that facilitates substrate binding (Fulton et al., 2019). Monomethylarginine (Rme1) is produced when peptidyl arginine methyltransferases (PRMTs) transfer the positively charged methyl group from SAM to a terminal nitrogen on the guanidinium part of residues of arginine. This occurs upon the first interaction of SAM and substrate. This first methyl transfer will be catalyzed by each and every PRMT. PRMT1, PRMT2, PRMT3, PRMT4 (CARM1), PRMT6, and PRMT8 are all examples of type I PRMTs. These PRMTs are responsible for the production of Rme1 and also catalyze a second reaction that leads to

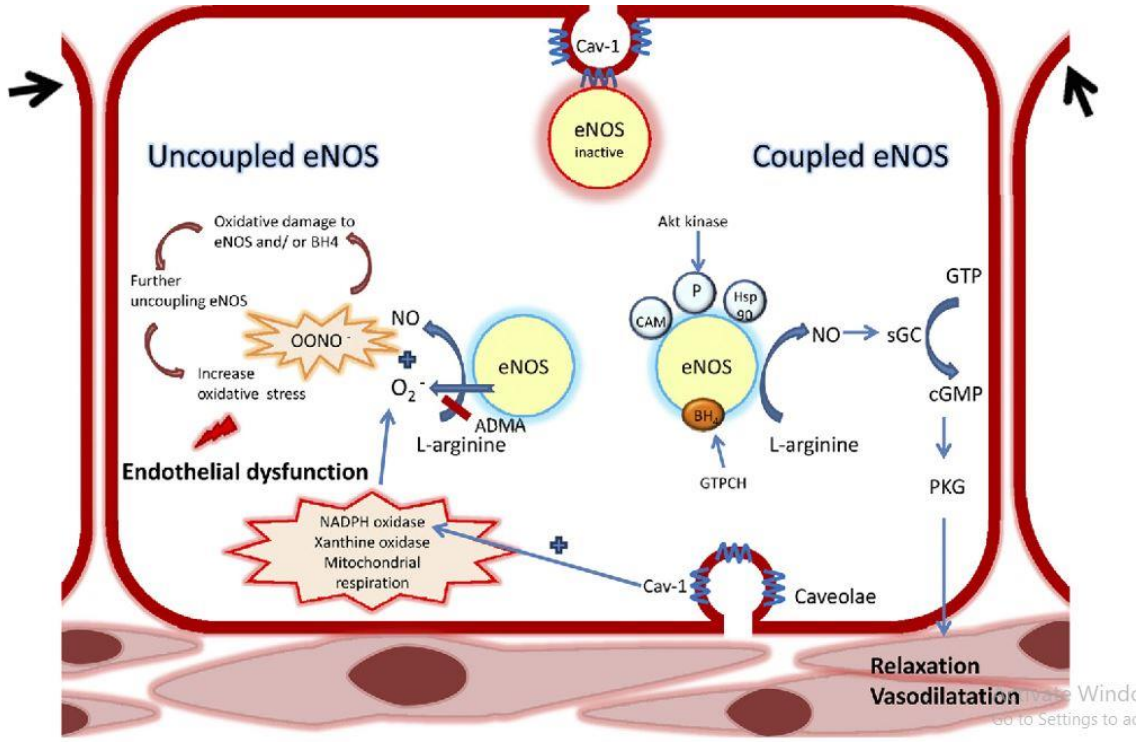


Figure 1.3 Implication of uncoupling of endothelial nitric oxide synthase (eNOS) in the etiology of endothelial dysfunction (Kietadisorn et al., 2012)

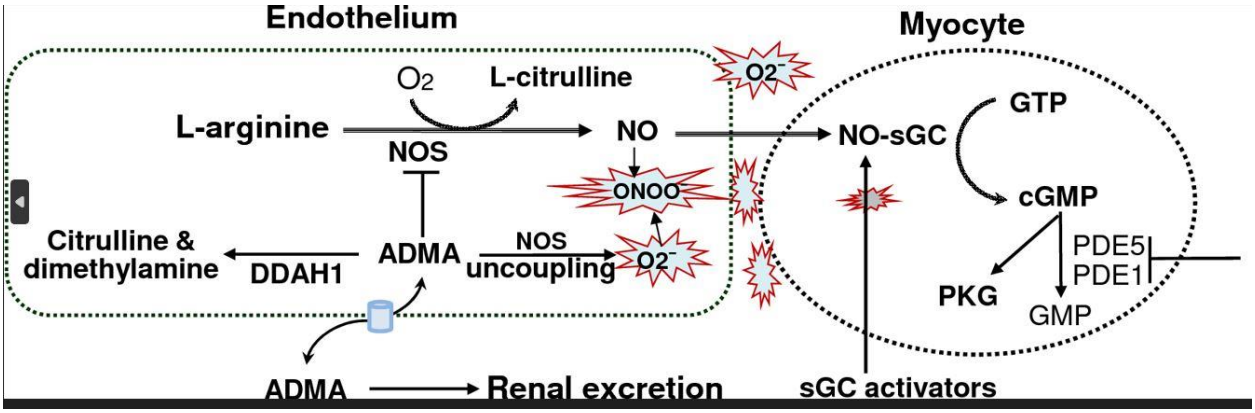


Figure 1.4 ADMA inhibits nitric oxide synthase (NOS)-induced NO/cGMP/PKG signalling in vascular endothelial cells and increases NOS-derived superoxide anion production. (Liu et al., 2016)

the production of asymmetric dimethylarginine residue (Rme2a) (Morales et al., 2016).

The kidney is the principal organ responsible for the elimination of ADMA from the blood, but the liver also plays a part in this process (Vallance & Leiper, 2004). In human beings, the DDAH enzyme catalyzes the hydrolysis of MMA and ADMA, resulting in the formation of citrulline, monomethylamine, and dimethylamine DMA, respectively. This enzyme was initially identified in rats, who were shown to convert ADMA into DMA. Rats were the source of this discovery. In adults, it is anticipated that around 90% of the daily ADMA production is hydrolyzed to DMA, with the remaining 10% being excreted in the urine in its original form.

The brain, kidney, pancreas and liver are the primary organs in which the enzyme DDAH is expressed (Tsikas, 2020). It is the primary enzyme that controls ADMA concentartion in the tissues as well as inside the cells themselves. DDAH I is found in greater abundance in tissues that produce neuronal nitric oxide synthase (nNOS), whereas DDAH II is located in tissues that produce endothelial (eNOS). Last but not least, ADMA can be converted to DMGV (-keto--(N, N-dimethylguanidino valeric acid) by a process known as transamination. The kidneys play a major role in the expression of the alanine glyoxylate aminotransferase 2 (AGXT2) enzyme, which is responsible for this conversion. Consequently, the kidneys are an extremely important organ in the process of getting rid of ADMA, both through the excretion of the substance in urine and through the metabolization of the substance by AGXT2 and DDAH. This might explain why individuals with greater degree of proteinuria have higher levels of ADMA, regardless of their glomerular filtration rate, as well as why ADMA emerges promptly in patients with IgA nephropathy or autosomal dominant polycystic kidney disease (Oliva-Damaso et al., 2019).

Endothelial cells of the inner arteries are responsible for converting l-arginine into nitric oxide. This NO then dissipates from cells of the endothelium to smooth muscle cells, which results in vasodilation of blood vessels and a reduction in blood pressure (Lorton & Shechter, 2019). Endothelium-dependent regulation of vascular tone is primarily mediated by NO. This regulation is regulated by metabolic, humoral, and mechanical variables, such

as an increase in blood flow. In addition to this, NO prevents the adherence and activation of aggregates of platelets, eliminates the harmful superoxide ions activity, and functions as a chemical that prevents blood clots and reduces the risk of atherosclerosis (Demir et al., 2012). The involvement of NO is also conventional in de novo formation of blood vessels, which is the outcome of the de novo production of vessels generated from pluripotent precursor cells, and in angiogenesis, which is the development of functional vessels from already formed capillaries. Both of these processes result in the formation of new blood vessels in the body. A crucial component in these processes is a protein known as vascular endothelial growth factor (VEGF). The release of NO is the mediator of its expression, which was necessary for the beginning stages of vasculogenesis. There are three distinct kinds of the NOS enzyme: neuronal NOS (nNOS), also known as type 1; inducible NOS (iNOS), which is known as type 2; and endothelial NOS (eNOS), which is recognized as type 3. It is generally agreed that NOS1 and NOS3 are continuously expressed. Caveolae in the plasma membrane are where endothelial NOS is stored. Endothelial NOS's distribution and activity are controlled by a wide variety of mechanisms. After being secreted by endothelial cells, nitric oxide (NO) is rapidly delivered to the nearest smooth muscle cells of the vascular system, where it plays its part by promoting the formation of cyclic guanosine monophosphate (cGMP) as a second messenger. This is how NO accomplishes its function. It is possible for reactive oxygen species to nullify its effects before it reaches its target cells. The three different isoforms of NOS are inhibited by ADMA, which acts as an intrinsic competitive inhibitor of L-arginine. The presence of high quantities of ADMA inhibits the production of NO and reduces the absorption of L-arginine by cells, which in turn contributes to oxidative stress and disrupts the subsequent production of NO biogenesis. ADMA inhibits the function of endothelial cells and contributes to the development of atherosclerosis in this way. As a consequence of this, it has been identified as a biomarker of endothelium diseases. Patients who suffer from a variety of metabolic and cardiovascular diseases, for example diabetes mellitus, hypertension, atherosclerosis, hypercholesterolemia, renal failure, chronic heart failure, hyperhomocysteinemia and stroke have been reported to have increased levels of ADMA. As a natural blocker of NOS, ADMA is demonstrated to have the effect of raising the level of systemic vascular resistance in human subjects (Dymara-Konopka & Laskowska, 2019).

In endothelial cells, DDAH-1 or DDAH-2 may convert ADMA to a less useful byproduct called citrulline. However, the enzymatic activity of DDAHs is lowered in the cells of endothelium of individuals who have cardiovascular diseases. It has been shown that elevating DDAH levels results in decreased ADMA levels while simultaneously enhancing eNOS activity. Overexpression of the DDAH-1 gene has been shown to improve cardiac stroke volume, reduce systolic blood pressure, and lower systemic vascular resistance. It also increases NO synthase activity, which has been documented. According to the findings of a research, the excessive synthesis of DDAH-2 in endothelial cells lowers levels of ADMA and increases the activity of eNOS that is produced by glycated protein. (Lee et al., 2021).

Imbalance in lipoprotein mechanism is a predominant cause and modifiable risk factor in coronary heart disease. It is reported that 56% of CHD occurs due to abnormal cholesterol worldwide (Vinay et al., 2020). The term dyslipidemia is coined for dysregulation in lipoprotein metabolism. In this context, it refers to increase in total cholesterol (TC) level, low-density lipoprotein (LDL) level, and triglycerides (TG) level, and reduction in high-density lipoprotein (HDL) level (Khan et al., 2021). Lipoproteins are classified into different types based on their density: chylomicrons, VLDL, intermediate-density lipoprotein (IDL), LDL and HDL. Substantial amounts of LDL and VLDL particles, sometimes known as "bad cholesterol" due to their association with a higher risk of heart disease, contain a large amount of cholesterol. Lipoproteins are responsible for transporting cholesterol throughout the body, however cholesterol itself is not a lipoprotein. In the early 20th century, researchers found that atherosclerotic plaque in aortic specimens had significantly higher cholesterol levels compared to plaque-free regions of the aorta (Shaya et al., 2022). High plasma LDL, IDL, VLDL, and Lipoprotein(a) levels promote thrombus and plaque formation, worsening CVD. Atherosclerotic plaques are caused by native LDL and oxLDL. Scavenger receptors on macrophages in atherosclerotic lesions such as SR-A1, CD36, and "lectin-like oxLDL receptor-1" recognise oxidation-specific epitopes on oxLDL such as cholesteryl ester modification, ApoB-100 modification, and PL oxidation and enhance the absorption of oxLDL, but they do not facilitate the uptake of native LDL. In vivo LDL oxidation occurs during a proinflammatory stage, although the mechanism is unknown. Liposomal acid lipase and acetyl-coenzyme A acetyltransferase (ACAT) convert

phagocytosed LDL by macrophages and modified form of LDL to free cholesterol, which is exported from the cells by cholesterol transporters. Modified LDL forms foam cells because macrophages cannot digest it. oxLDL and LDL aggregates increase atherosclerosis by stimulating arterial wall inflammatory cytokines and growth factors. S-adenosylmethionine-dependent methyltransferases activated by oxLDL produce asymmetric dimethylarginine, which decreases endothelial nitric oxide synthase and vascular tone. LDL and oxLDL increase foam cell, inflammation, and plaque development, promoting CHD. Hypertriglyceridemia diminishes hepatic TG lipase and HDL-binding protein 1 activity which is anchored by glycosylphosphatidylinositol, thus altering liver lipoprotein absorption and elevating plasma TG levels. 9-hydroxyoctadecadienoic acid and 13-hydroxyoctadecadienoic acid increase plaque formation, inflammation, and atherosclerosis by upregulating ROS, ICAM, and TNF via lipoprotein lipase (LPL) lipolysis (C. Zhu et al., 2019). HDL's major role is regarded to be reverse cholesterol transport, it is probable that its cardio protection is due to its involvement in other processes as well, such as the production of foam cells by macrophages, the activation of eNOS, monocyte adhesion, and platelet aggregation. The first and most important stage in reverse cholesterol transport, in which HDL picks up peripherally deposited cholesterol and transports it to the liver to be excreted, is HDL's capacity to accept cholesterol from macrophages (Cahill et al., 2019). In controlling concentration of plasma cholesterol, facilitating uptake of cholesterol by macrophages, making foam cells, and eventually resulting in formation of plaque and inflammation, the metabolism of lipids and liporproteins along with their transference are essential causative mechanisms of CVDs. Cardioprotective actions of lipids and lipoproteins include blocking the oxidation of proatherogenic compounds and suppressing the production of inflammatory proteins (Bhargava et al., 2022).

C-reactive protein, often known as CRP, is a kind of acute-phase reactant that is produced mostly in the liver. It is considered to be the most reliable indicator of systemic inflammation. CRP levels are raised in a variety of chronic non-communicable diseases. In addition, it contributes to the development of atherosclerosis by assisting in the process of the development of atheroma plaques as well as their rupture. CRP is currently a validated predictor of future cardiovascular events, with three relative CVD risk categories based on

high-sensitivity (hs) CRP serum concentrations: 1.0 mg/L corresponding to low risk, 1.0 to 3.0 mg/L corresponding to moderate risk, and >3.0 mg/L is designated as high risk of CVD (Da Venezia et al., 2021). Hepatocytes may be stimulated to produce CRP when exposed to inflammatory mediators that originate from periodontitis. Specifically implicated in this process are the mediators known as interleukin-1, interleukin-6, and tumour necrosis factor alpha. In this respect, periodontal infection may lead to systemic inflammation, which is often accompanied by a considerable rise in CRP levels (Esteves-Lima et al., 2020).

RESEARCH GAP/RATIONALE OF STUDY

The aim of this study is to examine the potential impact of ADMA on endothelial damage in individuals diagnosed with periodontitis, with the ultimate goal of identifying patients who are particularly vulnerable to cardiovascular disorders.

THEORETICAL GAP

The link between cardiovascular disease and periodontal disease has been explored in terms of inflammatory mediators that are produced as a result of oral pathogens that invade the endothelium subsequently and evoke an inflammatory response. However, the synergistic effect of oxidants and lipids in chronic periodontitis has not been elucidated in context to developing cardiovascular disease. Therefore, this study will help to explain the association between the two mechanisms thereby explaining the link between the above mentioned diseases.

CONTEXTUAL GAP

ADMA has been evaluated as a biomarker in the past in different regions of the world. But the levels varied due to geographical variations, genetic differences and life style. Moreover, the previous two studies conducted by Isola et al., 2020 and Sengul et al., 2022 had different findings due to varying severity of periodontal disease. Our study will validate either of the two studies in terms of establishing asymmetric dimethylarginine as a marker of identification of coronary heart disease in periodontitis patients in our population.

METHODOLOGICAL GAP

N/A

PROBLEM STATEMENT

Cardiovascular disease is the primary cause of mortality in the developing and already developed countries all over the world. The annual death rate has increased up to 17.9 million as reported by WHO report in 2019. On the other hand, prevalence of periodontitis has increased as reported by several local studies in Pakistan. The studies in the past has robust evidence that determine an association between the two diseases. Periodontitis increases the likelihood of an individual to develop cardiovascular events. The need to identify a common biomarker that can help identify the incidence of developing coronary heart disease is very important. The present study will help in early detection of coronary heart disease in periodontal patients through evaluation of ADMA in our geographical location.

HYPOTHESIS OF STUDY

- **ALTERNATE HYPOTHESIS:**

ADMA levels significantly increase in serum of chronic periodontitis (CP) and coronary heart disease (CHD) patients.

- **NULL HYPOTHESIS:**

ADMA levels do not significantly increase in serum of chronic periodontitis (CP) and coronary heart disease (CHD) patients.

OBJECTIVES

The objectives of this study are to:

- Determine the levels of serum ADMA levels in CP patients and CHD patients.
- Determine the levels of serum inflammatory mediators (hsCRP) in CP and CHD patients.
- Determine the levels of lipid profile (TC, TG, HDL, LDL) in CP and CHD patients.
- Determine the periodontal clinical parameters (BOP, PD, CAL, and PI) in CP and CHD patients.

SIGNIFICANCE OF STUDY

Our study focuses on early detection of cardiovascular diseases on the basis of serum ADMA levels in periodontal disease patients. According to the reported studies, the levels of ADMA are elevated in CHD population. To the best of our knowledge it will be the first study to assess the levels of ADMA in Periodontitis and Coronary heart disease patients.

Raised concentration of ADMA will validate the relation between periodontal and cardiovascular disease. It would be then advised to measure it in early stages of periodontitis to identify the risk of CVD in susceptible individuals. The study could be beneficial to reduce the burden of cardiovascular events in predisposed subjects. Similarly, adequate periodontal therapy could be provided to eliminate periodontitis thereby improving the inflammatory mediators in blood.

CHAPTER 2

LITERATURE REVIEW

Oral health is one of the essential components of general health. It is not possible to maintain a healthy system of the body without considering the health of oral cavity. Periodontal disease is a multifactorial inflammatory condition with several local and systemic risk factors playing an important part in its etiology and progression. Risk factors includes oral hygiene, smoking, ageing, genetics, gender, psychosocial stress, socioeconomic status, race, osteoporosis, osteopenia, and other clinical conditions including diabetes mellitus type 2 (T2DM) and obesity (Nagpal et al., 2015). It is characterized by the gradual loss of the tooth supporting structures and is caused by dysbiotic dental plaque biofilms, which commence the process. (Papapanou et al., 2018). Periodontitis represents one of the noncommunicable diseases that affects the most individuals worldwide. It is anticipated that 11.2% of the inhabitants suffers from severe periodontitis (Kassebaum et al., 2014). Around 45% of all adults in the UK are suffering from different periodontal stages and its occurrence is increasing with age (White et al., 2012). Periodontal disease is widely predominant in adult aged-populations with estimated prevalence of 50% in all parts of the world (Eke et al., 2012). Periodontal diseases represent 3.5 million years lived with disability (YLD) in the year 2016 (Tadjoedin et al., 2017). The incidence of chronic periodontitis was greatest (82%) in the elderly population, followed by (73%) adults, and (59%), teenagers in a recent epidemiological survey (M. Nazir et al., 2020). The more severe form of periodontitis is observed between the third and fourth decades of life, with an estimated global prevalence of 10% (Kassebaum et al., 2014). There are two types of PD: chronic periodontitis (CP) and aggressive periodontitis (AgP), which is a more severe and early-onset variant (Aarabi et al.,

2017). CP is the more common type. According to Frieden (2015), around 47% of persons in the United States aged 30 years have CP, with 30% having moderate and 8.5% having severe PD. According to Vieira and Albandar (2014), the genetic component of AgP is more significant than that of CP, despite the fact that AgP is far less common than CP (prevalence 0.1%). According to the outcomes of the Global Burden of Disease study, the prevalence of severe periodontitis over the world was 7.4% in 2015; however, the prevalence of periodontitis in its less severe forms may be as high as 50% (Billings et al., 2018). According to a research conducted by M. A. Nazir (2017), people who have periodontal disease have an increased risk of cardiovascular disease that is 19% higher than average, and this increase in relative risk rises to 44% among those who are 65 and older. Ayesha et.al executed a study where evidences were collected from various studies conducted in different parts of Pakistan. It was concluded that the total estimate of periodontitis was 56.62% (95% CI) at the national level (Fahim et al., 2022). It indicates that periodontitis is not caused simply as a result of plaque deposition but is also interrelated with several factors of the host which could modify the magnitude of plaque on a specific subject. Recent investigations have suggested the role of cytokines in response to the periodontal biofilms in a particular host which is similarly observed in a wide range of systemic conditions and diseases (Nagpal et al., 2015). It is estimated that between 45% and 50% of the world's population suffers from periodontitis, 11.2% of the world's population is affected with the disease's most severe form (Kassebaum et al., 2014). According to the 2017 Global Burden of illnesses, Injuries, and Risk Factors Study, among 354 illnesses and injuries across 195 countries, dental diseases (predominantly caries and periodontitis) caused the greatest YLD in terms of the age-adjusted prevalence rates (Vos et al., 2017). At present there is now a lot of evidence that serious periodontitis is linked to a number of NCDs, including diabetes, in a way that cannot be explained by chance (Chapple & Genco, 2013), cardiovascular disease (Tonetti & Dyke, 2013), chronic obstructive pulmonary disease (Linden et al., 2013) and chronic kidney disease (CKD) among the rest of them (Sharma et al., 2016). In fact, research on multiple populations have found that severe periodontitis is independently and strongly linked to both cardiovascular and overall mortality (Sharma et al., 2016). Possible causes include bacterial blood poisoning and the systemic inflammatory effects that follow, such as increased oxidative stress and C-reactive protein (Schenkein et al., 2020). Periodontitis has been linked to a considerable

decrease in survivability from death from all causes as well as death from cardiovascular disease in multimorbid groups, such as those with chronic kidney disease and concomitant diabetes (Sharma et al., 2016). Consequently, periodontitis may perhaps be an alternative, modifiable risk factor for cardiovascular disease. The American Academy of Periodontology and the European Federation of Periodontology organized a workspace in 2012 in order to discuss the links between systemic diseases and periodontitis including cardiovascular disease. The evidence demonstrating the epidemiological connections between periodontitis and emergent cardiovascular disease was examined in four separate technical articles, which were the basis for the consensus report, the biological acceptability of the links between periodontal bacteria and systemic inflammation, and the efficacy of periodontal interventions. The workshop came to the consensus that periodontitis raises the likelihood of atherosclerotic cardiovascular disease (ASCVD) in the future due to consistent and substantial epidemiological data. Even though in an artificial environment, experimental, and clinical studies confirmed the interface and the biological processes that went along with it, there weren't enough intervention trials at the time to draw any further conclusions. But it was decided that the link between periodontitis and CVD was scientifically possible. This was done through translocated circulating mouth bacteria, which may cause systemic inflammation that affects the development of atherothrombogenesis (Sanz, Marco del Castillo, et al., 2020).

Cardiovascular disease (CVD) is a multi-factorial disease found in middle-aged group over 55 years old. It has a high morbidity, and mortality rate, which exposes individuals to serious health consequences. They are one of the prominent causes of fatality globally. The rate of deaths from cardiovascular disease is more than 17 million, ranking primary among all other reasons of the death. Heart failure (HF), stroke, atrial fibrillation (AF), Ischemic heart disease and cardiomyopathy are accountable for greater than 95% of cardiovascular deaths (Zhou et al., 2021). In recent years, even though the mortality rate of CVD has reduced by 27.3%, the casualties have rose by 42.4% during the years of 1990 and 2015 (Mensah et al., 2015). The greatest cause of death and morbidity around the world continues to be coronary heart disease (CHD). Despite the fact that deaths from CHD has decreased in the United Kingdom, patients who had a non-fatal occurrence of CHD are now more likely to

suffer from multimorbidity and comorbidity. According to an analysis, the prevalence of CHD in the United Kingdom is roughly 4% in Wales, Scotland and Northern Ireland and 3% in Britain (Bhatnagar et al., 2016). Age, smoking, hypertension, and hypercholesterolemia are only a few of the risk elements for CVDs (including coronary heart disease) that have been identified through epidemiological studies. The discovery of such risk variables has sparked the creation of algorithms for the purpose of risk prediction and developing established models of cardiovascular risk for both males and females. At least a quarter of all potential future occurrences happening in people with just one of the traditional risk elements, these orthodox risk variables, nevertheless, cannot fully explain the increased cardiovascular risk. (Winning et al., 2020).

In terms of global mortality and morbidity, coronary artery disease (CAD) is first. By 2020, cardiovascular illnesses, especially atherosclerosis, are expected to overtake all other causes of death worldwide. Myocardial infarction and angina pectoris are both brought on by atherosclerosis of the coronary artery. Nitric oxide (NO), released by a healthy and functional endothelium, is crucial to the upkeep of vascular tone and architecture. Dysfunction of the endothelium, the first phase in the course of atherosclerosis, is caused by a drop in NO levels. As a structural analogue of L-arginine, endogenous asymmetric dimethylarginine (ADMA) reduces nitric oxide (NO) by competitively inhibiting the enzyme nitric oxide synthase. Serum ADMA/NO was investigated as a potential measure of CAD severity. A patient's risk of angina pectoris or myocardial infarction due to the severity of coronary atherosclerotic block may be better predicted by the serum ADMA/NO ratio than by individual blood ADMA levels (Shivkar & Abhang, 2014). ADMA was proven to be an effective measure for predicting subclinical atherosclerosis in a study conducted in Turkey. In addition, an ADMA cut-off value of 0.71 mol/L can aid in the identification of individuals who may benefit from preventative methods to lessen the progression of atherosclerosis (Gürel et al., 2015). Endothelial dysfunction and atherogenesis are linked to increased serum concentrations of asymmetric dimethylarginine. Serum levels of asymmetric dimethylarginine (ADMA) were compared to the occurrence of coronary atherosclerosis and the severity of the disease in individuals suspected of having coronary artery disease. It was first shown that ADMA levels are elevated in the presence of functionally significant stenosis, as measured by fractional

flow reserve (FFR), and a study conducted in Belgium validated this link. The elevated cardiovascular risk linked with high ADMA levels was explained by the identification of those patients with substantial and functionally significant atherosclerotic load (Mangiacapra et al., 2016). ADMA concentration in serum may be helpful in determining if coronary artery disease (CAD) corresponds significantly with the occurrence of coronary atherosclerosis and the severity of the disease in individuals, according to the findings of a separate study conducted in India. Since ADMA is linked to the severity of coronary atherosclerosis, it can serve as a prognostic marker (Goudhaman et al., 2021). In a 2021 analysis, Gao et al. observed a modest association between periodontitis and a higher risk of coronary heart disease in Chinese adults of middle age and older. However, demographic factors for instance gender, age, diabetes status, hypertension history, uric acid levels, and level of education can account for the vast majority of the correlation (Gao et al., 2021). Atherosclerotic load and Carotid intima medium thickness (CIMT) were shown to be strongly connected with each other in a study conducted in Turkey (Can et al., 2014). A few assessments done around the shared ailments among adult population of Pakistan take account of 41% hypertension, 21% obesity, 21% tobacco use, dyslipidemia (females 49%; males 34%), 17.3% high cholesterol, 8 10% diabetes mellitus (DM), and 16 and 2.8% stroke (Turin et al., 2013). It was reported 44.87% individuals belonging to Urdu speaking community had ≥ 7.5% chance of developing atherosclerotic events in around 10 years followed by Punjabi individuals with 19.23%; furthermore, high-risk people were in the minimum proportion in lieu of Sindhi community in their study of non-atherosclerotic Pakistani individuals. It is thereby concluded that Punjabi and Urdu speaking community have an increased tendency to develop atherosclerotic cardiovascular diseases (ASCVDs) as compared to other ethnic subgroups in Pakistan (Ashraf et al., 2016).

Periodontal disease has been linked to cardiovascular diseases since two decades. Both conditions share the same etiology and risk factors for example sex, age, blood pressure, diabetes, smoking, obesity, cholesterol levels, dietary patterns, race etc. The main presumed facts that suggest the biological link between systemic conditions and periodontitis and are (a) presence of infection in both diseases, (b) low-grade endotoxins and bacterial toxins in the blood caused by diseases of the periodontium, (c) systemic inflammation and immune

responses elicited by periodontal pathogens, (d) periodontal pathogenic organisms expressing virulence factors, and (e) presence of periodontal pathogens in non-oral parts of the body such as atheromatous plaques (Nagpal et al., 2015). The organisms involved in the pathogenesis of periodontitis include Porphyromonasgingivalis, Prevotella intermedia, Campylobacter rectus, Aggregatibacter, Tannerella forsythia, Fusobacteriumnucleatum and actinomycetemcomitans (Schulz et al., 2020). They are found in the dental plaque which deposits on the surface of the tooth. The mode of transportation of bacteria is the sulcular epithelium which is thin and epithelium of the oral cavity which is densely vascularized. The bacteria transcend the vasculature of the oral cavity and passes into the bloodstream to initiate inflammatory cascade in the arterial wall. Innate and adaptive immunity is triggered; through cells and cytokines which leads to oxidative stress production. Besides low density lipoproteins (LDL) gets oxidized which adds to atheromatous plaque formation. Antibodies in contrast to periodontal pathogenic organisms cross react with components of the intimal wall (tunica intima) due to similar molecular mechanisms causing mass destruction and promoting plaque progression. When the inflammation persists for a long time then it starts to destabilize the atheromatous plaque which then ruptures leading to cerebrovascular events and acute coronary syndromes (Carrizales-Sepúlveda et al., 2018). Multiple pathways, both direct and indirect, have been identified connecting periodontitis and CHD. It is generally agreed that atherosclerosis is the most prevalent pathophysiological substrate of CHD and that the illness can be best described as a persistent low-grade inflammatory syndrome (Golia et al., 2014) . Chronic periodontitis has been linked to low-grade inflammation that adds to the systemic inflammatory burden. An etiological association between periodontitis and CHD has been postulated due to data linking chronic inflammation to the development of CHD. Another possible reason for the link between periodontitis and CHD is the direct participation of periodontitis associated pathogenic bacteria in atherosclerosis (Mougeot et al., 2017). A link between periodontitis and incident coronary heart disease has been supported by various systematic evaluations from an epidemiological standpoint. People who had periodontitis had a considerably elevated risk of CHD incidents independent of known confounders, according to the findings of a meta-analysis that encompassed around 15 studies (Leng et al., 2015). Conversely, the association has not been consistently found in all of the studies, specifically if the group of participants has been categorized by age. Moreover, there

does not seem to be any evidence to support any kind of connection in those individuals over 65 years old. (Dietrich et al., 2013). Periodontal disease and coronary artery disease both have an inflammatory origin as the cause; there has recently been some worry about the examination of a link between these two illnesses. According to current viewpoints, cytokines and proteins play a crucial part in this development. Interleukin-6 and C-reactive protein are byproducts of inflammation that are synthesized as a result of periodontitis and in the course of coronary artery disease, respectively. The SNPs CRP + 1444 C > T and IL6-174 G > C have been linked to CAD in the literature. The existence of PCR and IL-6 polymorphisms was used to evaluate the relationship between periodontitis and coronary artery disease. The occurrence of the PCR + 1444 C > T mutation was found to be independently linked with CAD (Rocha et al., 2020).

Periodontal disease has been linked to angiographically validated coronary artery disease (CAD), according to a study conducted in Croatia. In individuals suffering from Acute Coronary Syndrome (ACS) and stable CAD, physical inactivity, poor oral hygiene, and periodontal inflammation were identified. Almost two-thirds of the patients examined had periodontitis, which puts them at risk of developing CAD (Vražić et al., 2015). In Pakistan, the link between individual periodontal characteristics and distinct systemic biomarkers was studied. In individuals who already had coronary heart disease and who had periodontitis, this research examined the connection between several clinical manifestations of periodontitis and circulating biomarkers of CHD risk. Bleeding on probing, a clinical indicator of inflammation of periodontal tissues, is most closely linked to CRP which is a biomarker for systemic inflammation that also plays a role as a risk factor for coronary heart disease. Patients with coronary illness who also had periodontitis showed increased signs of periodontal inflammation, which suggested that their systemic levels of risk biomarkers for coronary disease were enhanced (Bokhari et al., 2014). There was a study carried out which explored the relationship between periodontal disease and incident CHD. Incident CHD has been linked with a particular stage of periodontal disease that results in extensive tooth loss, but none of the CDC/AAP categories were significantly associated (Beck et al., 2020). A study conducted in Japan looked at the relationship between tooth loss, periodontal disease, and coronary heart disease. For the first time, a substantial link between a higher figure of

missing teeth and vascular obstruction was discovered, even after correcting for key confounders. It was also shown that tooth loss and coronary heart disease were linked to elevated levels of CRP concentrations, implying that the greater the number of teeth lost, the higher the concentration of CRP (Zanella et al., 2016). In Turkey, a study was done to evaluate the link between oral health (periodontally healthy/gingivitis, periodontitis, and edentulism) and the degree of CAD as determined by coronary angiography in a relatively large population. They discovered that periodontitis and edentulism were significant risk factors for CAD, and that the degree of CAD was also significantly associated with periodontitis and edentulism (Bilgin Çetin et al., 2020).

Asymmetric dimethylarginine (ADMA) is an analogue of amino acid L-arginine; produced by its post translational modification. It inhibits endogenous nitric oxide synthase (NOS) in the endothelial cells (Németh et al., 2017). By doing so it leads to vasoconstriction, elevated blood pressure, increased endothelial cell adhesions and impairs endothelium mediated relaxation (Sitia et al., 2010). L-arginine undergoes methylation on its guanidine residue of the side chain forming a covalent bond. S-adenosyl methionine (SAM) acts as a methyl donor. These methylated analogues of arginine are not the methylation of one part of arginine residue but mainly the breakdown of methylated side chains of amino acids in a protein molecule. Following methylation, these derivatives of arginine are released into plasma as asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and monomethyl arginine (MMA). PRMTs, short for protein arginine methyltransferases, are the enzymes responsible for catalyzing this process. PRMT-I expression is increased due to stress and oxidized low density lipoproteins (ox-LDL) as explained in hypercholesterolemia. ADMA is degraded to dimethylamine and citrulline by the help of enzyme dimethylarginine dimethylaminohydrolase (DDAH). Impairment in DDAH function in kidneys leads to elevated concentrations of ADMA (Dowsett et al., 2020). A systematic analysis was done to obtained a reference value of plasma ADMA in systemically healthy individuals. ADMA was reported to have reference value in between 0.34-1.10 with a mean value of 0.71 estimated by High-performance liquid chromatography (HPLC) and 0.25-0.92 with a mean of 0.57 by Enzyme-linked immunosorbent assay (ELISA) (Németh et al., 2017). Angiogenesis is regulated by ADMA through ADMA/DDAH pathway (Wieczór et al.,

2018). A study conducted in Italy on CP and CHD subjects concluded that; he found out ADMA levels were significantly raised in coronary heart disease and the coexistence of coronary heart disease and chronic periodontitis patients than CP patients and controls (Isola, et al., 2020). On the other hand, it was discovered that ADMA levels were significantly higher in the periodontitis group in comparison to the controls. (Şengül et al., 2022). An investigation was carried out on the levels of nitric oxide levels in periodontal disease and their association with CRP and lipid profile (cholesterol, triglycerides and lipoproteins). He found out that periodontal subjects had low NO metabolites and significantly elevated cholesterol, LDL and CRP than the control group (Andrukhov et al., 2013). It was determined that adrenomedullin (AM) and iNOS (Inducible Nitric Oxide Synthase) were three times higher in inflamed gingival tissues than healthy ones. NO and AM concentrations in saliva was elevated in aggressive periodontitis subjects than gingivitis and chronic periodontitis. On the contrary, NO levels determined in gingival crevicular fluid were markedly increased in all the three stages of periodontitis while AM were considerably higher in aggressive and chronic periodontitis subjects whereas lower in gingivitis patients (Hussain et al., 2015). It was first predicted by Ozer et al., (2011) that nitric oxide (NO), ornithine decarboxylase (ODC) and arginase were in higher concentrations in early periodontal lesion than advanced periodontal lesions (Ozer et al., 2011). Another study concluded that periodontal disease parameters are associated with raised ADMA concentration irrespective of low or high hsCRP levels suggesting that there must be alternate pathways which connects endothelial dysfunction and damaged periodontal tissue (Tsioufis et al., 2010). A randomized, double blind clinical trial was conducted to evaluate the levels of nitric oxide (NO) in GCF of CP subjects who were receiving nutritional supplements that include probiotics in addition to initial periodontal therapy. He concluded that nitric oxide levels in gingival crevicular fluid were markedly reduced in the test group compared to the control group up to 6 months of follow-up (Kuka et al., 2019). A trial study was conducted to bring into light the role of Endothelin 1 (ET-1) being one of the key players in relationship between periodontitis and coronary heart disease (CHD). He found out that individuals with coronary heart disease and periodontitis had elevated salivary and serum levels of ET-1 as compared to individuals with periodontitis and normal systemically healthy subjects Furthermore, CRP alone continued to

be the most important prognosticator of raised Endothelin-1 levels in serum and saliva both (Isola et al., 2020).

Dembowska et al. (2022) did a study to find out what similar risk factors there were for periodontitis and acute cardiac syndrome in the Polish community. He found that patients suffering from hypercholesterolemia had more severe inflammation of periodontium as measured by CAL and Community periodontal index (CPI) values, than those without hypercholesterolemia, and that lesser TC levels associate with comparatively less severe form of periodontitis. Furthermore, it was found that people with more progressive periodontitis had a greater amount of heart veins affected by atherosclerosis. This proves that periodontitis is bad for your overall health. In order to better understand the possible causative connection between periodontal disease and cardiovascular diseases (CVDs) in Thai adults, researchers decided to conduct a cohort study with the goal of prospectively evaluating the association between the two conditions. It has been proven that severe periodontitis is concomitant to an augmented incidence of coronary heart disorders, and this link exists regardless of recognized risk factors for cardiovascular disease. According to the findings of this prospective cohort analysis, there may be a causal association between periodontitis and CHD; however, this relationship does not exist for total CVD events or stroke (Tiensripojamarn et al., 2021). Based on the conclusions of the Polish study, people having sudden myocardial infarction had worse gum health than people without coronary heart disease. Acute cardiac infarction was linked to more severe periodontitis, more plaque buildup, and bleeding when the gums were probed. Periodontitis is a risk hazard for MI and also affects the amount of damage of left ventricles after a myocardial infarction. This means that there is a suspected inflammatory relationship between these two diseases. (Wojtkowska et al., 2021). A protein known as periostin is necessary for maintaining the integrity of periodontal tissues as well as their development and maturity. In order to investigate the importance of gingival crevicular fluid (GCF) periostin levels in the connection between CP and CHD, a study was carried out. Patients with coronary heart disease had reduced periostin levels, particularly those in the CHD CP group. It was hypothesized that periostin could play a part in the connecting mechanism that holds CHD and CP together as a relationship. Periostin has the potential to be both an effective CP preventive drug and a promising

biomarker for the identification of congestive heart failure in CHD patients (Rezaei et al., 2021). Researchers in the province of Zhejiang in China examined the levels of four traditional pro-inflammatory cytokines both systemically and locally (MCP-1, IL-1, VEGF, and PDGF) in people who had periodontitis and coronary heart disease (CHD). Their findings revealed that the inflammatory cytokines IL-1, MCP-1, and VEGF may have a function in the pathophysiology of CHD and chronic periodontal disease and that they can be employed as biomarkers. This was suggested by the fact that these cytokines were found to be present (H. Zhu et al., 2015). The results of a research that was carried out in China indicate that there may be a correlation between periodontitis and coronary heart disease (CHD), and the IL-23/IL-17 axis may possibly play a crucial part in the pathological process that both diseases undergo. Additionally, it was suggested that periodontitis could be a risk element for CHD (Qi et al., 2013). Malondialdehyde (MAA) is a byproduct of a lipid pathway that has been demonstrated to play a crucial part in dysfunction of endothelium, which in turn contributes to the progression of periodontitis and CHD. A study that was carried out in Italy discovered that patients with periodontitis and CAD had an elevated concentration of serum and salivary malondialdehyde than periodontitis subjects and healthy individuals, the fact that periodontitis and coronary artery disease functioned as an inducer to increase serum MAA levels through a mechanism that is regulated by hs-CRP. It also altered the amounts of MAA in the saliva. The study also found that periodontitis patients and healthy subjects had lower salivary and serum concentrations of MAA. Additionally, the study found that periodontitis and coronary heart disease both serve as a stimulation that raises blood MAA levels more than in healthy persons. It has been shown that MAA plays a role in the immunological response that occurs during periodontitis by means of the inflamed endothelium and the heat shock proteins contained inside it. These proteins are located on the surface of the endothelium and eventually excite some IgG, with a preference for antibodies that are triggered by the host. The induction defense mechanism, which is mediated by NO, is also affected by this process, which is controlled by MAA and other lipid indicators. This mechanism promotes endothelial cell excitability, which in turn raises the possibility of farther infection or inflammation throughout the body that is induced by periodontal disease (Isola et al., 2019).

The affiliation of coronary heart disease with periodontitis has been investigated in terms of various biomarkers. Similarly, when referring to the preclinical phases of a broad variety of inflammatory disorders, galectins play an important part as important regulators of cell surface activities and signaling. In Italy, a clinical study was performed to investigate the relationship and implications of periodontitis and coronary heart disease (CHD) in relation to Galectin-3 in individuals with coronary heart disease and periodontitis. It was discovered that individuals with periodontal disease and coexistence of periodontitis and coronary heart disease had considerably raised salivary and serum Galectin-3 levels than coronary heart disease patients and healthy individuals (Isola et al., 2021).

In order to explore the part that ADMA offers in the development of coronary artery disease (CAD) in people who have type 2 diabetes, a study was conducted on the population of Pakistan. All of the clinical (blood pressure and body mass index) and biochemical (blood glucose, total cholesterol, glycosylated hemoglobin, high-density lipoprotein, triglyceride, low-density lipoprotein, and asymmetrical dimethylarginine) indicators were found to be higher in diabetic patients who also had coronary artery disease (CAD). There was a substantial association between elevated serum ADMA levels and other biochemical indicators, with the exception of HDL-C, among patients who fit this description (Tariq & Khan, 2016). According to the findings of a research project that was carried out in Pakistan, serum ADMA levels were shown to be significantly higher in both the groups having disease, diabetic mellitus type 2 with CAD and diabetic mellitus type 2 without CAD, in contrast to those who are typically in good health. In addition, there was a strong positive association between ADMA concentrations and fasting blood glucose levels, glycosylated hemoglobin levels, TC levels, HDL-c levels, and TG levels in individuals with type 2 diabetes who did or did not have coronary artery disease (CAD). In addition, our results suggest that blood ADMA levels have a substantial role in the onset and development of CAD as well as the advancement of CAD (Tariq et al., 2017). Researchers have found a correlation between chronic periodontitis and the initiation of stable coronary artery disease. The purpose of the present study was to investigate the possible influence that periodontitis may play, if any, in the progression of CHD. Patients who have been diagnosed with stable coronary artery disease are the ones who have been shown to have a correlation between high levels of

cardiovascular markers and active periodontal inflammation and tissue disintegration such as blood fibrinogen, hsCRP, and lipids. This is in comparison to healthy individuals, who have lower levels of all three of these factors (Sanikop et al., 2022). In individuals who had periodontitis, coronary heart disease, or combined disease researchers looked into the role that asymmetric dimethylarginine, coenzyme Q10 and 3-nitrotyrosine, and played in oxidative/nitrosative stress and endothelial dysfunction. It is likely that there is a causal relationship between oxidative stress, higher levels of NT and ADMA, and the stimulation of the inflammasome, each of which might have had a role in the beginning and progression of CHD in patients with periodontal disease. This is because oxidative stress, raised levels of nitrotyrosine and ADMA, and the stimulation of the inflammasome are all associated with CHD. (Ferlazzo et al., 2021). ADMA was used in a study that was carried out in Germany, and the results showed a correlation between the severity of periodontal disease and endothelial dysfunction. According to the findings, periodontal inflammation has an effect on both the levels of nitric oxide synthase and methylated arginine metabolites that are found locally as well as those that are found systemically. It shed light on the connection between periodontal disease and the health of the body as a whole (Şengül et al., 2022).

A number of individuals with periodontal conditions have been found to have higher blood hs-CRP, which is a validated inflammatory test for cardiovascular risk assessment. This is something that has been noticed in these people. Although we did not find evidence linking risk classification based on high-sensitivity CRP and periodontal conditions in this study's sample of adult Chilean women, we did find a robust correlation between serum levels of CRP in gingival crevicular fluid, particularly in periodontitis, suggesting its potential relevance as an alternative non-invasive screening approach for systemic inflammation. (Da Venezia et al., 2021).

OPERATIONAL DEFINITIONS

1. CHRONIC PERIODONTITIS (CP)

It is a chronic inflammatory condition of periodontium caused by disruption of oral microbiota leading to a cascade of proinflammatory events including cells and mediators from adaptive and innate immunity. These series of events lead to prolonged inflammation of soft and hard tissues of the oral cavity (Cardoso et al., 2018).

2. CARDIOVASCULAR DISEASE (CVD)

Cardiovascular disease (CVD) is a group of disorders that involves both the blood vessels and heart, thus comprising coronary artery disease (CAD), coronary heart disease (CHD) and acute coronary syndrome (ACS) among a number of other conditions (Sanchis-Gomar et al., 2016).

3. CORONARY HEART DISEASE (CHD)

Coronary heart disease is a condition in which there is a blockage or narrowing of the coronary arteries. It is typically caused by atherosclerosis. It is a phenomenon in which fatty deposits and plaque are formed inside the coronary arteries (Vinay et al., 2020).

4. ATHEROSCLEROSIS

Atherosclerosis is a chronic, progressive illness that takes years to develop. It is distinguished by the presence of necrotic cores, calcified sections, changed lipid deposits, and inflammatory smooth muscle cells(SMCs), leukocytes, endothelial cells and foam cells

in the arterial wall. The atherosclerotic plaque begin as adipose streaks and progress to pathologic lesions as a consequence of genetic makeup and lifestyle changes (Mehu et al., 2022).

5. ASYMMETRIC DIMETHYL ARGININE (ADMA)

Asymmetric dimethyl arginine is synthesized from post translational modification of amino acid L-arginine. It is found in human plasma in small amounts. When it surpasses the limit of physiological concentration in which it is synthesized then it leads to various abnormalities in the body primarily affecting the cardiovascular system by inhibiting endogenous nitric oxide synthesis (Németh et al., 2017).

6. BLEEDING ON PROBING (BOP)

Bleeding occurs as a result of inflammation or ulceration inside a pocket. A BOP-positive [BOP (+)] site may point toward the existence of an inflammatory factor such as calculus (Räisänen et al., 2021).

7. CLINICAL ATTACHMENT LOSS (CAL)

It denotes the level of periodontal support that has been lost around a tooth. It is estimated with the periodontal probe as the distance from the cement-enamel junction (CEJ) to the base of the pocket. It can be assessed by adding probing depth to gingival recession (Zhao et al., 2019).

8. GINGIVAL RECESSION

The apical movement of the gingival margin towards the cementoenamel junction is termed as gingival recession. Subsequently the migration of the gingival margin towards apex is also accompanied with the injury to other components of the periodontal apparatus, it can be referred as “periodontal recession” (Recession & Considerations, 2018).

9. PROBING DEPTH (PD)

It is the most effective way to evaluate periodontal health. It measures clinical parameters such as gingival index (GI), level of clinical insertion (LCI) and clinical probe depth (CPD). These recordings are done by the use of a millimetric periodontal probe which is inserted in the gum supposing that in the measurement of the CPD the probe recognizes the most apical cells of the junctional epithelium (Reddy et al., 2020).

10. PLAQUE INDEX (PI)

The plaque index developed by Silness and Loe (1967) is a widely used index for evaluating plaque on the tooth surface. It uses an estimation of tooth area concealed by plaque. It scores plaque on the gingival third of the following tooth surfaces (buccal, lingual, mesial and distal) (Al-Asmar et al., 2023)

CHAPTER 3

METHODOLOGY

3.1 STUDY DESIGN

Case-control study

3.2 SUBJECT

88 individuals both males and females between the age group 30-60 years were enrolled in the study. Among them 66 individuals had established periodontal disease and coronary heart disease while remaining 22 individuals were systematically healthy people.

3.3 SETTING

This study was conducted in Biochemistry department of Bahria University Health Sciences Campus Karachi (BUHSCK) in collaboration with Periodontology out-patient department of Bahria University Dental College Hospital, Cardiac Care Unit (CCU) of PNS Shifa Hospital and Multidisciplinary Research Laboratory of Bahria University Health Sciences Campus Karachi (BUHSCK).

3.4 INCLUSION CRITERIA

The groups were selected on a prespecified age range (30-60 years).
The inclusion criteria for the Chronic Periodontitis (CP) group was:

- Presence of a minimum of 16 natural teeth
- Presence of at least 35% of sites with clinical attachment level (CAL) ≥ 3 mm
- Presence of probing depth (PD) ≥ 4mm
- Presence of ≥40% sites with bleeding on probing (BOP)

The inclusion criteria for the Coronary Heart Disease (CHD) group was:

- Individuals aged in between 30-60 years old with a diagnosis of Coronary Heart Disease (CHD) which included both stable ischemic disease and acute coronary syndrome (ACS)
- ≥50% of stenosis of at least one coronary artery verified by coronary artery bypass graft surgery (CABG), coronary angiography or current percutaneous coronary intervention (PCI)

The patients were asked about their underlying medical conditions, medications, cardiovascular risk factors, electrocardiography, echocardiography and coronary angiogram.

The inclusion criteria for chronic periodontitis and coronary heart disease group (CP+CHD) was based on the similar criteria of the individual CP and CHD groups.
The individuals for control group were

- Systemically healthy individuals
- No sites with PD ≥4 mm or CAL ≥ 4 mm
- ≤10% sites with BOP
- No radiographic findings of alveolar bone loss

3.5 EXCLUSION CRITERIA

The exclusion criteria for all the patients were:

- Patients suffering from Diabetes mellitus, Insulin resistance, Chronic kidney disease, Systemic lupus erythematosus (SLE).
- Patients taking contraceptives
- Patients on immunosuppressive drug therapy, antibiotics, or anti-inflammatory drugs since the last 3 months prior to enrollment in study
- Pregnant or lactating females
- Individuals having previous history of smoking or alcohol consumption
- Patients who had received periodontal therapy in the preceding 3 months of study
- Patients using medications that may cause gingival hyperplasia such as Cyclosporin A, Hydantoin, Nifedipine, or similar drugs

3.6 DURATION OF STUDY

Total duration of study was 6 months after approval from Bahria University Health Sciences Campus and Dental College Faculty Review Committee (FRC) FRC-BUHS-50-2022-508, 18/11/2022) and Ethical Review Committee (ERC) (ERC 04/2023, dated 27/1/23).

3.7 SAMPLE SIZE ESTIMATION

The sample size estimation was based on the primary outcome variable, which was represented by ADMA levels. Assuming an effect size of 0.379, an a priori power of 0.80, an alpha level probability, and a number of groups equal to 4, the sample size calculation using G Power version 3.1.9.2 (performed by mean of F test, one-way ANOVA with fixed effects) indicated that a minimum of 88 subjects divided into four groups were required. Each group had 22 individuals. [Reference article: Changes in the biomarkers of

oxidative/nitrosative stress and endothelial dysfunction are associated with cardiovascular risk in periodontitis patients. Current Issues in Molecular Biology, 43(2), 704-715]

3.8 SAMPLING TECHNIQUE

Consecutive sampling technique

3.9 HUMAN SUBJECTS AND CONSENT

Total number of patients enrolled in the study were 88 among which 22 patients were allocated to CP group, 22 patients to CHD group, 22 patients to CP+CHD and 22 systematically healthy individuals to control group. Written informed consent was obtained from each participant prior to enrolment in the study (Appendix-C).

3.10 MATERIALS

3.10.1 EQUIPMENT

3.10.1.1 DENTAL UNIT

The most fundamental piece of equipment needed to perform any dental surgery is the dental unit. It is made up of particular components that give drill attachments to the necessary electrical and air-operated mechanics. The seven components of a dental unit chair are the lamp, patient seat, foot controller, water dispenser and cuspidor, monitor, bracket table and controller, and dentist chair (Son et al., 2022). In the Bahria Dental Hospital,

patients were examined and samples were taken using a dental unit (WestTech, model: classic) (Figure 3.1(a))

3.10.1.2 WEIGHING MACHINE

- A calibrated digital scale (ZT-120) was used to collect weight measurements. The scale was tested for accuracy and recalibrated prior recording each measurement.
- The participants of the study were asked to remove shoes, heavy outerwear, and any objects from their pockets before the measurement. They were instructed to stand upright in the center of the scale platform with their weight evenly distributed on both feet.

3.10.1.3 HEIGHT MACHINE

- Height measurements were obtained using a stadiometer (ZT-120). Before each set of measurements, the stadiometer was adjusted and leveled. Each participant was instructed to take off their shoes and stand barefoot against the stadiometer with their feet flat on the floor, their backs straight, and their heels together.
- Participants were instructed to keep their heads in the Frankfurt horizontal plane and look straight ahead.
- The participant's height was recorded to the closest 0.1 centimeter after which it was converted to meter by dividing the length in centimeters by 100.

3.10.1.4 BLOOD PRESSURE MACHINE

- Blood pressure was measured using a calibrated mercurial sphygmomanometer (Yamasu Model-600, Japan) in accordance with the American Heart Association (AHA) recommendations.

- The participants sat comfortably, with their backs supported, and feet flat on the floor. The cuff size was determined by the participant's arm circumference.
- The cuff was worn on the upper arm, 2.5 cm above the antecubital fossa. The arm of the subject was supported and positioned at the level of heart.
- The pressure in the cuff was gradually increased until it exceeded the anticipated systolic pressure. The pressure was gradually released, and the pressure gauge was observed while listening to the Korotkoff noises using a stethoscope.
- The systolic blood pressure was measured by the appearance of clear, repetitive tapping noises (Korotkoff Phase I). The diastolic blood pressure was measured as the complete cessation of noises (Korotkoff Phase V).

3.10.1.5 TORNIQUET

A tourniquet is a tool that doctors use to momentarily prevent blood from flowing to a particular region of the body. During blood collection (phlebotomy), a tourniquet is used to make veins stand out and make venipuncture easier. By putting a tourniquet around the area where the needle will be inserted, blood flow is briefly cut off which makes the veins fill up and easier to reach.

3.10.1.6 SYRINGE

It is particularly useful to draw small quantities of blood for laboratory analysis. In our study phlebotomy was performed using a 5cc syringe.

3.10.1.7 VACUTAINER

A vacutainer is a blood collection tube system brand that is commonly used in medical settings for blood sampling and specimen collection. Its primary function is to aid in the safe

and efficient collection, storage, and transportation of blood samples for diagnostic purposes. A yellow top vacutainer of 5 ml was used for blood collection. In this type of vacutainer, the gel separator is placed. After centrifugation, a gel barrier is formed in the tube that separates the serum from the cellular components of the blood. It aids in the preparation of clear, separated serum for laboratory examination. The clot activator encourages coagulation, allowing the blood sample to clot prior to centrifugation (Figure 3.1(b))

3.10.1.8 MICROPIPETTE

A micropipette is a typical device used in laboratories that is used to measure small quantities of liquids with a volume range between 1 and 1000µl. In addition to this, a micropipette may be used to move an exact quantity of liquid from one container to another. We used micropipettes of 100µl to dispense the analyte, standards and substrate solution (Figure 3.1(c))

3.10.1.9 PIPETTE TIPS

Pipette tips are autoclavable disposable tools for transferring liquids with a pipette. The yellow pipette tips were used to accurately pipette liquids with volumes between 10 and 200 µl (Figure 3.1(c))

3.10.1.10 EPPENDORF TUBES

Eppendorf Tubes are polypropylene tubes that can be used only once for storing and transporting solid and liquid samples and reagents, as well as for mixing, centrifuging, and transporting them. A 1.5 ml eppendorf tube was used to prepare serum aliquots after centrifugation (Figure 3.1(d))

3.10.1.11 ULTRA LOW TEMPERATURE FREEZER

Long-term sample preservation is possible in ultralow-temperature upright and chest freezers because of their superior insulation and cabinet- and door-mounted gaskets. (Gumapas & Simons, 2013). Samples were stored in a VWR® ULT Upright Freezers (40086A-VWR International, USA) which is set at –86 °C to maintain their integrity and stability for a long term sample storage. Serum aliquots were appropriately labeled and securely sealed in eppendorf tubes prior to placement in the cabinet inside the freezer. They were placed in the designated storage boxes within the ultra-low temperature freezer (Figure 3.1(e))

3.10.1.12 CENTRIFUGE MACHINE

Centrifuge machine is a device that generates centrifugal forces required for the purpose of separation of various substances on the basis of their density. The samples were centrifuged at 3500rpm set at 20°C for 3 minutes prior to biochemical analysis using a centrifuge (Beckman Coulter Allegra® X-12, USA) (Figure 3.1(f))

3.10.1.13 VORTEX

A vortex is a piece of machinery that enables for the consistent mixing of different substances. Prior to biochemical analysis through ELISA machine the serum sample taken inside the eppendorf tubes was homogenized using a vortex (S02000-230V-EU) (Figure 3.1(g))

3.10.1.14 LABORATORY DRYING OVEN

Thermostatic lab dry oven is a device designed for low-temperature thermal treatments like heating, drying and testing at low temperatures in a setting with air flow. The microplate was incubated for 60 minutes at 37°C to allow the ADMA analyte to bind to the anti-ADMA antibody which is serving as the capture antibody. The microplate was incubated further after addition of substrate solution A and substrate solution B for 10 minutes at 37°C using a thermostatic laboratory drying oven (DNO9055-Leaidal Medical, England) (Figure 3.1(h))

3.10.1.15 ELISA MICROPLATE READER

ELISA plate reader is a device which detects and measure the changes in color in each well of the plate. They perform spectrophotometry, which is the measurement of the amount of light absorbed and reflected by an item, such as a protein. After addition of stop solution to the wells, the readings were obtained using microplate reader Synergy™ HTX Multi-Mode Microplate Reader (VT 05404-0998-BioTek, USA) set to 450 nm within 10 minutes (Figure 3.1(i))

3.10.2 INSTRUMENTS

- Dental examination set (mouth mirror, sickle probe)
- William’s periodontal probe

3.10.3 KITS

Human asymmetric dimethylarginine ELISA Kit (Cat.No E1887Hu) for quantitative detection of asymmetric dimethylarginine (ADMA) in serum (Figure 3.2(b))

Figure 3.1(a) to (i) Equipment

(a) Dental Unit (Periodontology Department of Bahria University Dental Hospital), (b) Vacutainer, (c) Micropipette, (d) Eppendorf tube, (e) Ultra Low Temperature Freezer, (g) Centrifuge Machine, (g) Vortex, (h) Laboratory Drying Oven (i) Elisa Microplate Reader (MDRL, Bahria University Health Sciences)

3.10.4 SPECIMEN COLLECTION AND PROCESSING

The samples were allowed to rest in the tubes for at least 30 minutes at room temperature so that they can form a clot. The clot was then removed from the serum by spinning the collection tube at 3500 RPM for 3 minutes at 20^{o}C (Figure 3.3(a)(d)).

3.10.4.1 STORAGE OF THE SAMPLES

Samples were stored as aliquots in labelled polypropylene tubes at -86^{o}C (Figure 3.3(b)).

3.10.4.2 REAGENTS OF THE ASSAY

- Standard Solution (8000ng/L)
- ELISA Plate (Pre-coated)
- Standard Diluent
- Streptavidin-HRP
- Stop Solution
- Substrate Solution labelled as A
- Substrate Solution labelled as B
- 25x concentrate Wash Buffer
- Human ADMA Antibody conjugated with Biotin
- Plate Sealer
- Distilled water

3.10.4.3 PRINCIPLE OF THE ASSAY

This was an ELISA kit, which stands for Enzyme-Linked Immunosorbent Assay. Human ADMA antibody was used to cover the plate. ADMA was introduced to the sample,

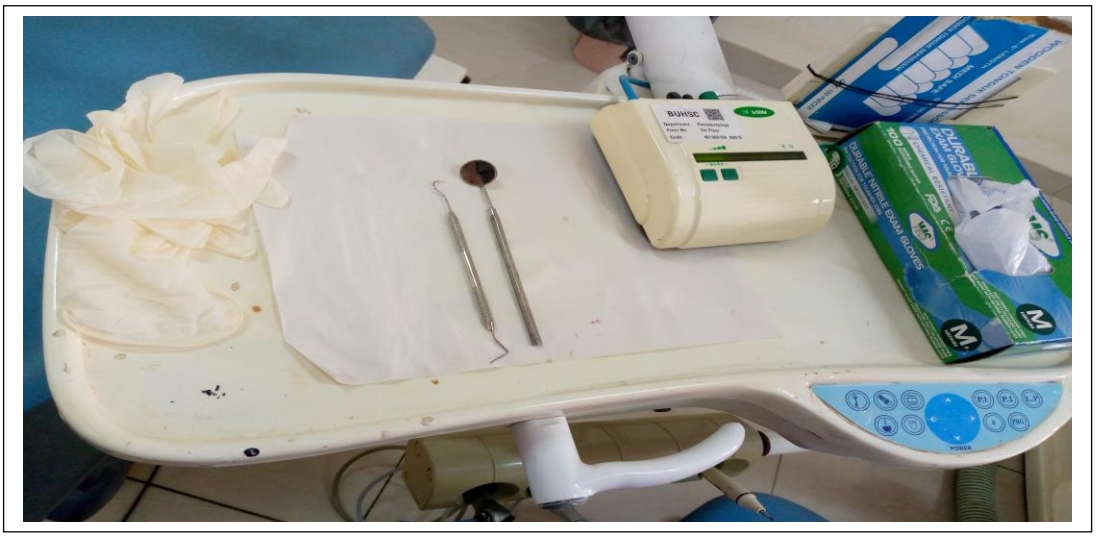


Figure 3.2(a) Tray set up showing all instruments (mouth mirror, sickle probe and William's periodontal probe)

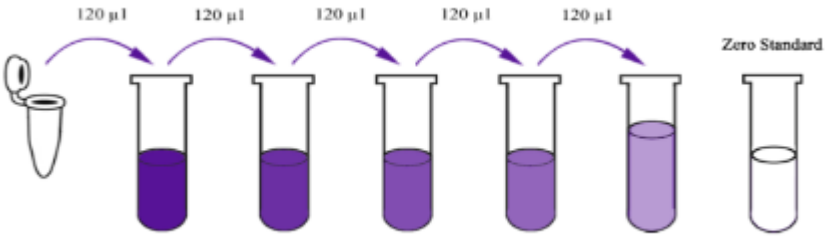
(i) **(ii)**

Figure 3.2(b) Kit

and the antibody-coated wells detected its presence. This was followed by the addition of a biotinylated Human ADMA antibody, which binds to any ADMA in the sample. Once the biotinylated ADMA antibody had been introduced, the Streptavidin-HRP attached to it. Unbound Streptavidin-HRP was removed by washing after an incubation time of one hour and before the addition of substrate solution. When a substrate solution was added, a color was developed that corresponded to the amount of human ADMA present. The process was stopped by adding an acidic stop solution, and the absorbance was read at 450 nm.

3.10.4.4 PREPARATION OF THE REAGENTS

All reagents were brought to room temperature 1 hour before use. The 120µl of the standard was adulterated with 120µl of standard diluent to make a standard stock solution. The standard was allowed to sit for 15 minutes with gentle perturbation prior to making dilutions. The duplicate standard points were prepared by serially diluting the standard stock solution at ratio of 1:2 with standard diluent to yield solutions. Standard diluent serves as the zero standard (0 ng/ml). The dilution of standard solutions according to the guidelines in the ELISA protocol are as follows (for the detection of E1887Hu Standard concentration (8000ng/ml).



20ml of Concentrate 25x Wash Buffer was diluted with distilled water to produce 500 ml of 1x Wash Buffer.

3.10.4.5 PROCEDURE OF THE ENZYMATIC ELISA ASSAY:

- One hour before use, all of the chemicals were brought to room temperature. The test was done at room temperature, which is 37°C.
- The number of micropipette tips needed for the test was worked out. The tips were put into the frames so they could be used.
- 50µl of standard was introduced to the well for standard.
- 40µl of sample were put into each sample well, and then 10µl of anti-ADMA antibody were put into each well. After that, we added 50µl of streptavidin-HRP to the sample wells and standard wells (but not to the blank control well). It was well mixed. A sealer was used to cover the plate. It was kept in the incubator at 37°C for an hour.
- The sealer was taken off, and the plate was washed with wash buffer five times. For each wash, at least 0.35 ml of wash buffer was left in each well for 30 seconds to 1 minute. The plate was wiped with paper towels or something else that could soak up liquid.
- Each well got 50µl of substrate solution A, and then each well got 50µl of substrate solution B. The plate was sealed with a new sealer, and then it was put in the dark at 37°C for 10 minutes.
- When 50µl of stop solution was added to each well, the blue color changed right away to yellow.
- The OD value of each well was measured right away with a microplate reader set to 450 nm. This was done 10 minutes after the stop solution was added.

3.10.5 OTHERS

- Subject Evaluation Proforma (Appendix-D)

3.11 PARAMETERS OF THE STUDY

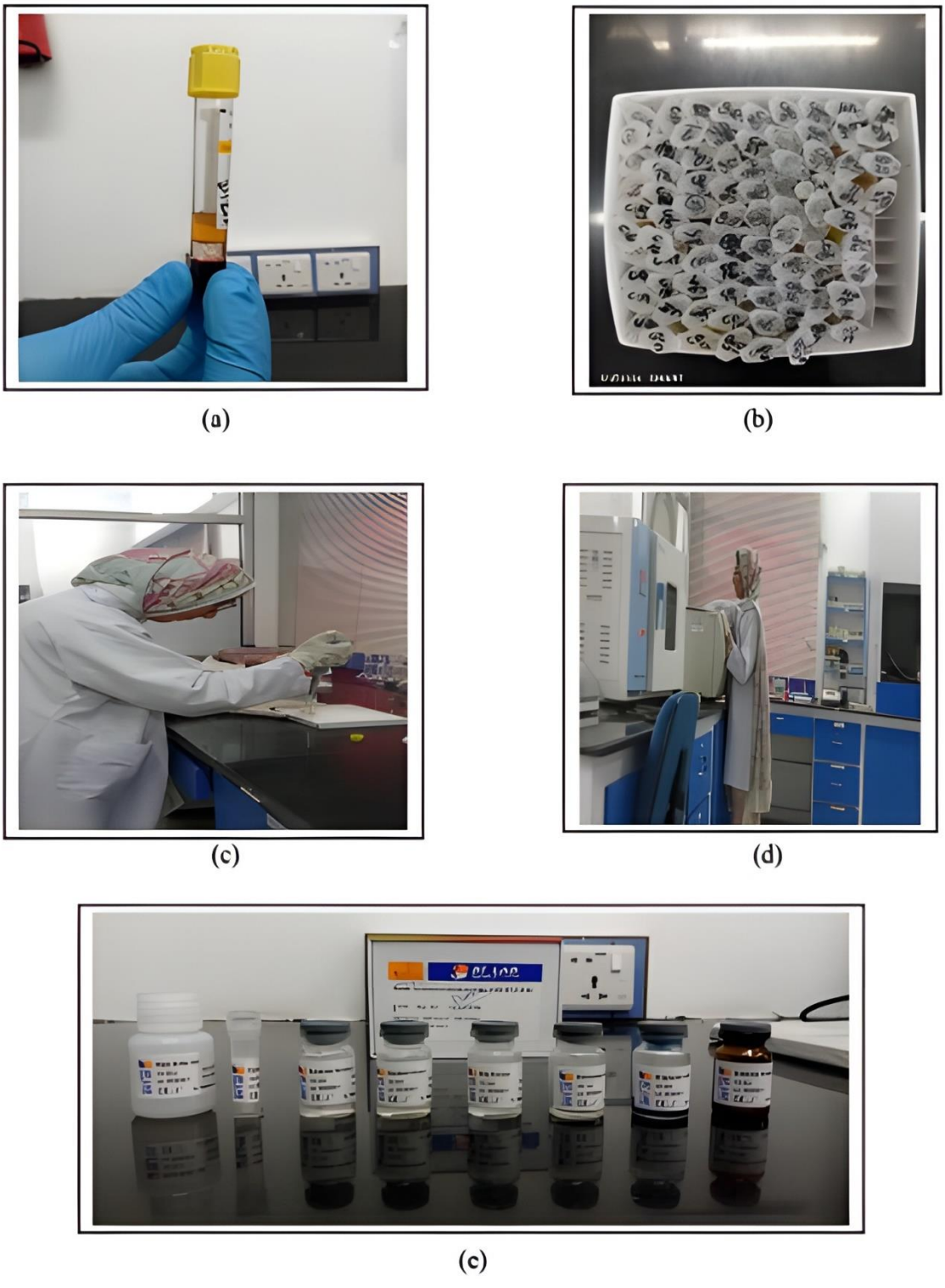


Figure 3.3 (a) to (e) Sample collection, processing and preparation

(a) Sample collection (b) Stored samples (c) Serum separation (d) Centrifugation (e) Reagents of Hu Asymmetric Dimethylarginine Kit

(i)

(ii)

(iii)

Figure 3.4 Human asymmetric dimethyl arginine ELISA Kit Performance

(i) Homogenized mixing of samples via vortex (ii) Incubation of ELISA plate covered with sealer (iii) Addition of samples to wells

3.11.1 CLINICAL PARAMETERS

3.11.1.1 PERIODONTAL PROBING DEPTH

The periodontal probing depth was determined by inserting William's periodontal probe into the sulcus at six distinct locations around the tooth which is being evaluated: mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual. Markings on the probe are used to measure the distance in millimetres from the base of the pocket to the gingival margin (Reddy et al., 2020) (Figure 3.5). PD was obtained based on the following criteria:

Mild: >3 &<5mm pocket depth

Moderate: ≥5- <7 mm pocket depth

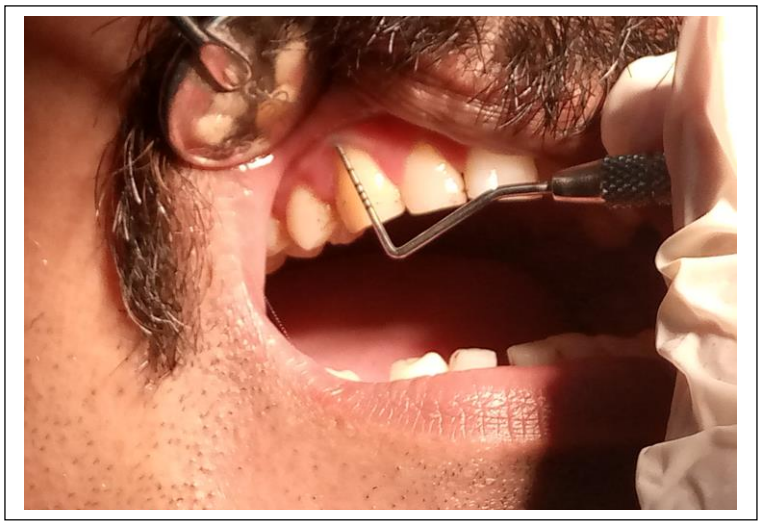
Severe: >7mm pocket depth

3.11.1.2 CLINICAL ATTACHMENT LEVEL (CAL)

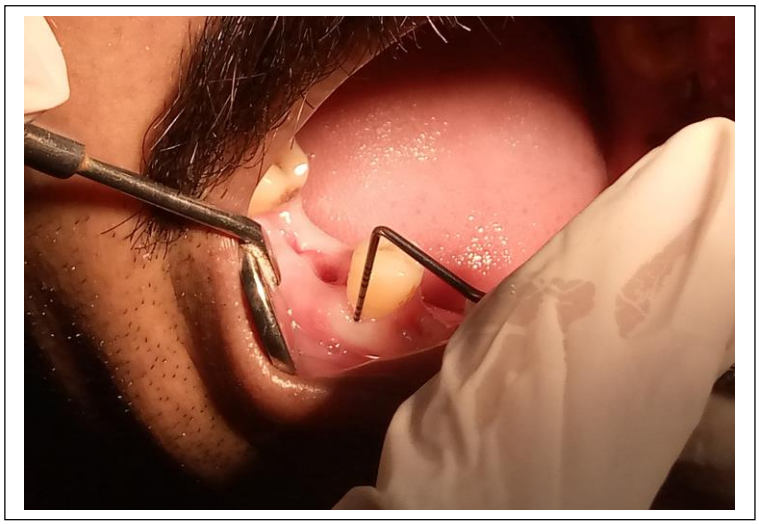
The clinical attachment level (CAL) was calculated by adding the distance from the cemento-enamel junction to the free gingival margin to the bottom of the gingival pocket/sulcus. To assist clinical measurements, a William's probe with 1-, 2-, 3-, 5-, 7-, 8-, 9-, and 10-mm gradations was positioned parallel to the long axis of all the present teeth. Measurements were taken in millimeters (mm) and rounded to the nearest millimeter (mm) (Germen et al., 2021). However, in the presence of gingival recession the CAL was calculated by adding the probing depth to the gingival margin level.

3.11.1.3 BLEEDING ON PROBING (BoP)

Bleeding on probing was assessed by placing William’s periodontal probe in the gingival sulcus at six different sites (Mesiobuccal, Buccal, Mesiolingual, Mesiolingual, Lingual, and Distolingual) of all teeth. It was calculated as number of bleeding sites/number



(i)



(ii)

Figure 3.5 Periodontal Probing Depth

(i) Maxillary arch (ii) Mandibular arch

of probed sites (%). Bleeding on probing was considered positive if the gingiva bleeds within 10 seconds of the probing (Zhao et al., 2019). According to the American Academy of Periodontology/European Federation of Periodontology consensus on the updated classification for periodontal and peri-implant diseases and conditions (G. Caton et al., 2018), the BoP cut-off for the classification of stable periodontal disease and the diagnosis of gingivitis in patients with intact or impaired periodontium is 10% of bleeding sites (Pietropaoli et al., 2020).

3.11.1.4 PLAQUE INDEX (PI)

The Plaque Index was assessed using the Silness and Loe Index (1964). It was recorded by examining 4 parts of teeth with a mouth mirror and a probe including distobuccal, buccal, mesiobuccal and lingual were examined (Al-Suwaidi et al., 2021). Based on the criteria, the scores were as follows

0: No visible plaque
1 A thin layer of plaque attached to the free gingival margin and adjacent area of the tooth which cannot be observed by the naked eye; it can be detected only after the periodontal probe has been ran on the surface of the tooth
2: Moderate layer of microbial plaque along the gingival margin, free interdental spaces or the surface of the tooth which is visible to the naked eye
3: Abundance of the plaque within the gingival pocket and/or on the tooth and gingival margin

The PI of the tooth was calculated by taking the mean of the area indices. The individual PI was computed by dividing the total number of inspected teeth by the sum of the recorded index tooth scores. The PI was employed as an oral hygiene indicator. The subject's plaque status was assigned as follows:

Excellent (<0.1)

Good (0.1-0.9)

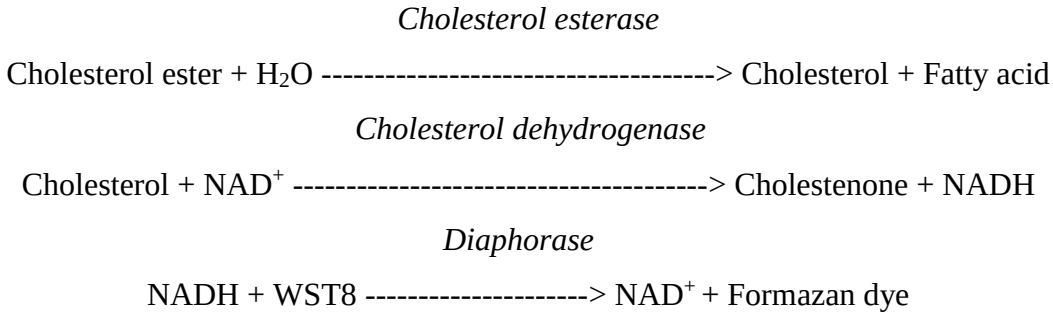
Fair (1.0-1.9)

Poor (2.0-3.0)

3.11.2 BIOCHEMICAL PARAMETERS

3.11.2.1 TOTAL CHOLESTEROL (TC)

The estimation of cholesterol was determined by enzymatic method. In the presence of water cholesterol esters were broken down in the sample into cholesterol and fatty acids. When cholesterol dehydrogenase was present, cholestenone and NADH are made from cholesterol and NAD+. Through an oxidation-reduction process, diaphorase and NADH turned WST8 into a form of dye called formazan. At a certain wave length, 460 nm, the strength of the color of formazan was directly related to the amount of total cholesterol in the sample. The sequence of the reaction is as follows:



High cholesterol levels raise the risk of coronary heart disease (CHD). Cholesterol levels are evaluated to help assess the patient's risk status and to track the patient's progress in lowering serum cholesterol concentrations. Cholesterol levels below 200 mg/dL (5.18 mmol/L) are considered desirable, whereas values between 200-239 mg/dL (5.18-6.19 mmol/L) are considered borderline high, and levels above 240 mg/dL (6.20 mmol/L) are considered increasingly high.

3.11.2.2 TRIGLYCERIDES (TG)

The estimation of triglycerides was performed through an enzymatic technique. In the presence of water lipoprotein lipase broke down triglycerides in the sample into glycerol and fatty acids. In the presence of glycerol dehydrogenase, glycerol and NAD+ produced dihydroxyacetone and NADH. Diaphorase and NADH catalyzed the oxidation reduction reaction that transformed WST8 into the formazan dye. At a wavelength of 460 nm, the intensity of the formazan color was directly proportional to the triglyceride concentration. The sequence of the reaction is as follows:

Lipoprotein lipase

Triglycerides + $3H_2O$ ----------> Glycerol + Fatty acids

Glycerol dehydrogenase

Glycerol + NAD+ ----------> Dihydroxyacetone + NADH

Diaphorase

NADH + WST8 ----------> NAD^+ + Formazan dye

Individuals who exhibit elevated levels of triglycerides in their serum are at a heightened risk of developing coronary heart disease and peripheral atherosclerosis. Triglyceride levels during fasting that are less than or equal to 150 mg/dL (1.70 mmol/L) are regarded as ideal and are further classified as follows: Borderline hypertension is regarded as 1.70-2.25 mmol/L or 150-199 mg/dL. 200–499 mg/dL (2.26–5.64 mmol/L) is considered high where as severe is 500 mg/dL (5.65 mmol/L) or higher.

3.11.2.3 HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C)

In the HDL test, a precipitation method is used, and the precipitant reagent is made up of Mg2+ and phosphotungstic acid. Precipitation and removal of the components occurs, with the exception of HDL cholesterol. An enzymatic approach is utilized by the cobas b 101 system in order to determined HDL cholesterol. High levels of HDL cholesterol lower the risk of coronary heart disease, while low levels of HDL cholesterol, especially when

combined with high levels of triglycerides, and raise the risk of cardiovascular disease. Low levels of HDL are considered as <40 mg/dL (<1.04 mmol/L) and high levels are considered as ≥60 mg/dL (≥1.55mmol/L).

3.11.2.4 LOW DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C)

LDL-cholesterol was measured from the obtained values of total cholesterol, triglycerides, and HDL-cholesterol. When the concentration of triglycerides is less than 400 mg/dL (4.52 mmol/L), the Friedewald formula is used to calculate LDL cholesterol (Singh et al., 2020).

$$LDL = TC - HDL - TG/5 \text{ (measured in mg/dL)}$$

Here [TG]/5 is a determinant of VLDL-cholesterol and all values are denoted in mg/dL.

LDL (Low Density Lipoproteins) play a major role in the initiation and advancement of atherosclerotic plaque and coronary artery disease. The cholesterol detected in atherosclerotic plaques is primarily derived from LDL. Among all single measures, LDL cholesterol is the most accurate clinical predictor of coronary atherosclerosis.

3.11.2.5 C-REACTIVE PROTEIN (CRP)

The Roche cobas b 101 is a diagnostic test method that uses photometric analysis to find out the amount of CRP is in human capillary serum and whole blood, EDTA K2/K3, and lithium heparin anticoagulated plasma and whole blood. The sample was adulterated with HEPES buffer and put into a reaction chamber of the machine, where it reacted with CRP antibody latex reagent. This step was repeated until the desired concentration of CRP antibody is achieved. The CRP in the plasma that has been diluted formed a complex with the CRP antibody that is present on the latex particle. The quantity of agglutination was directly proportional to the change in absorbance that is observed at 525 nm and 625 nm. The concentration of CRP was computed as a function of this change in absorbance. In healthy

people, CRP is a small amount of protein that can be up to 5 mg/L. Changes can be seen in 6-8 hours, and the highest point is reached in 24-48 hours. Levels that are up to a thousand times higher than usual can be caused by conditions such as a myocardial infarction, surgery, trauma or cancerous tumors.

3.12 PROTOCOL OF THE STUDY

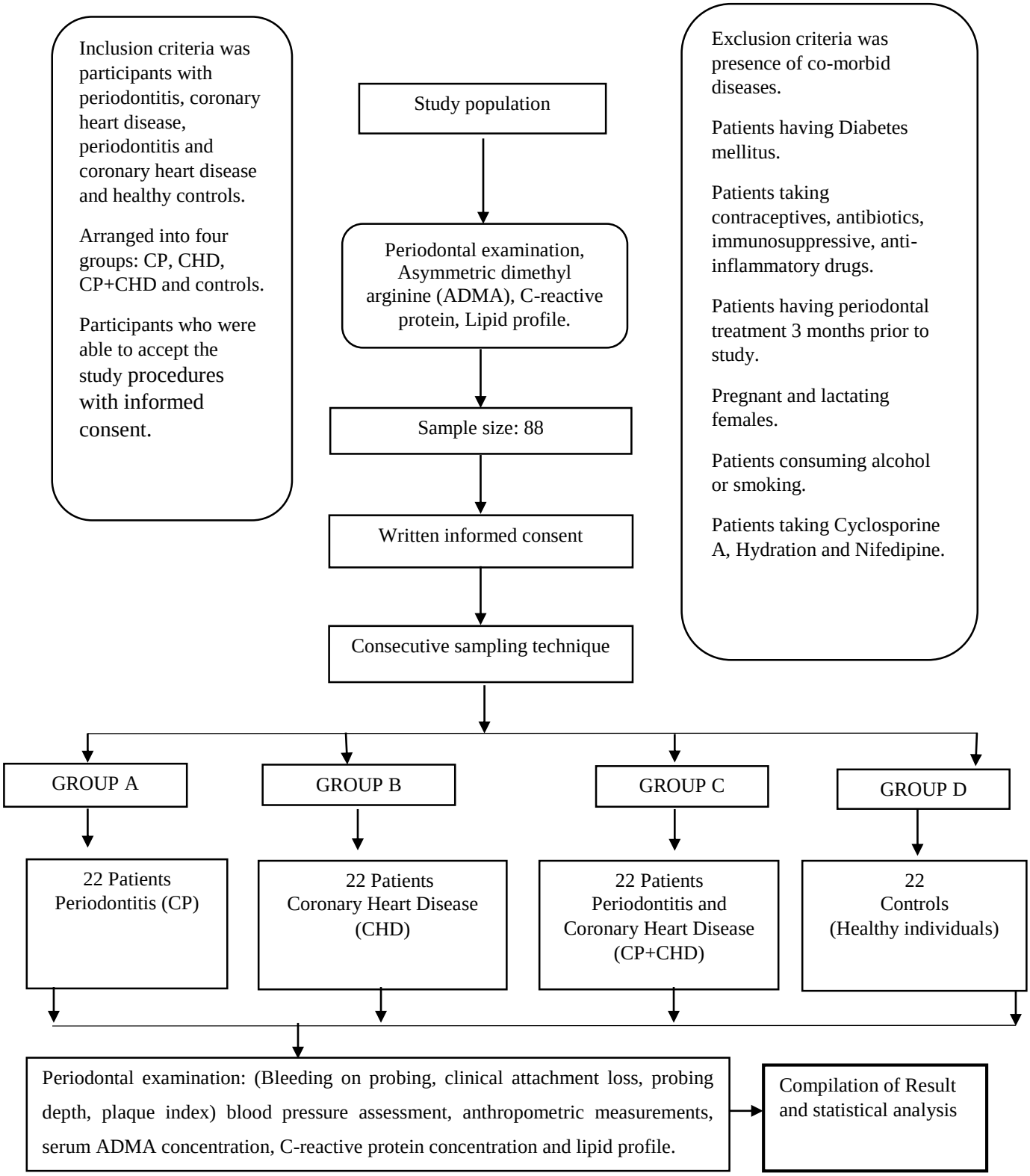
This case-control study was conducted after obtaining FRC (18/11/2022, FRC-BUHS-50-2022-508) and ERC approval (27/1/23, ERC 04/2023) of Bahria University Health Sciences Campus Karachi (BUHSCK). All the male and female patients of periodontitis and coronary heart disease presenting at the Periodontal Department of Bahria Dental College Hospital and Cardiac Care Unit (CCU) of PNS Shifa Hospital Karachi as per the inclusion criteria of the study were recruited prior taking written informed consent. The participants were divided into four groups based on a predetermined age range (30 to 60 years) and sex, in order to ensure that an equivalent number of cases belonged to each of the categories established by the selection variable (age and sex).

- C= Controls
- CP= Chronic Periodontitis
- CHD= Coronary Heart Disease
- CP+CHD= Chronic Periodontitis and Coronary Heart Disease

All the demographic information of patients (such as name, sex (male / female), age (years), occupation, address, contact number), anthropometric measurements (height and weight), co-morbidities (diabetes and dyslipidemia), family history for hypertension, diabetes, ischemic heart disease/coronary heart disease, asthma/ chronic obstructive pulmonary disease and hyperlipidemia, angiographic findings, history of cardiovascular procedures, cardiac medicines were obtained. The anthropometric measurements (height and weight) were performed to assess BMI of the participants. The SBP and DBP readings were obtained from the recruited subjects. The dental clinical parameters which includes probing

depth, clinical attachment level, bleeding sites and the plaque index score were assessed for all the teeth (six sites per tooth namely buccal, mesiobuccal, distobuccal, lingual, mesiolingual, distolingual) in the study participants. Fasting blood samples of the patients were obtained for serum analysis of the following biochemical parameters; ADMA, C-reactive protein and lipid profile. The biochemical parameters which includes hs-CRP, TC, TG, HDL-C and LDL-C were routinely performed for CHD and CP+CHD patients while they were determined for Controls and CP individuals at PNS Shifa Laboratory. Blood was collected and transported safely in an insulated box to the laboratory for the analysis of serum ADMA. The samples were centrifuged to separate out the serum. Later, it was stored at -80^{o}C in the freezer. After all the samples were collected. They were thawed and homogenously mixed on vortex machine. The protocol of ELISA was followed to obtain the readings of the analyte ADMA. The biochemical parameters were then compared among the four studied groups.

3.13 ALGORITHM OF THE STUDY



3.14 STATISTICAL ANALYSIS

Data were stored and analyzed using IBM-SPSS version 23.0; counts with percentages were reported on baseline demographic, medical history, co morbidities, family history and drug treatment across four studied groups. The Shapiro-Wilk test was used to determine if continuous variable distributions were normal. Mean with standard deviation was reported on age (years), weight (kg), height (m), BMI (kg/m^2), SBP (mm/hg) and DBP (mm/hg). Median with Interquartile range (75^{th} percentile – 25^{th} percentile) was reported for CRP, lipid profile, ADMA, and periodontal parameters. Association of baseline characteristics was tested using Pearson Chi Square test for categorical variables, one-way ANOVA was used to compare mean of baseline quantitative parameters, Kruskal Wallis test was used to compare median of periodontal and other studied parameters across control, CP, CHD and CP+CHD samples. Multiple comparisons between groups was done using Mann Whitney U test. Spearman Rank correlation analysis was done to study correlation of ADMA with other studied variables. Multinomial Logistic Regression analysis was used predict the risk of endothelial dysfunction using univariate and multivariate model. The multivariate logistic regression model then incorporated the factors that were significantly associated with endothelial dysfunction in univariate model. Scatter plots were used to display the correlation of ADMA with significant parameters. Box plots were used to display the median of ADMA and other parameters. ROC analysis was used to estimate the area under the curve of ADMA among periodontal cases. Sensitivity, specificity and cutoff value of ADMA was also derived for ADMA using ROC curve. All p-values less than 0.05 were considered statistically significant.

CHAPTER 4

RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS AND MEDICAL HISTORY OF GROUP CONTROLS, CP, CHD AND CP+CHD (Table 4.1)

Age in Years

In Control group there were 59.1% were aged less than or equal to 40 years old, in CP group there were 59.1% were aged 41 - 50 years old, in CHD group there were 54.5% were aged more than 50-years old while in CP+CHD groups there were 68.2% were aged more than 50-years old. The difference was significant statistically.

Gender

In Control group 40.9% were females while 59.1% were males, in CP group 18.2% were females while 81.8% were males, in CHD group 4.5% were females while 95.5% were males while in CP+CHD group 100% were males with difference statistically significant among the groups.

Extent of Coronary Heart Disease (Number of coronary vessels with stenosis)

The percentage of individuals in both groups having coronary artery stenosis was more than 50%. Among the groups CHD and CP+CHD, 54.5% and 27.3% patients had stenosis in 1 vessel, 22.7% 36.4% had stenosis in 2 vessels, 22.7% and 36.4% patients had stenosis in 3 vessels. There was no statistical difference reported in the angiographic findings between the two groups.

History of Medications or Cardiac procedures (PCI, CABG)

The status of cardiac procedures such as percutaneous coronary intervention (PCI) (n=27) or coronary artery bypass grafting (CABG) (n=3) or medications (n=14) was monitored in study individuals of CHD and CP+CHD groups. It was observed that in CHD group 27.3% patients were on medications, 68.2% patients undergone PCI and 4.5% had CABG while in CP+CHD group 36.4% patients were using medicines, 54.5% had PCI and 9.1% had CABG. There was no statistical difference found for the cardiac procedures among the two groups.

Comorbidities

In controls 13.6% individuals presented with hypertension and none was found with hyperlipidemia, in CP group 9.1% patients presented with hypertension and none was found with hyperlipidemia, in CHD group 50.0% patients were found to be hypertensive and 4.5% with hyperlipidemia while in CP+CHD group 63.6% patients had hypertension while 18.2% patients had hyperlipidemia. Pearson Chi Square test did give a significant association of co morbidities with studied groups.

Table 4.1: Demographic characteristics and medical history of study participants

Characteristics		***Groups***								***p-value***
		Controls (n=22)		***CP (n=22)***		***CHD (n=22)***		***CP+CHD (n=22)***		
		n	***%***	***n***	***%***	***n***	***%***	***n***	***%***	
Age Group	≤40 years	13	59.1	0	0.0	4	18.2	2	9.1	<0.01*
	41 - 50 years	7	31.8	13	59.1	6	27.3	5	22.7	
	>50 years	2	9.1	9	40.9	12	54.5	15	68.2	
Gender	Male	13	59.1	18	81.8	21	95.5	22	100.0	<0.01*
	Female	9	40.9	4	18.2	1	4.5	0	0.0	
Angiographic results	1 vessel	0	0.0	0	0.0	12	54.5	6	27.3	0.18
	2 vessel	0	0.0	0	0.0	5	22.7	8	36.4	
	3 vessel	0	0.0	0	0.0	5	22.7	8	36.4	
Cardiac Procedures	Medicines	0	0.0	0	0.0	6	27.3	8	36.4	0.62
	PCI	0	0.0	0	0.0	15	68.2	12	54.5	
	CABG	0	0.0	0	0.0	1	4.5	2	9.1	
Hypertension	Yes	0	0.0	2	9.1	11	50.0	14	63.6	<0.01*
	No	22	100.0	20	90.9	11	50.0	8	36.4	
Hyperlipidemia	Yes	0	0.0	0	0.0	1	4.5	4	18.2	0.02*
	No	22	100.0	22	100.0	21	95.5	18	81.8	

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, Test applied: chi square test, *p<0.05 considered statistically significant

4.2 ANTHROPOMETRIC MEASUREMENTS AND VITALS OF GROUP CONTROLS, CP, CHD AND CP+CHD (TABLE 4.2)

Weight

In control group mean weight was 71.23 (SD=±11.90) kg, in CP group mean weight was 70.18 (SD=±9.73) kg, in CHD group, mean weight was 71.4 (SD=±10.90) kg, in CP+CHD group mean weight was 76.18 (SD=±11.04) kg. The difference was non-significant statistically.

Height

In control group mean height was 1.64 (SD=±0.09) m, in CP group mean height was 1.68 (SD=±0.09) m, in CHD group mean height was 1.65 (SD=±0.14) m and in CP+CHD group mean height was 1.67 (SD=±0.05) m. The difference was non-significant statistically.

Body mass index (BMI)

In control group mean BMI was 26.41 (SD=±4.13) kg/m^2, in CP group mean BMI was 25.02 (SD=±3.72) kg/m^2, in CHD group mean BMI was 26.82 (SD=±6.47) kg/m^2 and in CP+CHD group mean BMI was 27.36 (SD=±4.81) kg/m^2. There was no statistical difference found for this parameter across the four groups.

Systolic Blood Pressure (SBP)

In control group mean SBP was 118.9 (SD=±2.29) mm/hg, in CP group mean SBP was 147.8 (SD=±5.04) mm/hg, in CHD group mean SBP was 120.77 (SD=±13.48) mm/hg

and in CP+CHD group mean SBP was 139.0 (SD=±2.38) mm/hg. One-way ANOVA showed a significant mean difference for systolic blood pressure across the four studied groups.

Diastolic Blood Pressure (DBP)

In control group mean DBP was 78.27 (SD=±2.05) mm/hg, in CP group mean DBP was 93.59 (SD=±4.18) mm/hg, in CHD group mean DBP was 76.45 (SD=±8.16) mm/hg while in CP+CHD group mean DBP was 90.14 (SD=±12.90) mm/hg. One-way ANOVA showed a significant mean differences for this parameter across the four studied groups.

4.3 FAMILY HISTORY OF PARTICIPANTS AND DRUG TREATMENT OF CHD OF GROUP CHD AND CP+CHD (TABLE 4.3)

Family History

In control group 27.3% individuals were reported for diabetes, 13.6% were reported for hypertension, none was reported for asthma /COPD, CAD/IHD and Hyperlipidemia. In CP patients for family history 22.7% were reported for diabetes, 13.6% were reported for hypertension, 9.1% were reported for asthma/COPD, 4.5% were reported for CAD/IHD and none was reported for Hyperlipidemia. In CHD patients for family history 27.3% were reported for diabetes, 31.8% were reported for hypertension, 4.5% were reported for asthma/COPD, 27.3% were reported for CAD/IHD and 4.5 were reported for Hyperlipidemia. In CP +CHD group for family history 18.2% were reported for diabetes, 31.8% were reported for hypertension, none was reported for asthma/COPD, 13.6% were reported for CAD/IHD and none was reported for Hyperlipidemia.

Cardiac Medicines

The cardiac medicines being used by the patients in CHD and CP+CHD groups were recorded. In CHD group, it was found that 90.9% uses Antiplatelets, 4.5% on Anticoagulants,4.5% on Angiotensin receptor blockers, 18.2% on Beta-blockers, 9.1% on Calcium channel blockers, 90.9% on Statins, 18.2% uses Anti hypertensives, 4.5% uses Analgesics, 54.5% uses Proton pump inhibitors, and 31.8% uses Diuretics.

4.4 COMPARISON OF BIOCHEMICAL PARAMETERS IN GROUP CONTROLS, CP, CHD AND CP+CHD (TABLE 4.4)

C-reactive Protein (hs-CRP)

Median of CRP in control group samples was 1.05 [IQR=(1.5-0.7)] mg/L, in CP it was 3.55 [IQR=(4.4-2.9)] mg/L, in CHD it was 3.6 [IQR=(7.15-0.82)] mg/L, in CP+CHD it was 6.85 [IQR=(9.2-4.36)] mg/L. Kruskal Wallis test did give a significant difference in the median values of CRP across the four studied groups.

Total Cholesterol (TC)

Median Total Cholesterol in Control group samples was 185 [IQR=(195-180)] mg/dL, in CP it was 185.85 [IQR=(190-182.35)] mg/dL, in CHD it was 160.61 [IQR=(205.11-127.32] mg/dL and in CP+CHD it was 147.45 [IQR=(172.67-135.45)] mg/dL. Kruskal Wallis test did give a significant difference in the median values of total cholesterol of high density lipoprotein across the four studied groups.

Table 4.2: Descriptive statistics of Clinical Parameters of study participants

Parameters	***Groups***								***p-value***
	Controls		***CP***		***CHD***		***CP+CHD***		
	Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	
Weight (kg)	71.23	11.90	70.18	9.73	71.41	10.90	76.18	11.04	0.27
Height (m)	1.64	0.09	1.68	0.09	1.65	0.14	1.67	0.05	0.59
BMI (Kg/m^2)	26.41	4.13	25.02	3.72	26.82	6.47	27.36	4.81	0.43
SBP mm/hg	118.91	2.29	147.82	5.04	120.77	13.48	139.00	2.38	<0.01*
DBP mm/hg	78.27	2.05	93.59	4.18	76.45	8.16	90.14	12.90	<0.01*

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, Test applied: one-way annova test, *p<0.05 considered statistically significant

Table 4.3: Descriptive statistics of Family History and Drug Treatment of CHD

Family History and Drug Treatment of CHD		***Controls***		***CP***		***CHD***		***CP+CHD***	
		n	***%***	***n***	***%***	***n***	***%***	***n***	***%***
Diabetes	Yes	6	27.3	5	22.7	6	27.3	4	18.2
	No	16	72.7	17	77.3	16	72.7	18	81.8
Hypertension	Yes	3	13.6	3	13.6	7	31.8	7	31.8
	No	19	86.4	19	86.4	15	68.2	15	68.2
Asthma/COPD	Yes	0	0.0	2	9.1	1	4.5	0	0.0
	No	22	100.0	20	90.9	21	95.5	22	100.0
CAD/IHD	Yes	0	0.0	1	4.5	6	27.3	3	13.6
	No	22	100.0	21	95.5	16	72.7	19	86.4
Hyperlipidemia	Yes	0	0.0	0	0.0	1	4.5	0	0.0
	No	22	100.0	22	100.0	21	95.5	22	100.0
Antiplatelet	Yes	0	0.0	0	0.0	20	90.9	18	81.8
	No	22	100.0	22	100.0	2	9.1	4	18.2
Anticoagulants	Yes	0	0.0	0	0.0	1	4.5	0	0.0
	No	22	100.0	22	100.0	21	95.5	22	100.0
Nitrates	Yes	0	0.0	0	0.0	0	0.0	9	40.9
	No	22	100.0	22	100.0	22	100.0	13	59.1
Angiotensin-converting enzyme inhibitors	Yes	0	0.0	0	0.0	0	0.0	2	9.1
	No	22	100.0	22	100.0	22	100.0	20	90.9
Angiotensin receptor blockers	Yes	0	0.0	0	0.0	1	4.5	2	9.1
	No	22	100.0	22	100.0	21	95.5	20	90.9
Beta-blockers	Yes	0	0.0	0	0.0	4	18.2	5	22.7
	No	22	100.0	22	100.0	18	81.8	17	77.3
Calcium channel blockers	Yes	0	0.0	0	0.0	2	9.1	1	4.5
	No	22	100.0	22	100.0	20	90.9	21	95.5
Statins	Yes	0	0.0	0	0.0	20	90.9	16	72.7
	No	22	100.0	22	100.0	2	9.1	6	27.3
Antihypertensive	Yes	0	0.0	0	0.0	4	18.2	1	4.5
	No	22	100.0	22	100.0	18	81.8	21	95.5
Analgesics	Yes	0	0.0	0	0.0	1	4.5	5	22.7
	No	22	100.0	22	100.0	21	95.5	17	77.3
Proton pump inhibitors	Yes	0	0.0	0	0.0	12	54.5	5	22.7
	No	22	100.0	22	100.0	10	45.5	17	77.3
Diuretics	Yes	0	0.0	0	0.0	7	31.8	3	13.6
	No	22	100.0	22	100.0	15	68.2	19	86.4

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, COPD= chronic obstructive pulmonary disease, CAD= coronary artery disease, IHD= Ischemic heart disease

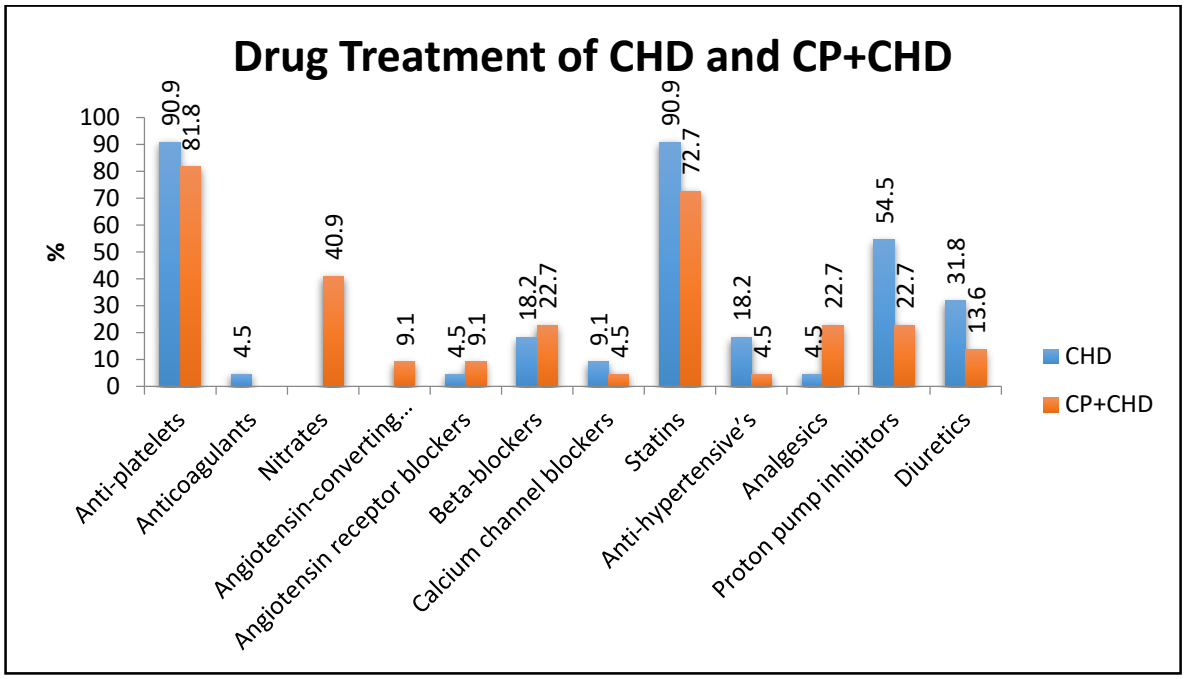


Figure 4.3: Descriptive statistics of Drug Treatment of CHD and CP+CHD Patients

Triglycerides (TG)

Median Triglycerides (mg/dL) in Control group samples was 131 [IQR=(140-122)] mg/dL, in CP it was 116.1 [IQR=(137.8-109.4)] mg/dL, in CHD it was 140.36 [IQR=(170.72-100.32)] mg/dL and in CP+CHD it was 135.45 [IQR=(158.4-110.88)] mg/dL. Kruskal Wallis test did give a significant difference in the median values of triglycerides of high density lipoprotein across the four studied groups.

HDL Cholesterol (HDL-C)

Median HDL Cholesterol (mg/dL) in Control group samples was 50 [IQR=(55-45)] mg/dL, in CP it was 37.6 [IQR=(41.9-35.6)] mg/dL, in CHD it was 34.64 [IQR=(42.96-29.41)] mg/dL and in CP+CHD it was 37.75 [IQR=(48.02-30.19)] mg/dL. Kruskal Wallis test did give a significant difference in the median values of high density lipoprotein across the four studied groups.

LDL Cholesterol (LDL-C)

Median LDL Cholesterol (mg/dL) in Control group samples was 120 [IQR=(125-110)] mg/dL, in CP it was 104.6 [IQR=(108.2-101.2)] mg/dL, in CHD it was 116.49 [IQR=(144.35-81.27)] mg/dL and in CP+CHD it was 85.95 [IQR=(95.2-77.4)] mg/dL. Kruskal Wallis test did give a significant difference in the median values of low density lipoprotein across the four studied groups.

Asymmetric Dimethylarginine (ADMA)

Median ADMA in Control group samples was 16.39 [IQR=(21.3-5.76)] µmol/L , in CP it was 24.16 [IQR=(26.39-19.95)] µmol/L, in CHD it was 37.46 [IQR=(184.98-23.51)]

Table 4.4: Comparison of CRP, Lipid Profiles and ADMA across Studied Groups

Parameters	***Controls***	***CP***	***CHD***	***CP+CHD***	***p-value***
	Median (Q3 – Q1)	***Median (Q3 – Q1)***	***Median (Q3 – Q1)***	***Median (Q3 – Q1)***	
CRP mg/L	1.05 (1.5-0.7)	3.55 (4.4-2.9)	3.6 (7.15-0.82)	6.85 (9.2-4.36)	<0.01*
TC (mg/dL)	185 (195-180)	185.85 (190-182.35)	160.61 (205.11-127.32)	147.45 (172.67-135.45)	<0.01*
TG (mg/dL)	131 (140-122)	116.1 (137.8-109.4)	140.36 (170.72-100.32)	135.45 (158.4-110.88)	0.38
HDL-C (mg/dL)	50 (55-45)	37.6 (41.9-35.6)	34.64 (42.96-29.41)	37.75 (48.02-30.19)	<0.01*
LDL-C (mg/dL)	120 (125-110)	104.6 (108.2-101.2)	116.49 (144.35-81.27)	85.95 (95.2-77.4)	<0.01*
ADMA (µmol/L)	16.39 (21.3-5.76)	24.16 (26.39-19.95)	37.46 (184.98-23.51)	59.53 (206.24-25.67)	<0.01*

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, TC= total cholesterol, TG= Triglycerides, HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein Cholesterol, ADMA= Asymmetric dimethylarginine, Test applied: kruskal wallis test, *p<0.05 considered statistically significant

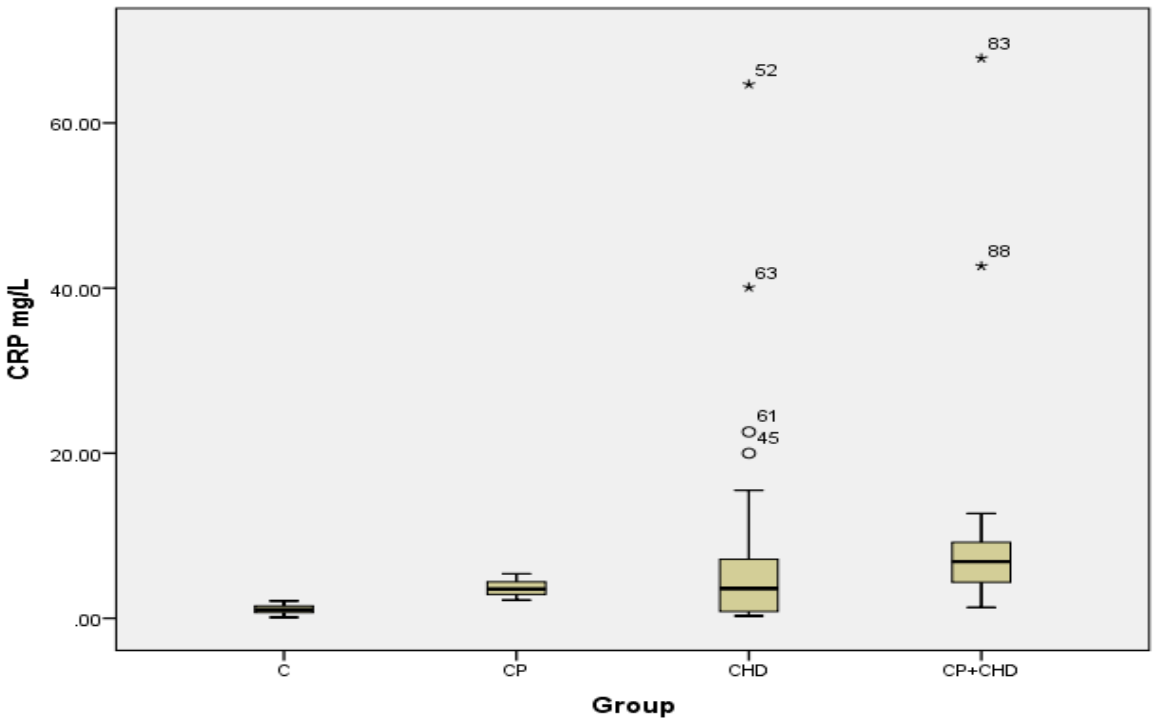


Figure 4.4(a) Box plot of CRP showing skewed distributions with some of the extreme values in CHD and CP+CHD group

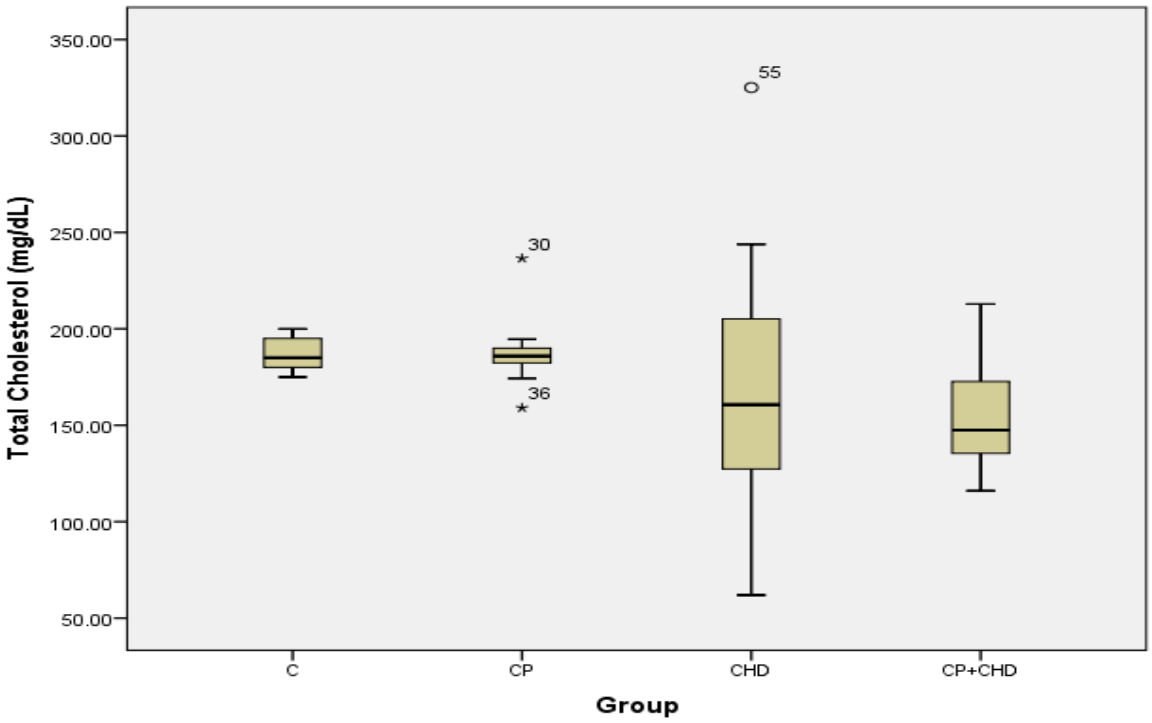


Figure 4.4(b) Box plot of TC showing skewed distributions with some of the extreme values in CHD and CP group

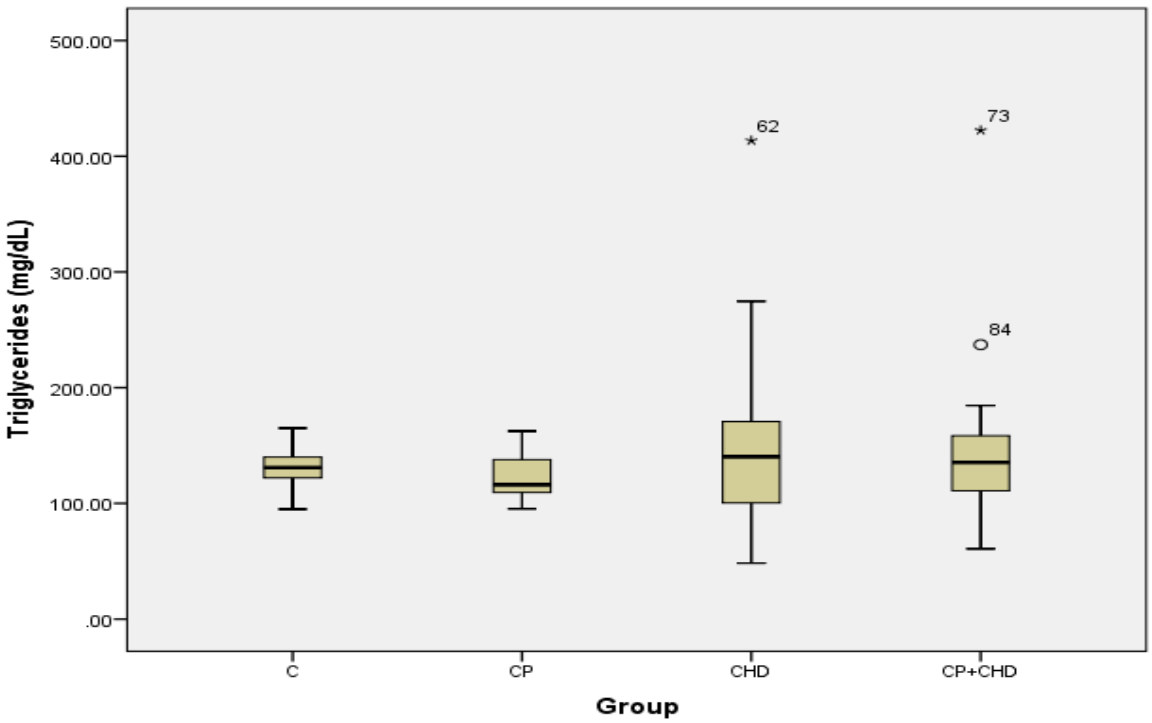


Figure 4.4(c) Box plot of TG showing skewed distributions with some of the extreme values in CHD and CP+CHD

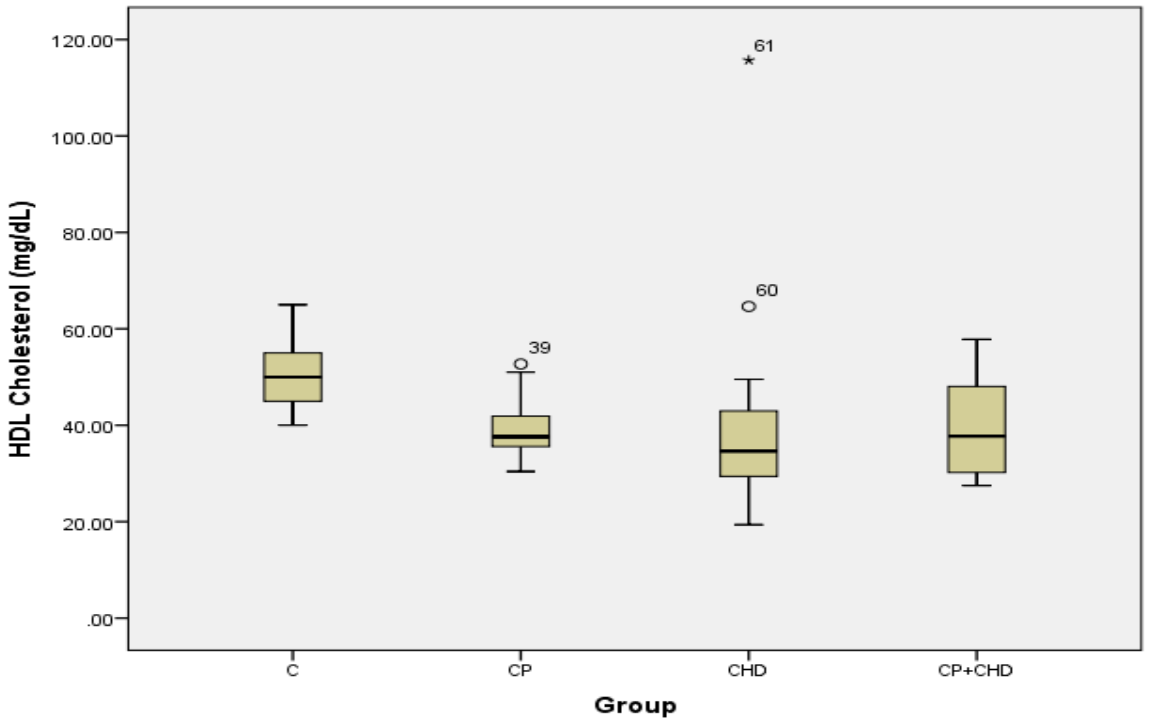


Figure 4.4(d)Box plot of HDL showing skewed distributions with some of the extreme values in CP and CHD group

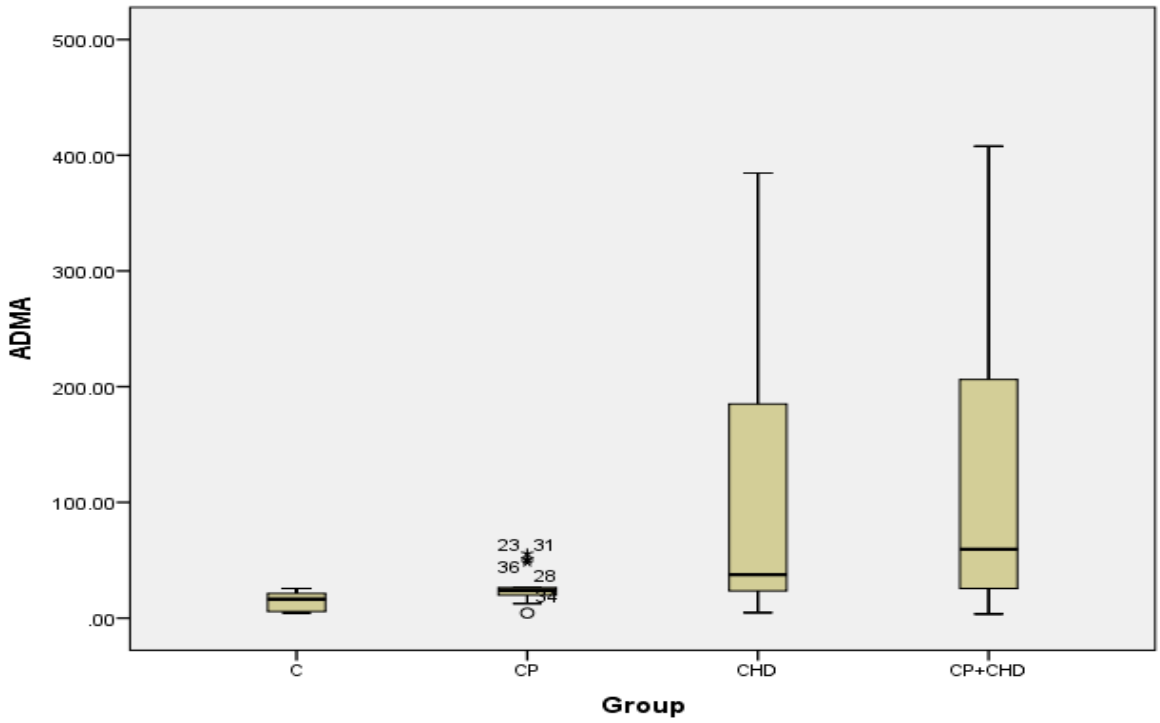


Figure 4.4(e) Box plot of ADMA showing skewed distributions with some of the extreme values in CP group patients, the values of ADMA were found litter higher in CHD and CP+CHD patients.

µmol/L and in CP+CHD it was 59.53 [IQR=(206.24-25.67)] µmol/L. Kruskal Wallis test did give a significant difference in the median values of asymmetric dimethylarginine across the four studied groups.

4.5 INTERGROUP COMPARISON OF BIOCHEMICAL PARAMETERS IN GROUP CONTROLS, CP, CHD AND CP+CHD (TABLE 4.5)

Table-5 reports the multiple comparison of CRP, Lipid profile and ADMA between studied groups using Mann Whitney U test.

C-reactive Protein (hs-CRP)

Our study results showed CRP did give significant difference between controls vs. CP, controls vs. CHD, controls vs. CP+CHD and CP vs. CHD+CP ($p<0.05$).

Total Cholesterol (TC)

Total cholesterol did gives significant differences between controls vs. CHD, Controls vs. CP+CHD, CP vs. CHD and CP vs. CP+CHD ($p<0.05$).

Triglycerides (TG)

Triglycerides did not give any significant differences between groups.

HDL-Cholesterol (HDL-C)

HDL did gives significant differences between controls vs. CP, controls vs. CHD and controls vs. CP+CHD ($P<0.05$).

LDL-Cholesterol (LDL-C)

LDL did gives significant difference between controls vs. CP, controls vs. CP+CHD and CP vs. CHD+CP ($P<0.05$).

Asymmetric Dimethylarginine (ADMA)

ADMA did give significant differences between all pairs ($p<0.05$) except CHD vs. CP+CHD found statistically insignificant ($p=0.57$).

4.6 DENTAL PARAMETERS ACROSS STUDIED GROUPS - CONTROLS, CP, CHD AND CP+CHD

No of teeth

Median No. of teeth in Control group samples was 27 [IQR=(28-26)], in CP it was 19 [IQR=(22 -17)], in CHD it was 24.5 [IQR=(26-22)] and in CP+CHD it was 17 [IQR=(19-16)]. The difference was significant statistically.

Table 4.5: Multiple Comparisons of CRP, Lipid Profiles and ADMA between Groups

Parameters	***Controls vs. CP***	***Controls vs. CHD***	***Controls vs. CP+CHD***	***CP vs. CHD***	***CP vs. CHD+CP***	***CHD vs. CP+CHD***
CRP mg/L	<0.01*	<0.01*	<0.01*	0.99	<0.01*	0.051
TC (mg/dL)	0.74	0.03*	<0.01*	0.03*	<0.01*	0.45
TG (mg/dL)	0.25	0.27	0.60	0.13	0.29	0.63
HDL-C (mg/dL)	<0.01*	<0.01*	<0.01*	0.11	0.90	0.25
LDL-C (mg/dL)	<0.01*	0.90	<0.01*	0.67	<0.01*	0.09
ADMA (µmol/L)	<0.01*	<0.01*	<0.01*	0.02*	<0.01*	0.57

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, TC= total cholesterol, TG= Triglycerides, HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein Cholesterol, ADMA= Asymmetric dimethylarginine, Test applied: mann whitney u test, *p<0.05 considered statistically significant

Probing depth (PD)

Median PD (mm) in Control group samples was 1.5 [IQR=(2-1)], in CP it was in CP it was 4.5 [IQR=(5 - 4)], in CHD it was 2 [IQR=(2.5-1)] and in CP+CHD it was 5.5 [IQR=(6-5)]. The difference was significant statistically.

Clinical Attachment Level (CAL)

Median CAL (mm) in Control group samples was 2.25 [IQR=(2.5-2)], in CP it was 7 [IQR=(8 - 6)], in CHD it was 2.75 [IQR=(3.5-2.5)] and in CP+CHD it was 7.75 [IQR=(9-7)]. The difference was significant in all the groups.

Bleeding on probing (BoP%)

Median BoP % in Control group samples was 3.7 [IQR=(4.32-2.56)], in CP it was 42.07 [IQR=(46.08-39.22)], in CHD it was 15.77 [IQR=(18.12-10.26)] and in CP+CHD it was 53.03 [IQR=(62.75-47.37)]. Kruskal Wallis test did gives significant difference in the median values of all these parameters across studied groups ($p<0.05$).

4.7 PLAQUE INDEX SCORES AMONG GROUP PARTICIPANTS – Controls, CP, CHD and CP+CHD (Table 4.7)

Tooth no 16 (T1)

Our study results demonstrated Median T1 (16) in Control group samples was 3.5 [IQR=(5-3)], in CP it was 8 [IQR=(9-7)], in CHD it was 5 [IQR=(6-3)] and in CP+CHD it was 7 [IQR=(8-6)].

Table 4.6: Comparison of Dental Parameters across studied group of participants

Parameters	***Controls***	***CP***	***CHD***	***CP+CHD***	***p-value***
	Median (Q3 – Q1)	***Median (Q3 – Q1)***	***Median (Q3 – Q1)***	***Median (Q3 – Q1)***	
No. of teeth	27 (28-26)	19(22-17)	24.5 (26-22)	17 (19-16)	<0.01*
PD (mm)	1.5 (2-1)	4.5 (5-4)	2 (2.5-1)	5.5 (6-5)	<0.01*
CAL (mm)	2.25 (2.5-2)	7 (8-6)	2.75 (3.5-2.5)	7.75 (9-7)	<0.01*
BoP %	3.7 (4.32-2.56)	42.07 (46.08-39.22)	15.77 (18.12-10.26)	53.03 (62.75-47.37)	<0.01*

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, PD= probing depth, CAL= clinical attachment level, BoP= bleeding on probing, Test applied: kruskal wallis test, *p<0.05 considered statistically significant

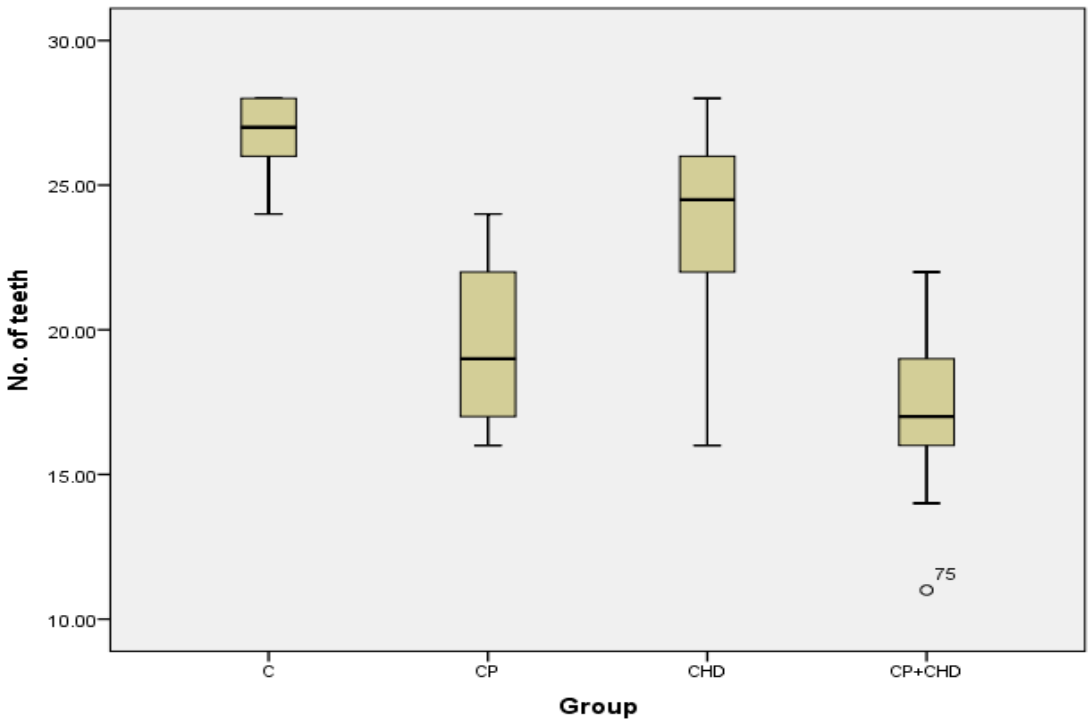


Figure 4.6(a) Box plot of no. of teeth showing skewed distributions with some of the extreme values in CP+CHD

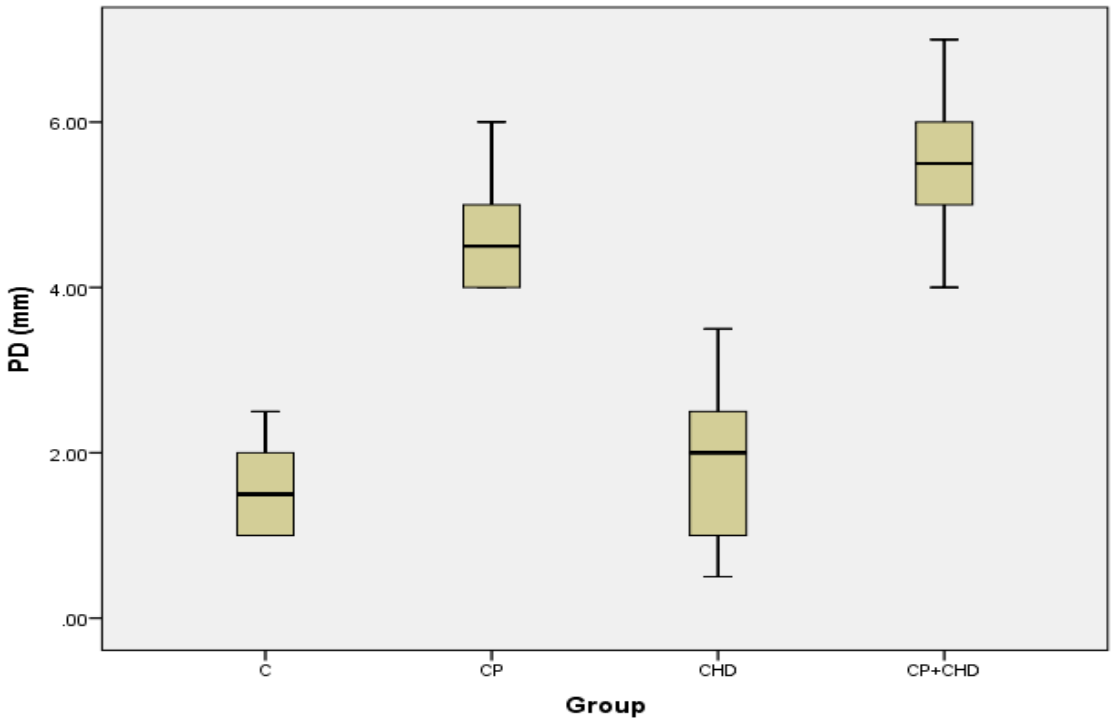


Figure 4.6(b) Box plot of PD showing skewed distributions with no outlier in any group

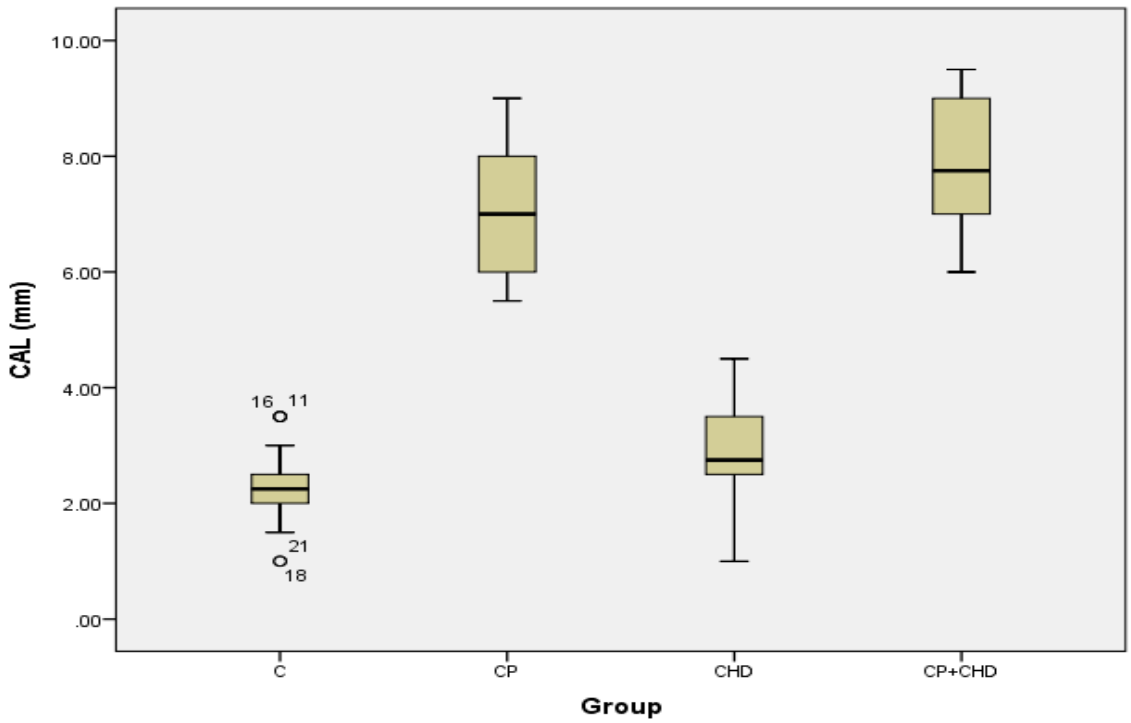


Figure 4.6(c) Box plot of CAL showing skewed distributions, higher values were observed in CP patients

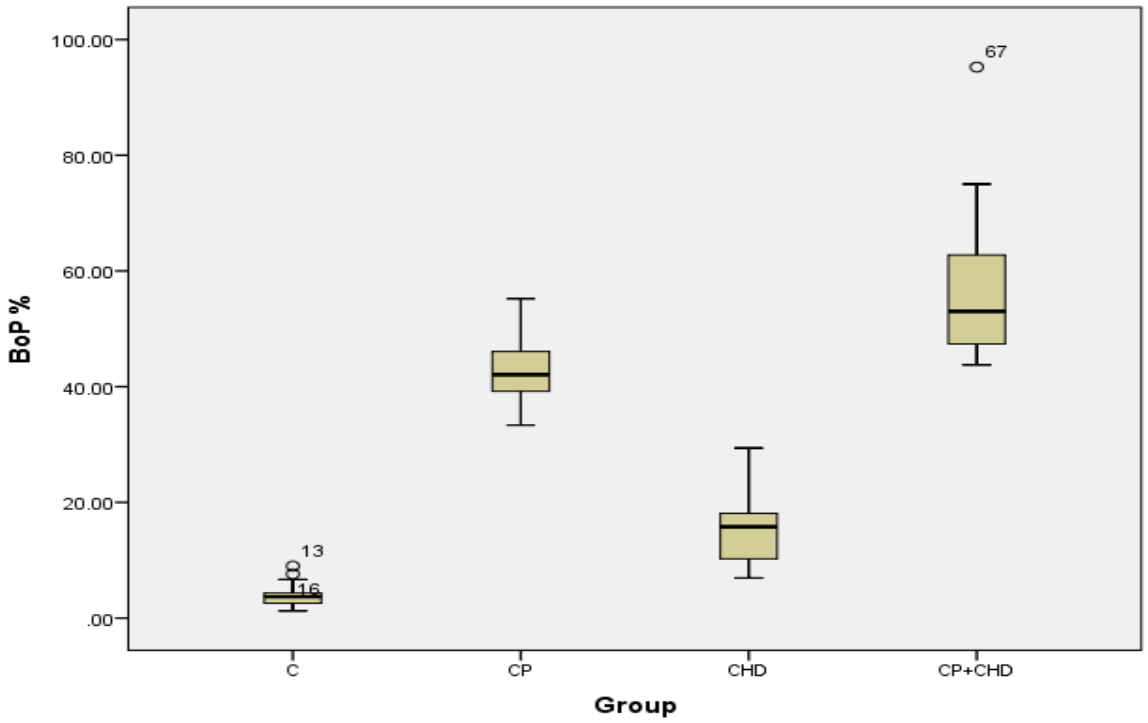


Figure 4.6(d) Box plot of BOP (%) showing skewed distributions with some extreme values in CP+CHD group

Tooth no 12 (T2)

Median T2 (12) in Control group samples was 3.5 [IQR=(5-3)], in CP it was 12 [IQR=(14-11)], in CHD it was 4.5 [IQR=6-3)] and in CP+CHD it was 6.5 [IQR=(8-6)].

Tooth no 24 (T3)

Median T3 (24) in Control group samples was 3 [IQR=(5-3)], in CP it was 5 [IQR=(6-5)], in CHD it was 4 [IQR=(6-3)] and in CP+CHD it was 7 [IQR=(8-6)].

Tooth no 36 (T4)

Median T4 (36) in Control group samples was 3.5 [IQR=(5-3)], in CP it was 7 [IQR=(8-7)], in CHD it was 3 [IQR=(6-2)] and in CP+CHD it was 8 [IQR=(9-6)].

Tooth no 32 (T5)

Median T5 (32) in Control group samples was 3 [IQR=(5-3)], in CP it was 10 [IQR=(10-9)], in CHD it was 3 [IQR=(7-2)] and in CP+CHD it was 7 [IQR=(9-6)].

Tooth no 44 (T6)

Median T6 (44) in Control group samples was 3 [IQR=3], in CP it was 10 [IQR=22], in CHD it was 3 [IQR=4] and in CP+CHD it was 7 [IQR=7.5].

Total Plaque Index Scores (TPIS)

Median TPIS in Control group samples was 23 [IQR=(23-22)], in CP it was 64.5 [IQR=(67-62)], in CHD it was 24 [IQR=(26-24)] and in CP+CHD it was 44.5 [IQR=(48-36)].

Plaque Index Score (PI)

Median PI Score in Control group samples was 0.96 [IQR=(0.96-0.92)], in CP it was 2.69 [IQR=(2.79-2.58)], in CHD it was 1 [IQR=(1.08-1)] and in CP+CHD it was 1.85 [IQR=(2-1.5)]. Kruskal Wallis test did gives significant median difference for all these parameters across studied groups ($p<0.05$).

4.8 INTERGROUP COMPARISON OF DENTAL PARAMETERS IN GROUP CONTROLS, CP, CHD AND CP+CHD (TABLE 4.7)

No of teeth

Number of teeth gives significant differences between all groups ($p<0.05$) using Mann Whitney U test.

Probing depth (PD)

Non-significant differences were observed between controls vs. CHD for PD (mm) while all other comparisons were found statistically significant ($p<0.05$).

Table 4.7: Comparison of Plaque Index Scores across studied groups

Parameters	***Controls***	***CP***	***CHD***	***CP+CHD***	***p-value***
	Median (Q3 – Q1)	***Median (Q3 – Q1)***	***Median (Q3 – Q1)***	***Median (Q3 – Q1)***	
T1 (16)	3.5(5-3)	8(9-7)	5(6-3)	7(8-6)	<0.01*
T2 (12)	3.5(5-3)	12(14-11)	4.5(6-3)	6.5(8-6)	<0.01*
T3 (24)	3(5-3)	5(6-5)	4(6-3)	7(8-6)	<0.01*
T4 (36)	3.5(5-3)	7(8-7)	3(6-2)	8(9-6)	<0.01*
T5 (32)	3(5-3)	10(10-9)	3(7-2)	7(9-6)	<0.01*
T6 (44)	3(4-3)	22(25-20)	4(6-2)	7.5(10-5)	<0.01*
TPIS	23(23-22)	64.5(67-62)	24(26-24)	44.5(48-36)	<0.01*
PI Score	0.96(0.96-0.92)	2.69(2.79-2.58)	1(1.08-1)	1.85(2-1.5)	<0.01*

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, T1= teeth 1(upper right first molar), T2= teeth 2 (upper right lateral incisor), T3= teeth 3 (upper left first premolar), T4= teeth 4 (lower left first molar), T5= teeth 5 (lower left lateral incisor), T6= teeth 6 (lower right first premolar), Test applied: kruskal wallis test, *p<0.05 considered statistically significant

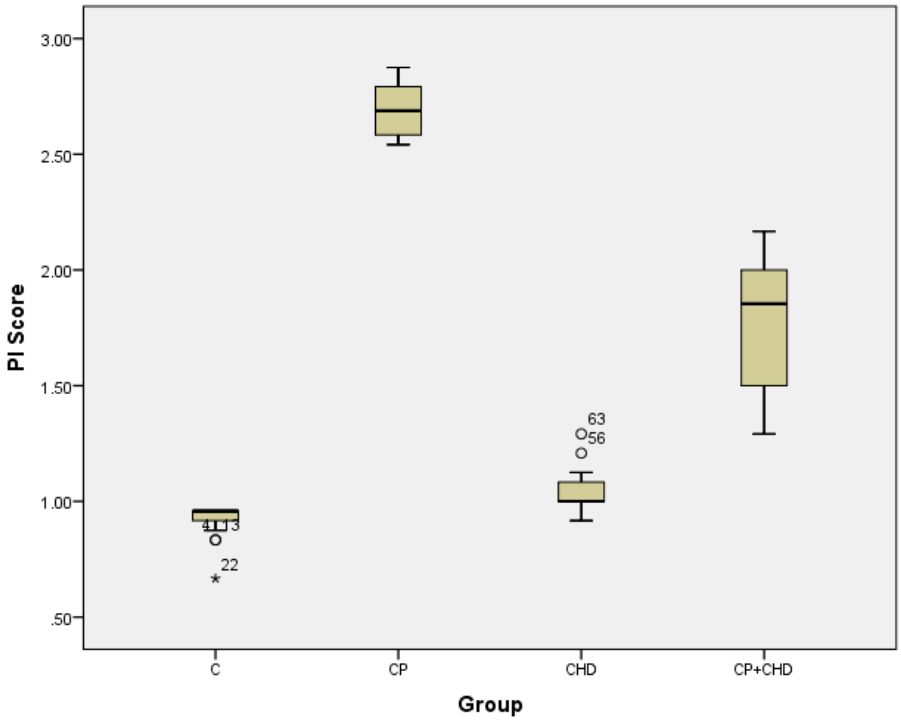


Figure 4.8 Box plot of PI scores showing skewed distribution, extreme values were on CHD patients

Clinical Attachment Level (CAL)

CAL was statistically significant among all the groups ($p<0.05$).

Bleeding on Probing (BoP)

BoP% was significant across all the studied groups ($p<0.05$).

Plaque Index Score (PI)

PI score was significant across all the studied groups using Mann Whitney U test ($p<0.05$).

4.9 CORRELATION OF ADMA WITH DEMOGRAPHIC, CLINICAL, BIOCHEMICAL AND DENTAL PARAMETERS (TABLE 4.9)

The correlation analysis of ADMA with studied parameters was performed using spearman rank correlation. The correlation was insignificant for the parameters age, BMI, SBP, DBP, TC, LDL, TG, PD and CAL. ADMA gave 59% positive correlation with studied groups, 49% negative correlation with gender, 21% positive correlation with CRP, 26% negative correlation with HDL, 33% negative correlation with no. of teeth, % positive correlation with BoP and 22% positive correlation with PI scores. All these correlations were found statistically significant with $p<0.05$.

4.10 UNIVARIATE LOGISTIC REGRESSION ANALYSIS FOR THE ASSOCIATION OF ADMA AND OTHER BIOCHEMICAL PARAMETERS WITH CP, CHD AND CP+CHD CASES (TABLE 4.10)

Our study reports the risk for periodontal cases using studied parameters. In univariate model patients with higher CRP and ADMA were found more likely to be case of CP, CHD or CP+CHD, whereas samples with higher HDL were found significantly less likely to be periodontal case. Total cholesterol also gives significant negative association with CP and CP+CHD patients. LDL gives significant negative association with CP+CHD cases. Risk for CHD and CP+CHD were increase with increase in CRP and ADMA of patients.

4.11 MULTIVARIATE LOGISTIC REGRESSION ANALYSIS FOR THE ASSOCIATION OF ADMA AND OTHER BIOCHEMICAL PARAMETERS WITH CP, CHD AND CP+CHD CASES (TABLE 4.11)

Our study reports the risk for periodontal cases using multivariate model after adjusting for age and gender. Results shows for CP, CHD and CP+CHD cases CRP and ADMA gives significant positive association, whereas HDL did give a negative association, samples with higher CRP and ADMA will be more likely to be found with case of CP, CHD or CP+CHD, and with higher HDL will be at less risk of periodontal case. Odds for CP+CHD were higher for CRP and ADMA and HDL parameters as compare to odds estimated for CHD or CP patients.

Table 4.8: Comparison of Dental Parameters between studied Groups

Parameters	***Controls vs. CP***	***Controls vs. CHD***	***Controls vs. CP+CHD***	***CP vs. CHD***	***CP vs. CHD+CP***	***CHD vs. CP+CHD***
No. of teeth	<0.01*	<0.01*	<0.01*	<0.01*	0.01*	<0.01*
PD (mm)	<0.01*	0.39	<0.01*	<0.01*	<0.01*	<0.01*
CAL (mm)	<0.01*	0.03*	<0.01*	<0.01*	0.02*	<0.01*
BoP %	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*
PI Score	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*	<0.01*

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, PD= probing depth, CAL= clinical attachment level, BoP= bleeding on probing, PI= plaque index, Test applied: mann whitney u test, *p<0.05 considered statistically significant

Table 4.9: Correlation Analysis of ADMA with Studied Parameters

Parameters	***r-value***	***p-value***
Group	0.59	<0.01*
Gender	-0.49	<0.01*
Age (years)	0.21	0.06
BMI (Kg/m2)	0.17	0.11
SBP mm/hg	0.12	0.27
DBP mm/hg	0.08	0.44
CRP mg/L	0.21	0.04*
TC (mg/dL)	-0.10	0.37
HDL-C (mg/dL)	-0.26	0.014*
LDL-C (mg/dL)	-0.05	0.64
TG (mg/dL)	0.14	0.18
No. of teeth	-0.33	<0.01*
PD (mm)	0.15	0.16
CAL (mm)	0.20	0.06
BoP %	0.43	<0.01*
PI Score	0.22	0.03*

BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, CRP= c-reactive protein, TC= Total Cholesterol, HDL-C= high density lipoprotein cholesterol, LDC-C= low density lipoprotein cholesterol, PD= probing depth, CAL= clinical attachment level, BoP= bleeding on probing, PI= plaque index, Test applied: spearman rank correlation, *Correlation with $p<0.05$ considered statistically significant

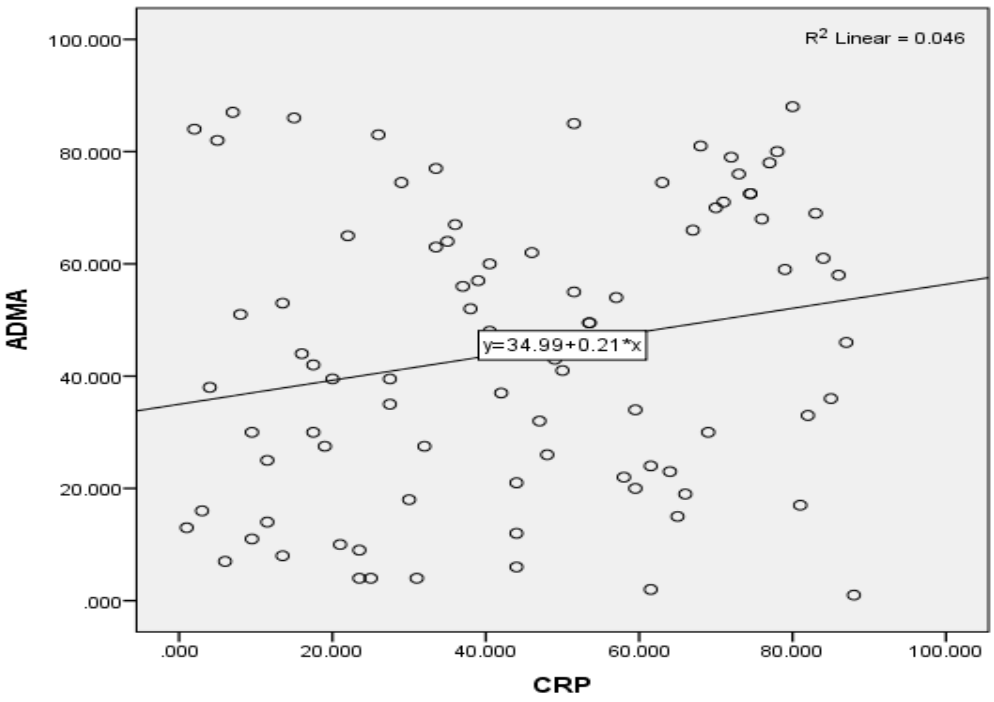


Figure 4.9a ADMA gives positive correlation with CRP, R-square showed 4.6% variation in ADMA was explained by CRP.

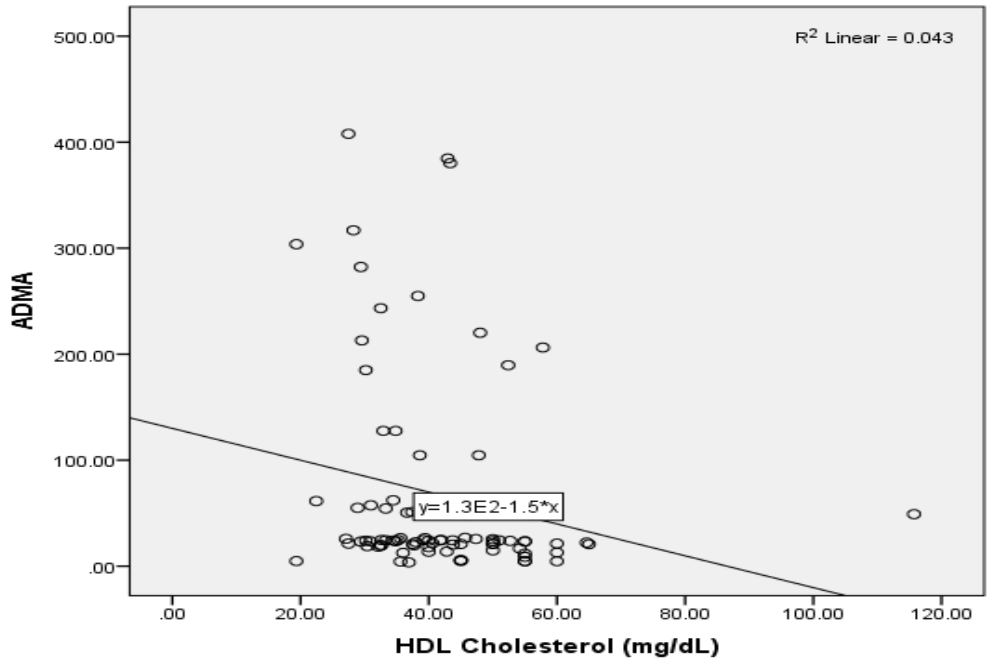


Figure 4.9b ADMA gives negative correlation with HDL, R-square showed 4.3% variation in ADMA was explained by HDL.

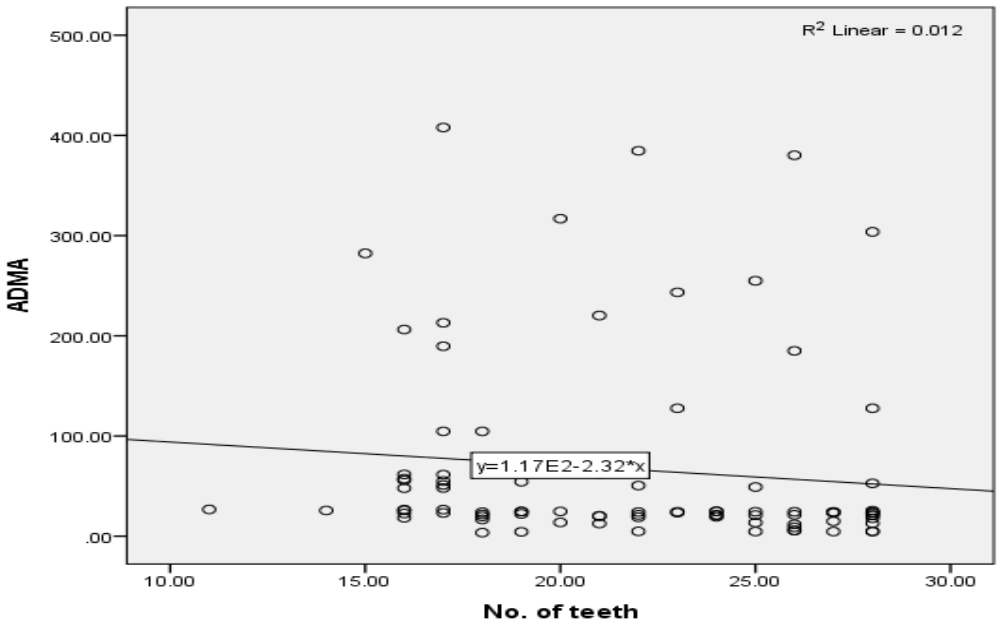


Figure 4.9c ADMA gives negative correlation with no of teeth, R-square showed 1.2% variation in ADMA was explained by no. of teeth.

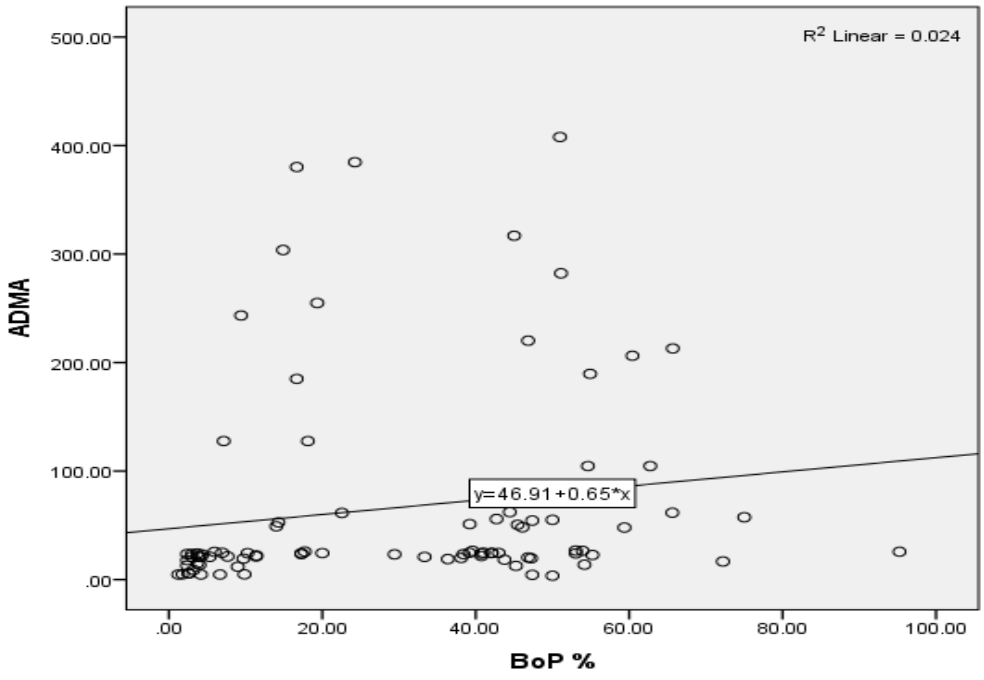


Figure 4.9d ADMA gives positive correlation with BOP (%), R-square showed 2.4% variation in ADMA was explained by BOP (%)

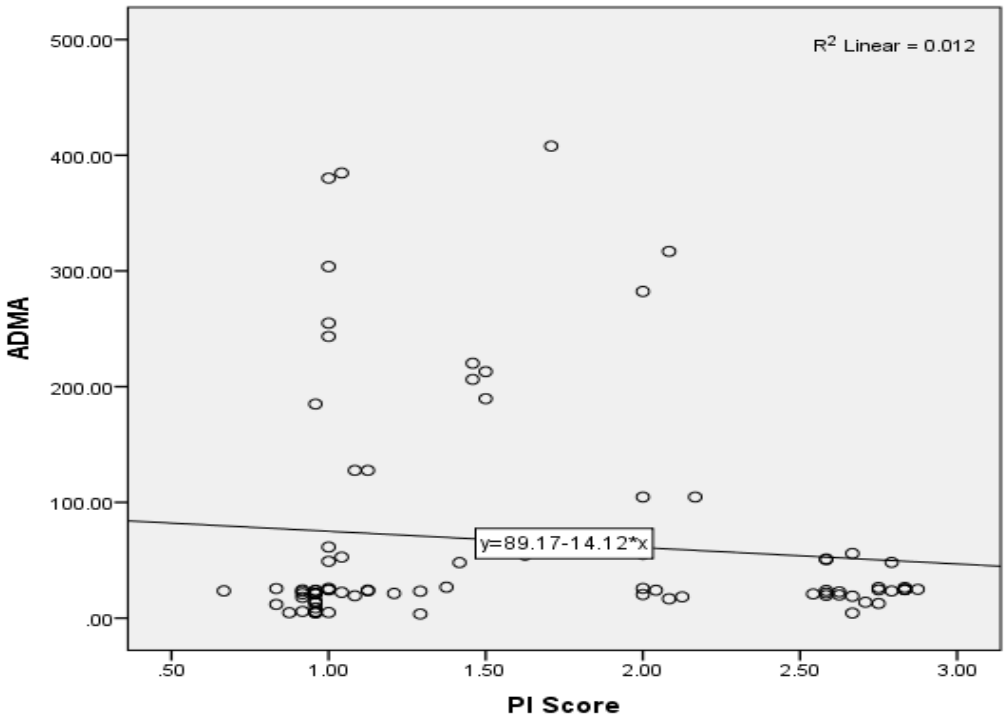


Figure 4.9e ADMA gives negative correlation with PI scores, R-square showed 1.2% variation in ADMA was explained by PI Scores

Table 4.10: Risk Estimation for Endothelial Dysfunction using Univariate Model

Parameters	***Univariate Model***		
	CP	***CHD***	***CP+CHD***
	Odds Ratio (95% C.I)	***Odds Ratio (95% C.I)***	***Odds Ratio (95% C.I)***
CRP mg/L	3.44* (1.75-6.76)	4.13* (2.09-8.15)	4.15* (2.11-8.19)
TC (mg/dL)	1.00 (0.98-1.01)	0.97* (0.95-0.99)	0.96* (0.93-0.98)
HDL-C (mg/dL)	0.91* (0.85-0.97)	0.91* (0.85-0.97)	0.92* (0.87-0.98)
LDL-C (mg/dL)	0.98 (0.96-1.00)	1.00 (0.98-1.01)	0.95* (0.93-0.98)
TG (mg/dL)	0.99 (0.98-1.01)	1.00 (0.99-1.01)	1.00 (0.99-1.01)
ADMA (μmol/L)	1.11* (1.02-1.20)	1.14* (1.06-1.24)	1.14* (1.06-1.24)

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, CRP= c-reactive protein, TC= total cholesterol, TG= Triglycerides, HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein Cholesterol, ADMA= Asymmetric dimethylarginine, Test applied: univariate logistic regression test, *odds ratio considered statistically significant with $p<0.05$

4.12 RECEIVER OPERATOR CHARACTERISTICS CURVE FOR ADMA IN DISEASED INDIVIDUALS - CP, CHD AND CP+CHD (TABLE 4.12)

Our study reports the results of ROC curve for ADMA, area under the curve of ADMA for cases was 85.3% considered statistically significant ($p<0.01$), the sensitivity was 92.4% and specificity was 45.5%, cutoff value of ADMA for cases suggested by ROC was 14.30 or greater.

4.13 RECEIVER OPERATOR CHARACTERISTICS CURVE ANALYSIS FOR ADMA IN CP PATIENTS (TABLE 4.13)

Our study reports the results of ROC curve for ADMA CP cases only, area under the curve of ADMA for CP cases was 77% considered statistically significant ($p<0.01$), the sensitivity was 95% and specificity was 13.6%, cutoff value of ADMA for CP cases suggested by ROC was 4.7 or greater.

4.14 RECEIVER OPERATOR CHARACTERISTICS CURVE ANALYSIS FOR ADMA IN CHD PATIENTS (TABLE 4.14)

Our study reports the results of ROC curve for ADMA CHD cases only, area under the curve of ADMA for CHD cases was 89% considered statistically significant ($p<0.01$), the sensitivity was 95% and specificity was 28%, cutoff value of ADMA for CHD cases suggested by ROC was 7.30 or greater.

Table 4.11: Risk Estimation for Endothelial Dysfunction using Multivariate Model

Parameters	***Multivariate Model***		
	CP	***CHD***	***CP+CHD***
	Odds Ratio (95% C.I)	***Odds Ratio (95% C.I)***	***Odds Ratio (95% C.I)***
CRP mg/L	3.30* (1.48-7.37)	3.96* (1.77-8.84)	3.93* (1.76-8.77)
TC (mg/dL)	1.00(0.98-1.02)	0.98(0.96-1.01)	0.97(0.95-1.00)
HDL-C (mg/dL)	0.91* (0.86-0.98)	0.91* (0.85-0.98)	0.94* (0.88-0.99)
LDL-C (mg/dL)	0.99 (0.96-1.01)	1.00 (0.98-1.02)	0.97 (0.94-1.00)
TG (mg/dL)	0.99 (0.97-1.01)	1.00 (0.99-1.01)	1.00 (0.98-1.01)
ADMA (μmol/L)	1.13* (1.01-1.26)	1.17* (1.04-1.30)	1.17* (1.05-1.31)

Group CP= chronic periodontitis, Group CHD= coronary heart disease, Group CP+CHD= chronic periodontitis and coronary heart disease, CRP= c-reactive protein, TC= total cholesterol, TG= Triglycerides, HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein Cholesterol, ADMA= Asymmetric dimethylarginine, Test applied: multivariate logistic regression test, *odds ratio considered statistically significant with $p<0.05$, Multivariate odds adjusted for age and gender

Table 4.12: ROC analysis of ADMA for cases (CP, CHD, CP+CHD)

Area	*S.E*	*p-value*	*Asymptotic 95% Confidence Interval*		*Sensitivity, Specificity, Cutoff value*
			Lower Bound	*Upper Bound*	
0.853	0.040	<0.01*	0.774	0.932	Sensitivity=92.4 Specificity=45.5 Cutoff =14.30
Test Result Variable(s): ADMA					

Table 4.13: ROC analysis of ADMA for CP patients

Area	*S.E*	*p-value*	*Asymptotic 95% Confidence Interval*		*Sensitivity, Specificity, Cutoff value*
			Lower Bound	*Upper Bound*	
0.77	0.07	<0.01*	0.63	0.91	Sensitivity=0.95 Specificity=13.6 Cutoff =4.7
Test Result Variable(s): ADMA					

4.15 RECEIVER OPERATOR CHARACTERISTICS CURVE ANALYSIS FOR ADMA IN CP+CHD PATIENTS (TABLE 4.15)

Our study reports the results of ROC curve for ADMA CP+CHD cases only, area under the curve of ADMA for CP+CHD cases was 88% considered statistically significant ($p<0.01$), the sensitivity was 95% and specificity was 32%, cutoff value of ADMA for CP+CHD cases suggested by ROC was 10.3 or greater.

Table 4.14: ROC analysis of ADMA for CHD patients

Area	*S.E*	*p-value*	*Asymptotic 95% Confidence Interval*		*Sensitivity, Specificity, Cutoff value*
			Lower Bound	*Upper Bound*	
0.89	0.05	<0.01*	0.79	0.99	Sensitivity=0.95 Specificity=0.28 Cutoff =7.30
Test Result Variable(s): ADMA					

Table 4.15: ROC analysis of ADMA for CP+CHD patients

Area	*S.E*	*p-value*	*Asymptotic 95% Confidence Interval*		*Sensitivity, Specificity, Cutoff value*
			Lower Bound	*Upper Bound*	
0.88	0.05	<0.01*	0.77	0.99	Sensitivity=0.95 Specificity=0.32 Cutoff =10.3
Test Result Variable(s): ADMA					

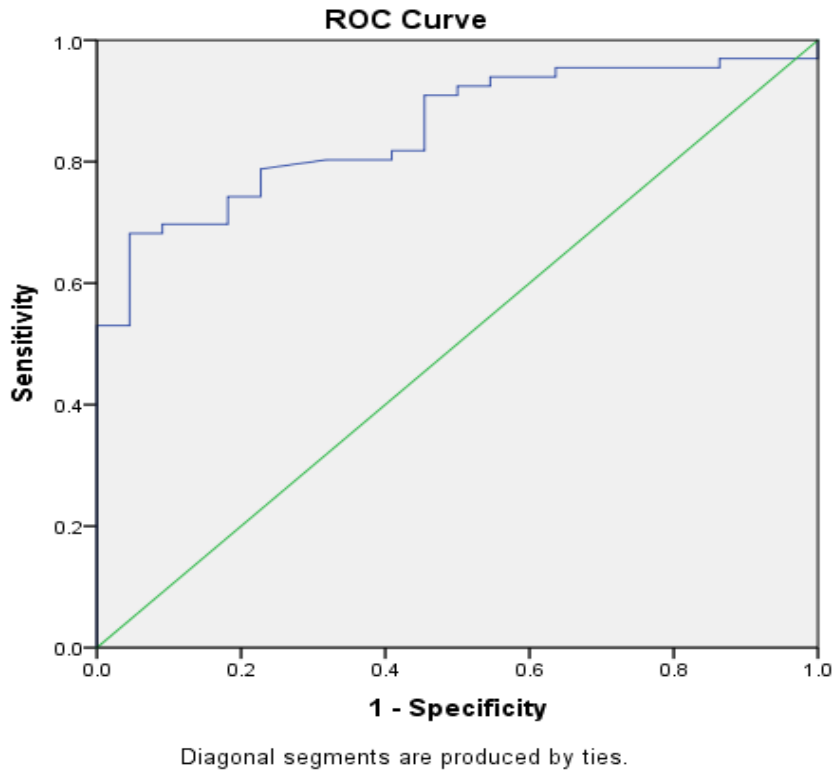


Figure 4.12 ROC curve of serum ADMA in diseased individuals

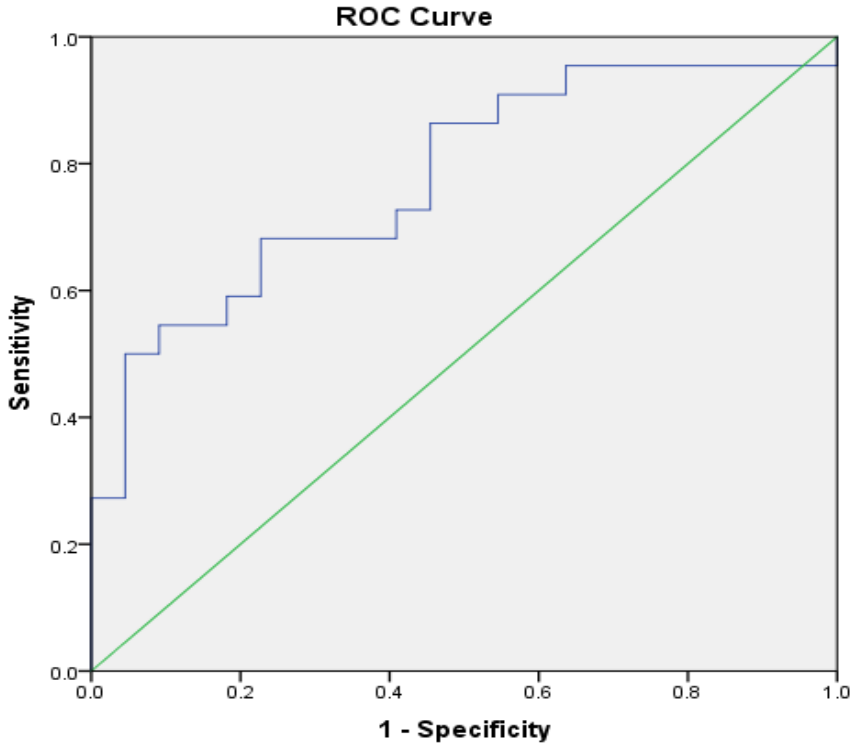


Figure 4.13 ROC curve of serum ADMA in CP patients

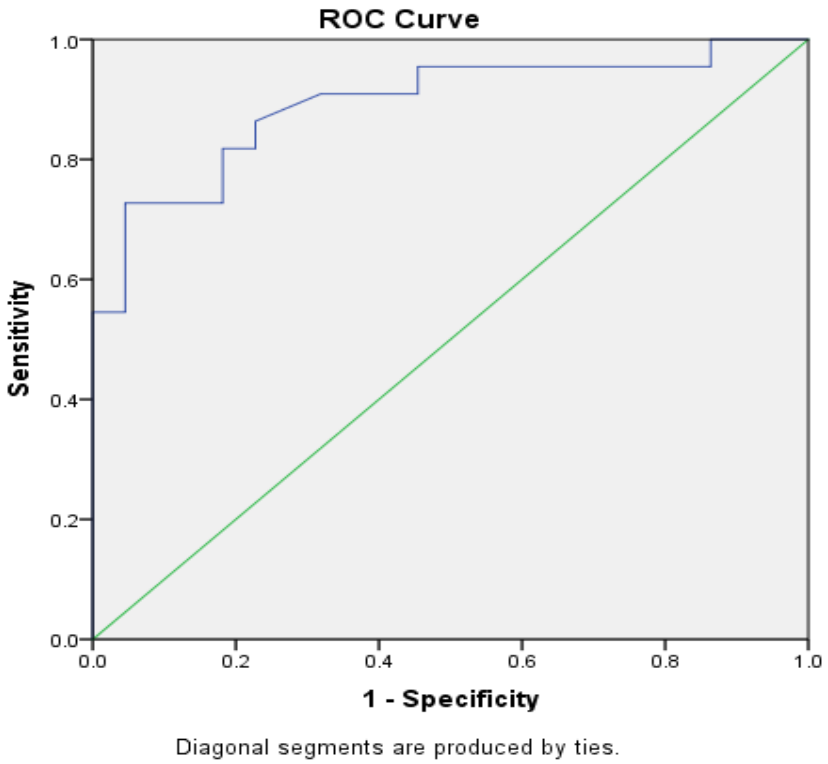


Figure 4.14 ROC curve of serum ADMA in CHD patients

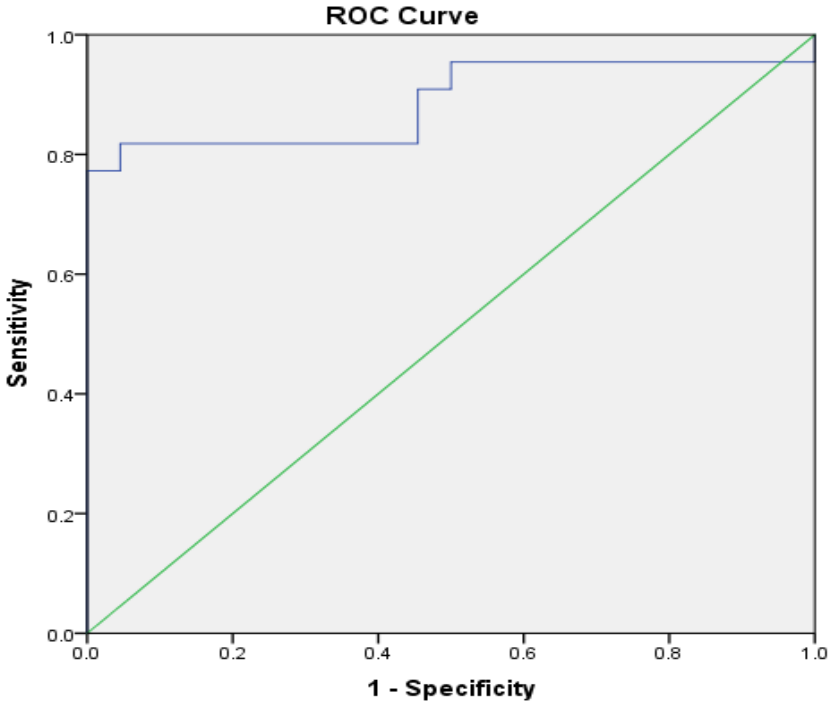


Figure 4.15 ROC curve of serum ADMA in CP+CHD patients

CHAPTER 5

DISCUSSION

5.1 SEQUENCE OF DISCUSSION EXPERIMENT

Endothelial dysfunction is essential to the pathog12enesis of numerous systemic diseases, mainly coronary heart disease (CHD). It is the initial stage for the development of an atherosclerotic lesion which is the hallmark of various cardiovascular disorders. Endothelium, which lines the interior surface of blood vessels, is a critical regulator of vascular homeostasis, as has been well established. Numerous cardiovascular disorders have been linked to impaired endothelial function, highlighting the need for dependable biomarkers to evaluate endothelial dysfunction.

ADMA has emerged as a promising candidate for assessing endothelial dysfunction in a number of clinical conditions. ADMA is a non-proteinogenic amino acid endogenous inhibitor of nitric oxide synthase, the enzyme accountable for the production of vasodilator and anti-inflammatory molecule nitric oxide (NO). Multiple cardiovascular diseases, such as CHD, hypertension, and atherosclerosis, have been associated with elevated ADMA levels. Recent studies have also implicated ADMA in the course of chronic periodontitis, a chronic inflammatory condition that affects the tooth support structures.

Chronic periodontitis and coronary heart disease share associated risk factors such as smoking, diabetes, and dyslipidemia, and have bidirectional relationships. Endothelial dysfunction is regarded as a crucial mediator in this interaction, potentially contributing to

the elevated cardiovascular risk observed in chronic periodontitis patients. Consequently, there is a growing interest in investigating ADMA as a possible biomarker for assessing endothelial dysfunction in both chronic periodontitis and CHD.

Multiple studies have examined the relationship between ADMA levels and endothelial dysfunction in these two conditions. Sengul et al., 2022 found elevated levels of ADMA in patients with chronic periodontitis compared to healthy controls, indicating a potential role for ADMA in assessing endothelial dysfunction in periodontal disease. Likewise, Isola et al., 2022 found an association between elevated ADMA levels and impaired endothelial function in CP and CHD patients. Guney et al., 2023 implied that methylated arginine metabolites which included ADMA is involved in the pathogenesis of periodontal disease. Since physiological levels of ADMA regulates endothelial dysfunction, abnormal levels attributed to periodontal inflammation may indirectly contribute to damage of the tissues (Güney et al., 2023).

Despite these findings, however, the evaluation of ADMA as a marker of endothelial dysfunction for chronic periodontitis and CHD remains an active area of research. To establish the clinical efficacy of ADMA in this context, a number of crucial concerns and knowledge deficiencies must be addressed. First, the temporal relationship between ADMA levels and the development of endothelial dysfunction in both diseases must be investigated. Valuable insights could be gained from longitudinal studies examining ADMA levels over time and their association with changes in endothelial function.

In addition, potential confounding factors and their effect on ADMA levels must be carefully considered. Traditional cardiovascular risk factors, such as age, gender, smoking, and comorbidities, may independently influence ADMA levels and endothelial function. Understanding the interaction between these factors and ADMA would enhance its utility as a marker of endothelial dysfunction in chronic periodontitis and CHD.

In addition, it is crucial to investigate the potential of ADMA as a biomarker predictive of cardiovascular events in patients with chronic periodontitis. Significant clinical

implications would result from determining the prognostic value of ADMA in identifying individuals at high risk of developing CHD or experiencing adverse cardiovascular outcomes. This would not only allow for early intervention and risk stratification, but it would also shed light on potential therapeutic targets for enhancing cardiovascular outcomes in this population.

In the present study, 59.1% individuals were aged between 41 - 50 years old in the CP group which is similar to a study conducted by Shazam et al. (2020). Among the CHD and CP+CHD groups, 54.5% and 68.2% were aged above 50 years. This is in coherence with the study conducted by Babiker et al. (2021). In control group there were 59.1% participants who were aged less than or equal to 40 years. Males were predominant among all the four groups (59.1%, 81.8%, 95.5%, 100%) than females (40.9%, 18.2%, 4.5%, 0.0%). This could potentially be attributed to the fact that oral hygiene and periodontal health in males failed to satisfy the necessary criteria for a healthy periodontium during enrollment. In regard to this, a greater number of male subjects were recruited in comparison to females. This corresponds to a study by Aoyama et al. (2018) in which the periodontal status was more unsatisfactory among participants in the male cohort. However, this is in contrast to the findings reported by Isola et al. (2021). Overall, the demographic parameters such as age and gender were statistically significant among all the four groups.

High blood pressure has long been recognized as a risk element for the initiation of heart disease. A strong association between blood pressure and cardiovascular disease has been suggested in individuals with lower BP values SBP (120–139 mmHg) and DBP (80–89 mmHg) who are not exposed to CHD (Fuchs & Whelton, 2020). The vital signs which were assessed in the study participants included diastolic blood pressure and systolic blood pressure. In the control group mean SBP was 118.9 ± 2.29 mm/hg, and mean DBP was 78.27 ± 2.05 mm/hg. In the CP group the mean SBP was 147.82 ± 5.04 and mean DBP was 93.59 ± 4.18 which is regarded as elevated blood pressure according to the blood pressure guidelines (Flack & Adekola, 2020). Aguilera et al. (2021) reported similar findings in their study where they found out that individuals with periodontitis presented with elevated systolic and diastolic blood pressure as compared to controls. This explains the increased

bleeding of gums in periodontitis individuals. The findings of elevated systolic blood pressure were independent of traditional cardiovascular risk factors. Periodontitis may increase the occurrence of hypertension through several mechanisms. First, systemic inflammation from periodontitis may alter endothelial function through CRP, IL-6, and TNF-α (D'Aiuto et al., 2018). Clinical evidence shows periodontitis impacts systemic endothelial function and hypertension. Second, oral microbiota-related bacteremia may also directly cause vascular dysfunction. An immunological response to Porphyromonas gingivalis (Pg), a common periodontal infection, vascular inflammation, increased BP, and endothelial dysfunction in experimental animals (Czesnikiewicz-Guzik et al., 2019). Inflamed periodontium may prime B cells, T cells, and monocyte/macrophages to chemotactically recruit to perivascular adipose tissue and adventitia, which has been linked to vascular dysfunction, hypertension, and atherosclerosis (Guzik et al., 2017; Mikolajczyk et al., 2016). In the CHD group the mean SBP was 120.77 ± 13.48 and mean DBP was 76.45 ± 8.16. These results are line up with the findings reported by Jorge-Galarza et al. (2020). This in part explains that majority of the patients were undergoing treatment for hypertension. In the CP+CHD group the mean SBP was 139.00 ± 2.38 and mean DBP was 90.14 ± 12.90. These findings are in contrast to the findings reported by Bokhari et al. (2014). However, the SBP and DBP was statistically significant among all the groups.

In CP patients for family history 22.7% were reported for diabetes which is contradictory to the findings reported by Romano et al., 2021. He reported 68.3% periodontitis individuals had a positive family history for diabetes. 13.6% were reported for hypertension which is in accordance to the literature reported by Heji et al., 2021. 4.5% were reported for family history of CAD/IHD. These results are in contrast to the findings reported by Machado et al., 2021. In CHD patients for family history 27.3% were reported for diabetes, 31.8% were reported for hypertension, 4.5% were reported for asthma/COPD which is aligned with the literature reported by Andriani et al., 2022. 27.3% were reported for CAD/IHD which is aligned with the findings of Swarnkar et al., 2022. 4.5% were reported for Hyperlipidemia.

While for the drug treatment of CHD 90.9% uses Antiplatelets, 4.5% on Anticoagulants,4.5% on Angiotensin receptor blockers, 18.2% on Beta-blockers, 9.1% on Calcium channel blockers, 90.9% on Statins, 18.2% uses Anti-hypertensives, 4.5% uses Analgesics, 54.5% uses Proton pump inhibitors, and 31.8% uses Diuretics medicines. This is in contrast with the literature reported by Isola et al. (2021). In his study he found that 46.2% CHD patients were taking antihypertensive, 33.3% were on statin therapy, 38.5% were consuming low-dose aspirin while 35.90% were taking beta blockers. Another study conducted by Isola et al. (2019) reported that 42.4% were on antihypertensive therapy, 39.4% were on statins, 36.4% were consuming low dose aspirin while 39.4% were on beta blockers. In CP +CHD group for drug treatment 81.8% uses Antiplatelets, 40.9% uses Nitrates, 9.1% uses Angiotensin-converting enzyme inhibitors, 22.7% uses Beta-blockers, 4.5% uses Calcium channel blockers, 72.7% uses Statins, 4.5% reported for Anti-hypertensives, 22.7% reported for Analgesics, 22.7% were reported for Proton pump inhibitors and 13.6% uses Diuretics medicines. Conversely, Isola et al. (2021) found that among CP+CHD patients, 50% were on antihypertensive therapy, 36.8% were on statins, 42.1% were consuming low dose aspirin while 36.8% were on beta blockers. Similarly, Isola et al. (2019) reported in his study that among CP+CHD group 44.1% patients were taking antihypertensive, 38.2% were on statin therapy, 35.3% were consuming low-dose aspirin while 41.2% were taking beta blockers

C-reactive protein is an acute phase reactant protein and an imprecise marker of inflammation synthesized by liver. It is strongly associated with risk of endothelial dysfunction and is significantly found elevated in the course of various inflammatory conditions (Colantonio et al., 2022). The median values of hsCRP were significantly higher in CP patients as opposed to controls. Esteves-Lima et al. (2020) reported similar findings in their study when hsCRP was assessed in CP and normal healthy individuals. In another study carried out by S. Gupta et al. (2020) it was observed that the patients having healthy periodontium had lesser mean hsCRP levels contrasted with the patients with chronic periodontitis. Similarly, Isola et al, 2019 found out that CP patients had increased hsCRP levels compared to controls. In another study conducted by Isola et al. (2021) it was found that CP individuals had increased hsCRP levels in contrast to controls. C-reactive protein

was considerably raised in CHD patients in contrast to controls. This is in coherence to the conclusions reported by Tayefi et al. (2017) which indicated that hsCRP is intensely related to CHD in contrast to the traditional biomarkers such as LDL and FBG. Conversely low hsCRP levels were reported in a study conducted by Aoyama et al. (2018). Winning et al. (2020) also reported low values for hsCRP in incident and prevalent CHD cases. Similarly, patients in CP+CHD group had significantly increased c-reactive protein values as compared to controls and CP groups. These findings are in line with the findings reported by studies conducted by Isola et al. (2019), Isola et al. (2020) and Isola et al. (2021) in which hsCRP was considerably raised in CP+CHD patients as compared to CP,CHD and controls. A study conducted on periodontitis and coronary heart disease patients demonstrated that hsCRP was significantly increased in CHD+CP patients (Kumar et al., 2014). Another study conducted on chronic periodontitis and coronary heart disease patients in an ethnic Han population demonstrated that CP+CHD patients have the highest mean hsCRP levels and the controls the lowest (Liu et al., 2010). These studies are in coherence with findings of our study.

Total cholesterol comprises of HDL and LDL which are considered as good and bad cholesterol for health. Any imbalance between the two leads to the formation of coronary heart disease. Based on the findings of our investigation, it was found that TC was predominantly increased in CP+CHD group contrasted to controls. Our results further demonstrated that TC levels were considerably increased in CP+CHD group as opposed to CP patients. These findings are in contrast to the study conducted by Isola et al. (2020). Furthermore, it was found that TC levels were significantly low in CHD patients as opposed to controls. These findings are in contrast with the data reported by Al-Rawi & Shahid. (2017) he reported that the mean level of serum TC was lower in CHD patients compared to healthy controls, although the difference was not significant statistically. It was found that CHD and CP+CHD patients had lower cholesterol levels compared to CP individuals and controls. It is attributed to the fact that these individuals were on cholesterol lowering therapy. Similar findings were observed in the literature reported by Isola et al. (2021). However, Isola et al. (2021) found out in another study that CP+CHD individuals had slightly higher cholesterol levels compared to CP, CHD and controls. A study conducted by on CP patients with normal

weight reported that periodontitis individuals had normal cholesterol levels which is similar to the findings of our study (Cury et al., 2017).

HDL has an atheroprotective effect which is mediated by reverse cholesterol transport. It's the release of cholesterol into the bloodstream from peripheral tissues (such as foam cells in atherosclerotic plaques) and subsequent elimination in the stool. Conventionally, the cardiovascular protective effect of HDL is attributed to its ability to receive cholesterol from cells and transport cholesterol along the RCT pathway, including to the liver. The HDL-C was significantly elevated in CP patients as opposed to controls in our study. This is contradictory to the research conducted by Han et al. (2020), he found lower HDL-C concentrations in CP patients as compared to controls. It is demonstrated in studies that individuals have more tendency to develop cardiovascular disease due to decreased HDL levels more than the total cholesterol. Subsequently, HDL levels were considerably low in CHD patients as contrasted with controls. This is in line with the findings reported by Shabana et al. (2020). In our study the concentrations of HDL-C were significantly increased in CP+CHD patients as opposed to controls. Conversely, Sanikop et al. (2022) reported low HDL levels in coronary heart disease patients presented with periodontitis in contrast to controls.

LDL cholesterol is regarded as the bad cholesterol for health because it contributes to the development of atherosclerotic plaque in coronary arteries. In our study it was found that there was an essential disparity between the two in the concentrations of LDL-C in CP patients as contrasted with controls. The levels of LDL-C were ominously decreased in CP patients compared to controls which is contrast to the findings reported by Sanikop et al. (2022). However, HDL was higher in healthy individuals as well as those having mild periodontitis, but it was lesser in people with moderate or severe periodontitis. LDL was higher in people with severe or moderate periodontitis and lower in individuals who were systematically healthy (O et al., 2022). Likewise, LDL-C levels were considerably low in CP+CHD group as compared to the individuals in CP group. It is contradictory to findings reported by (Tang et al., 2011).Similarly the levels of LDL-C were also low in CP+CHD patients compared to controls. During viral and inflammatory disorders, changes in

lipoprotein metabolism and structure occur, with a rise in TG and LDL levels and a decrease in HDL levels. These changes initially defend against infections, but if they persist, they lead to atherogenesis. In addition to boosting excess cholesterol removal from peripheral tissues and transferring it to the liver for the process of excreation from the body, HDL plays a role in immunity via its antibacterial, anti-inflammatory and antioxidant characteristics. As a result, lower HDL levels and, as a result, higher levels of pro-atherogenic lipids, which are typical of dyslipidemia, may trigger a severe proinflammatory state (Cury et al., 2018).

No of teeth was significant in individuals in group CP, CHD and CP+CHD as compared to controls. This finding was similar to the study conducted by Isola et al. (2020). Subsequently this parameter was significant in CP group compared to CHD patients, CHD group compared to CP+CHD group and CP group in contrast to CP+CHD group. This is consistent to the literature reported by Ferlazzo et al. (2021). The highest median values for parameters PD, CAL and BoP was recorded in individuals having combined disease (CP+CHD). Rezaei et al. (2021) conducted a study on periostin levels to evaluate the association between coronary heart disease and chronic periodontitis. He found out that the highest mean values were found in the group CHD-CP for periodontal parameters. Another study conducted by Kumar et al. (2014) demonstrated the highest values for these parameters in combine disease patients. Furthermore, our findings are in coherence with the literature reported by Sanikop et al. (2022) which demonstrated highest mean values for PPD and CAL in CP+CHD patients. The PD,CAL and BoP was considerably high in the CP+CHD individuals in contrast to CP Yagnik et al. (2019). The difference between the parameters PD, CAL and BoP was significant in CP compared to CHD patients. Isola et al. (2020) reported a similar findings while evaluating the role of vitamin D in periodontitis individuals.

Plaque index is used to assess the oral health of individuals. It is one of the determinants of periodontal health. The plaque index assessed for all the individual index teeth (T1-T6) were significant for all the groups. Furthermore, the median plaque index (PI) score was statistically significant among all the groups. Findings comparable to these were reported by Mahmood & Omer (2016). Raheem & Ahmed (2014) studied the link between periodontitis and ASCVD through assessment of MMP-8 and hs-CRP in periodontitis and

heart patients. Among the other periodontal parameters plaque index was statistically significant between the CP group, ATH+CP (atherosclerotic cardiovascular disease & chronic periodontitis) and controls. Majdiah M & Norsuryani (2017) carried out a study to determine the relation between hypertension with periodontal parameters in patients. He found that that the difference between the case groups were insignificant which is contradictory to our findings.

The correlation of ADMA with the studied parameters was insignificant for age, BMI, DBP, SBP, TC, LDL, TG, PD and CAL. However, the correlation analysis of ADMA with studied parameters reported that ADMA gives positive correlation with studied groups and negative correlation with gender. This is aligned with the findings reported by Gruber et al. (2008). Subsequently our study found a negative correlation with the number of teeth supporting the fact that ADMA may be a causative factor in the etiology of periodontits promoting loss to alveolar bone and thereby loosening and ultimately loss of teeth. However, there is scarcity of evidence to support this theory. It was found in our study that ADMA has a correlation in a positive direction with CRP. Similarly, a clinical study by Isola et al. (2020) found that ADMA was positively correlated with CRP in all the enrolled individuals in his study. Subsequently, Tsioufis et al. (2010) found that ADMA and CRP levels in serum were shown to be related with one another in a dose-varying manner in CP patients with untreated hypertension. Consequently, our study findings demonstrated negative correlation with HDL. These findings are consistent with the literature reported by Pase et al. (2020). ADMA demonstrated positive correlation with BoP and PI scores. These findings are parallel with the literature reported by Güney et al. (2023). Findings comparable to these were reported by Şengül et al. (2022) for correlation between ADMA and clinical periodontal parameters.

Multinomial logistic regression model was used to evaluate the probability of a person to develop endothelial dysfunction while accounting for factors such as age and gender. The results pointed out the associations between certain biomarkers (ADMA, CRP, HDL, LD and TGs) and the odds of developing heart disease by means of endothelial dysfunction. It was indicated that elevated levels of CRP and ADMA are significantly associated with a higher probability of having endothelial dysfunction in patients with CP, CHD, or both conditions

occurring simultaneously (CP+CHD). This correlation suggests that elevated CRP and ADMA levels may contribute to endothelial dysfunction, a condition characterized by impaired endothelial function (the inner lining of blood vessels). Endothelial dysfunction is a crucial precursor to the onset of numerous cardiovascular diseases. According to our study findings, higher HDL concentrations are correlated with a lower risk of endothelial dysfunction. Therefore, individuals with CP+CHD combined who have greater HDL levels are at lower risk for developing endothelial dysfunction. In terms of cardiovascular health, HDL (the "good cholesterol") is well recognized as a protective factor. Due to this inverse correlation, it is possible that patients with greater HDL levels may less likely to develop endothelial dysfunction. In addition, we evaluated the odds ratios for CP+CHD, CHD, and CP cases for three biomarkers (CRP, ADMA, and HDL). The odds ratio shows the probability of a certain result happening in response to exposure to a specific risk factor. When CRP, ADMA, and HDL levels are included, the probabilities of developing endothelial dysfunction in individuals with both Chronic Periodontitis and Coronary Heart Disease (CP+CHD) are greater than in patients with either CHD or CP alone. When analyzing these particular indicators, this shows that the combination of CP and CHD may have a greater influence on endothelial dysfunction than each disease alone. The clinical parameters (CRP & HDL) of present study coincide with the study by Liu et al. (2010). CRP and HDL cholesterol were found to be significantly associated with an increased frequency of CHD in this regression model in their study.

Based on the findings of our investigation, serum ADMA concentration were considerably raised in individuals with both CP and CHD in contrast to those with CP and controls. This finding is consistent with previous studies that have suggested a link between ADMA, endothelial dysfunction, and cardiovascular disease (Ferlazzo et al., 2021; Isola et al., 2020). The concurrent existence of CP in CHD patients may contribute to the observed decline in endothelial function via elevated ADMA levels. It was found in a clinical trial, plasma concentrations of ADMA were lower in patients who received intensive periodontal treatment after 3 and 6 months than in controls (Rapone et al., 2022). Similarly, the levels of ADMA were significantly increased in CHD patients compared to controls. Roomi et al. (2023) conducted a study was to find out how effectively serum ADMA levels may be used

to diagnose cardiovascular risk in people with coronary heart disease. Serum ADMA levels, he found, had good potential as a biomarker for measuring cardiovascular risk. Subsequently our study demonstrated significantly increased ADMA levels in CP patients as in contrast to controls. Şengül et al. (2022) conducted a study on Stage III Grade B generalized periodontitis and healthy individuals to evaluate the role of periodontal inflammation in systemic disease. Consequently, he found out that ADMA concentration were significantly increased in periodontitis patients in contrast to controls. Besides it was found out in our study that the median values of ADMA were significantly elevated in CP+CHD patients compared to CP and controls. These results are in line with the literature reported by Ferlazzo et al. (2021).

Receiver operator characteristics curve illustrates the ability of a binary classifier to discriminate between true positive (sensitivity) and false positive (specificity). It is a graphical depiction of how well a binary classifier does when the threshold for differentiation is changed. In our study it was plotted to assess the ability of ADMA to predict endothelial dysfunction in patients with disease. The ROC curved reported for ADMA in diseased individuals (CP, CHD and CP+CHD) reported the sensitivity of 92.4% and specificity of 45.5%. The cutoff value of ADMA determined for cases was 14.30µmol/L as per the ROC analysis. There seems no evident literature to support or contradict the above findings. Subsequently, the ROC curve reveled the sensitivity of ADMA was 95% and specificity was 13.6% for individuals with chronic periodontitis. The threshold value of ADMA for CP cases suggested by ROC was 4.7 µmol/L. However, there seems no literature is available to support or contradict these findings. Consequently, the ROC curve for CHD cases demonstrated the sensitivity was 95% and specificity was 28%. The cutoff value of ADMA for CHD cases suggested by ROC was 7.30 µmol/L. These findings are in contrast with Roomi et al. (2023). His study demonstrated a threshold value of 1.05 µmol/L. In addition, the sensitivity was 95% and specificity was 32% for ADMA when the diagnostic efficacy was determined for CP+CHD cases. Furthermore, the threshold of ADMA for CP+CHD cases suggested by ROC was 10.3 which is contradictory to the optimal cut off value reported by Ferlazzo et al. (2021). He reported the optimal value of 1.25 µmol/L.

5.2 IMPLICATIONS OF THE STUDY

5.2.1 THEORETICAL IMPLICATIONS

The link between ADMA levels (as a measure of endothelial dysfunction) in patients with periodontitis (CP) and coronary heart disease (CHD) subjects that was identified in the research lends credence to the concept that periodontitis may be a risk factor or contributing factor in the development or progression of coronary heart disease. The deterioration of the endothelium is one of the most important contributors to the formation of atherosclerosis, which is one of the most important underlying processes in coronary heart disease. Since the research found a substantial correlation between ADMA levels and endothelial dysfunction in CP individuals as well as CHD, this lends credence to the theory that endothelial dysfunction may be a shared mechanism that links periodontitis and coronary heart disease.

5.2.2 PRACTICAL IMPLICATIONS

The results of our study has proposed that ADMA has a potential therapeutic target due to its involvement in endothelial dysfunction and inflammatory processes in periodontitis individuals. Reducing ADMA levels or augmenting endothelial function may have positive effects on periodontal health and contribute to better cardiovascular outcomes in periodontitis patients.

5.2.3 POLICY IMPLICATIONS

The study underscores the importance of maintaining good oral health as a preventive measure for cardiovascular disease. Healthcare providers may emphasize the significance of regular dental check-ups and periodontal care in patients with cardiovascular risk factors. Furthermore, since serum ADMA is determined to be a reliable marker of endothelial

dysfunction, it could be readily measured in clinical settings and become a standard component of cardiovascular risk assessment.

5.3 LIMITATIONS AND STRENGTHS OF THE STUDY

(A) LIMITATIONS

Lack of randomization and generalizability

Consecutive sampling lacks randomization since it depends on participant availability. The sample acquired by this technique could not be as varied as the sample obtained through random sampling, which limits generalizability. This can affect how well the results of the research can be applied to different demographics, environments, or patient groups.

Causality

The study design is another limitation to find causal associations among ADMA levels, endothelial dysfunction, periodontal disease, and coronary heart disease.

Effects of medications

We did not take into consideration the possible influence of medication usage on our findings which might have effects on the results of our study.

Heterogeneity of participants

Individuals with periodontitis and CHD can have distinct characteristics, disease intensity, and reactions to treatment. The study might not take these differences into account, which could hide important subgroups where ADMA and vascular dysfunction are linked in different ways.

(B) STRENGTHS

Less confounding factors

Diabetes and smoking are well-established risk factors for periodontitis and coronary heart disease, respectively. By eliminating individuals with these disorders from the research, the confounding effects of these variables on ADMA levels and endothelial function were reduced. This enabled a more direct examination of the link between ADMA and the study's findings.

Clinical Relevance

The study's results have immediate practical importance since they established ADMA as a possible biomarker for detecting endothelial dysfunction in individuals with periodontitis and CHD. This might aid healthcare providers in identifying persons at high risk of cardiovascular problems, allowing for earlier intervention and treatment.

Translational Implications

The study findings revealed a relationship between oral health (periodontitis) and cardiovascular health (CHD) through the shared cause of endothelial dysfunction. Collaborations between dentistry and cardiovascular professionals may be encouraged as a result, leading to a more holistic approach to patient treatment and a better knowledge of the link between the two disorders.

Patient Risk Stratification

The study's results might help with risk stratification for periodontitis patients. Those with increased ADMA levels may be at a greater risk of developing or suffering CHD problems. This data might be used to develop personalized treatment regimens and prevention measures.

Potential Therapeutic Targets

The research might reveal ADMA-related pathways as therapeutic targets by establishing ADMA as a measure of endothelial dysfunction in both periodontitis and CHD patients. Developing strategies to control ADMA levels or its downstream effects might lead to new therapy options for periodontitis patients and those at risk of cardiovascular disease.

Contribution to Public Health

Understanding the relationship between periodontitis, ADMA, endothelial dysfunction, and CHD might have far-reaching consequences for public health. It may raise awareness of the relevance of oral health in the prevention of cardiovascular disease, encouraging preventative behaviors and dental care among at-risk groups.

Measurement of ADMA

In our study we have used well-established and validated methods to measure ADMA levels, which increases the validity and reliability of our results.

5.4 FUTURE RESEARCH DIRECTIONS / RECOMMENDATIONS

- Conduct longitudinal studies in larger and more varied populations to investigate the temporal correlations between serum ADMA levels, endothelial dysfunction, periodontal disease, and coronary heart disease.
- Assess various endothelial dysfunction and inflammatory biomarkers in individuals with periodontal disease and coronary heart disease to better understand the complicated interaction between these factors.
- In future research, consider correcting for medication usage to more properly analyze the link between ADMA and endothelial dysfunction in this group.
- Investigate the possible therapeutic implications of utilizing ADMA as an endothelial dysfunction biomarker in patients with periodontal disease and coronary heart disease, such as its capacity to predict future cardiovascular events and guide treatment choices.
- Investigate the processes that drive the link between periodontal disease, coronary heart disease, and endothelial dysfunction, particularly the role of oral bacteria in vascular function and inflammation.
- Explore the possibility of therapies like periodontal therapy to enhance endothelial function and lower the risk of cardiovascular events in individuals with periodontal disease and coronary heart disease.

- In future research, consider incorporating additional biomarkers, such as oxidative stress indicators and endothelial progenitor cells, to give a more thorough evaluation of vascular health in individuals with periodontal disease and coronary heart disease.

5.5 CONCLUSION

Our results indicate that serum ADMA levels may be a valuable measure of endothelial dysfunction in individuals with periodontal disease and coronary heart disease. Our research contributes to the expanding body of knowledge on the interconnected nature of periodontal disease, cardiovascular disease, and endothelial dysfunction. The therapeutic consequences of employing ADMA as a biomarker in this group, however, need to be confirmed in future studies. To further understand the temporal correlations between these factors and the effect of drug usage, longitudinal studies with bigger sample numbers and numerous biomarker assessments are required. Our findings support the idea that endothelial dysfunction may play a role in the aetiology of both periodontal disease and coronary heart disease, and they advocate for more investigation into this topic.

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A

Bahria University
Discovering Knowledge
Health Sciences Campus, Karachi

FACULTY RESEARCH COMMITTEE
BAHRIA UNIVERSITY HEALTH SCIENCES CAMPUS

LETTER OF APPROVAL

Date: 18th November, 2022

To,
Dr. Afsheen Zehra
M.Phil - Student
Department of Biochemistry
BUHS-Karachi

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

Title of Study: "Evaluation of Serum Asymmetric Dimethylarginine (ADMA) as a marker of Endothelial Dysfunction in patients with Periodontal Disease and Coronary Heart Disease"

Principal Investigator: **Dr. Afsheen Zehra**

Reference No: **FRC-BUHS 50- 2022-508**

Dear Dr. Afsheen Zehra,

Thank you for submitting research proposal to FRC-BUHS. The committee has approved your project.

This letter is referred to ERC for approval.

Regards

Dr. Mehreen Lateef,
Principal BUCAHS,
CO- CHAIRPERSON FRC-BUHS

Faculty Research Committee, Bahria University Medical College
Sailor's Street, Adjacent PNS-SHIFA DHA
Webmail: frc-bumdc@bahria.edu.pk

B

Bahria University
Discovering Knowledge
Health Sciences Campus Karachi

ETHICAL REVIEW COMMITTEE
LETTER OF APPROVAL

Date: 27-Jan-2023

Reference:
FRC-BUHS-50/2022-508

PATRON
Prof. Ambreen Usmani
Principal & Dean
Health Sciences(BU)

CHAIRPERSON
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Ms Shabina Arif
Mr M Amir Sultan
Prof Dr Rafat Murad
Ms NajmusSahar Ilyas

Dr. Afsheen Zehra
Lecturer
Department of Biochemistry
BUHS-Karachi

Subject: Institutional approval of research study

Title of Study: Evaluation of serum asymmetric dimethyl arginine (ADMA) as a marker of endothelial dysfunction in patients with periodontal disease and coronary heart disease

Principal Investigator: Dr. Afsheen Zehra

Reference No: ERC 04/2023

Dear Dr. Afsheen Zehra,

Thank you for submitting the above mentioned study proposal. ERC Bahria University Health Sciences Campus has reviewed this project in the meeting held on 26-January-2023 and gives approval. Kindly notify us when the research is complete.

Regards,

27/1/2023

DR. AMBREEN SURTI
Secretary, ERC
BUHS

27/1/2023

DR. QURATULAIN JAVAID
Chairperson, ERC
BUHS

Cc:
Principal BUHS

C

INFORMED CONSENT (ENGLISH)

You are giving your consent to participate voluntarily and at your own will in this research project that aims to detect early diagnostic marker of cardiovascular disease in periodontitis patients. The project will evaluate marker for diagnosing cardiovascular disease in periodontitis patients and simultaneously confirming the presence of the marker in established cardiovascular disease 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 blood samples at the beginning and end 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. Afsheen Zehra on mobile number: 0331-2357207 or visit PNS Shifa Hospital/ 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 blood 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.

Name of Patient: _____
S/D/ W/o _____
Signature / Thumb impression of patient: _____
Name of Researcher: _____
Signature of Researcher: _____
Date: _____

C

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 دل کی بیماری کے ابتدائی تشخیصی نشان کا پتہ لگانا ہے۔ یہ پروجیکٹ پیریڈونٹائٹس کے مریضوں میں دل کی بیماری کی تشخیص کے لیے مارکر کا جائزہ لے گا اور ساتھ ہی ساتھ قلبی امراض کے قائم 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 ڈاکٹر افشین زہرہ سے موبائل نمبر: 0331-2357207 پر رابطہ کریں یا اپنی بیماری سے متعلق کسی بھی سوال/ایمرجنسی کی صورت میں پی این ایس شفا ہسپتال/ 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 اپنے خون 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۔

مریض کا نام: _____

والد/شوہر کا نام: _____

مریض کے دستخط / انگوٹھے کا نشان: _____

محقق کا نام: _____

محقق کے دستخط: _____

تاریخ: _____

D

SUBJECT EVALUATION PROFORMA

Serial No: _____ Reg. No: _____ Date: _____

Patient's Name: _____

S/D/W/O _____

Sex: _____

Age: _____

Address: _____

Phone No: _____

Occupation: _____

Income:_____ (Rs <10,000, 10,000-20,000, >20,000)

Height: _____ Weight:_____

Medical history:

1. Hypertension: -----No (0) Yes (1)

2. SBP/DBP: _____

3. Date of angiography: _____

4. Angiographic results: 1 vessel (0), 2 vessel (1), ≥3 vessel (2)

5. Cardiac Procedures: Medicines (0), PCI (1), CABG (2)

Medicines: _____

Other Co-morbidity: _____

Family History: _____

Periodontal Examination:

GINGIVAL STATUS

	Upper right posterior	Upper anterior	Upper left posterior
Color			
Contour			
Size			
Consistency			
Stippling			
Position			
Bleeding on probing			
Exudation			
	Lower right posterior	Lower anterior	Lower left posterior
Color			
Contour			
Size			
Consistency			
Stippling			
Position			
Bleeding on probing			
Exudation			

PERIODONTAL CHARTING

Teeth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
Bleeding on Probing (Ainamo & Bay classification)																
Mobility																
Plaque Index (Silness-Löe)																
Probing depth (William's Probe)																
Recession (Miller's classification)																
Pockets Upper arch																
Pockets Lower Arch																
Recession (Miler's classification)																
Probing depth (William's Probe)																
Plaque Index (Silness-Löe)																
Mobility																
Bleeding on Probing (Ainamo & Bay classification)																
Teeth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

E

BAHRIA UNIVERSITY DENTAL HOSPITAL

BAHRIA UNIVERSITY MEDICAL & DENTAL COLLEGE
Sailer Street, Adjacent PNS Shifa
DHA Phase II, Karachi. Tel: 021-35319491-9

APPOINTMENT CARD

Name: _____ OPD No: _____

S. No.	Date	Time	Initial

Note : Cancellation shall be made 24 hours before appointment time.

THESIS Dr. Afsheen Zehra

ORIGINALITY REPORT

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