Republic of Iraq
Ministry of Higher Education
And Scientific Research
University of Baghdad
College of Dentistry

Evaluation of High Sensitivity C- reactive Protein, IgM Anticardiolipin Antibody and IgG Antiphosphoryl choline levels in Serum of Patients have Chronic Periodontitis with and without Atherosclerotic Cardiovascular Disease

A Thesis Submitted to the Council of the College of Dentistry/University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Master of Science in Periodontics

BY

Reham Adnan Radhi

B.D.S

Supervised by

Assist. Prof. Dr. Alaa Omran Ali ALmosawi

B.D.S., MSC. Periodontics

2019 A.D 1441 A.H

Certification of the Supervisor

I certify that this thesis entitled "**Evaluation of High Sensitivity C-reactive Protein, IgM Anticardiolipin Antibody and IgG Antiphosphoryl choline levels in Serum of Patients have Chronic Periodontitis with and without Atherosclerotic Cardiovascular Disease**" was prepared by **Reham Adnan Radhi** under my supervision at the College of Dentistry/ University of Baghdad in partial fulfilment of the requirements for the degree Master of Science in Periodontics.

Signature
Assistant Professor
Dr. Alaa Omran Ali ALmosawi
B.D.S., M.Sc. (Periodontics)
(The Supervisor)

Linguistic certification

This is to certify that the thesis entitled **"Evaluation of High Sensitivity C-reactive Protein, IgM Anticardiolipin Antibody and IgG Antiphosphoryl choline levels in Serum of Patients have Chronic Periodontitis with and without Atherosclerotic Cardiovascular Disease"** was prepared under my linguistic supervision. Its language was amended to meet the style of the English language.

Signature:

Prof. Dr. Abdulkarim Fadhil Jameel

Linguistic Reviewer / / 2020

Certification of the examination committee and the dean

We, the members of the examining committee, certify that after reading the thesis and examining the student **"Reham Adnan Radhi"** in its contents, it is adequate for the award of the Master of Science in Prosthodontics.

Signature
Professor
Dr. Saif Sehaam Saliem Juma
MSc. Periodontology
(Chairman of Examination Committee)

Signature
Professor
Dr. Kadhim Jawad Hanau
MSc. Periodontology
(Member)

Signature
Professor
Dr.Batool Hasson Hashim
Ph.D. Medical Microbiology
Clinical Immunity
(Member)

Approved by the council of the College of Dentistry/University of Baghdad.

Signature
Assistant Professor
Dr. Nada Jafer MH. Radhi
B.D.S, M.Sc., Ph.D.
Dean of the College of Dentistry
University of Baghdad

Dedication

To my beloved parents

To my dear husband who was always with me

To my lovely child JOJO

To all my family who supported me with all love

To all people who helped me during my research

Acknowledgement

My deepest sense of gratitude to "Allah", who gave me the opportunity and surrounded me by wonderful people who helped to accomplish this research.

I would like to express my special thanks of gratitude for Assist ***Prof. Dr. Nada Jafer M.H Radhi*** *the Dean of the college of Dentistry/ University of Baghdad.*

Special thanks to the Assistant Dean for scientific affairs and students ***Prof. Dr. Nidhal H. Ghaib*** *for her support.*

Special thanks to the previous Dean of the college of Dentistry/ University of Baghdad ***Prof. Dr. Hussain Faisal Al-Huwaiz.i***

Grateful thanks and appreciation are expressed to ***Prof. Dr. Saif Sehaam saliem Juma*** *Head of the department of periodontics for his support and kindness.*

A million thanks, love and gratitude to my supervisor ***Assist. Prof. Dr. Alaa Omran Ali*** *for her advice, support, and guidance throughout the study*

My thanks and best regards to all the seniors of Periodontics Department for their efforts and kindness.

I would like to extend my thanks and appreciation to ***Prof. Dr. Batool Hassan Al-Ghurabi*** *for her advices at the beginning of my research.*

Grateful thanks and respect to all members at college of dentistry, university of Basra for their cooperation during sample collection.

My thanks and respect to the staff members of the Iraqi national blood bank for their help during sample collection.

My thanks and respect to all the medical staff of cardiology center in Al Sadr Teaching Hospital who provided all the facilities and practical help to me.

I gratefully acknowledge to the cooperation of 85 persons who took part in this study.

*Special thanks to **Dr. Ahmed Qasim** for his help in statistical analysis.*

Finally, my deepest appreciation to my great mother and father who provided me with the best support and positive energy , my dear husband who devoted all his time and effort to help me finish my study , I would like to express grateful thanks to my lovely family, my brothers , ,my uncle and my aunt. Thank you for your patience and continuous support.

Abstract

Background: Periodontitis and atherosclerosis cardiovascular disease are chronic inflammatory diseases which are highly prevalent. Over the last two decades, the amount of evidence supporting an association between dental plaque bacteria and coronary diseases that develop as a result of atherosclerosis has increased.

Aims of the study: To determine and compare the periodontal health status in study groups (Atherosclerotic cardiovascular disease patient with chronic periodontitis and patient having chronic periodontitis) and control group, to estimate the serum levels of high sensitivity C- reactive protein, IgM anticardiolipin antibody and IgG antiphosphoryl choline in study and control groups and compare between them , to test the correlation among the biomarkers and between biomarkers and clinical periodontal parameters.

Materials and methods: 85 subjects, males and females were included in this study with age range (35-64) years old, they were divided into three groups: Atherosclerotic cardiovascular disease with chronic periodontitis group (30 patients), chronic periodontitis group (30 patients) and control group (25 systemically healthy subjects, have healthy periodontium). Periodontal health status was determined by measuring the following clinical periodontal parameters: Plaque index (P.I), Gingival index (GI), Bleeding on probing (BOP), Probing pocket depth (PPD) and Clinical attachment loss (CAL) for all teeth except third molar. After the clinical examination, 5ml of venous blood were collected from study and control groups. After centrifusion, serum samples were kept frozen at (- 20) °C. IgM

anticardiolipin antibody and IgG antiphosphoryl choline were determined by mean of enzyme – linked immune-sorbent assay(ELISA), while high-sensitivity C-reactive protein serum levels was determined by mean of immunoturbidimetry.

Results: The results showed that the mean values of clinical periodontal parameters (P.I, GI, and CAL), were higher in the Atherosclerotic cardiovascular disease with Chronic periodontitis group than in the Chronic periodontitis group with a significant differences at ($p \leq 0.01$, $P \leq 0.01$, $p \leq 0.05$) respectively. Mean value of PPD was higher in Chronic periodontitis group than in the Atherosclerotic cardiovascular disease with Chronic periodontitis group with highly significant differences ($P \leq 0.01$). A higher percentage of score one of BOP sites demonstrated by Chronic periodontitis group than in the (Atherosclerotic cardiovascular disease with chronic periodontitis) group with highly significant difference.

Furthermore, the levels of serum high sensitivity C-reactive protein was higher in Atherosclerotic cardiovascular disease with chronic periodontitis group when compared with Chronic Periodontitis group and control group, with highly significant differences between them($P \leq 0.01$).

Regarding serum IgM anti-cardiolipin antibody level, the median value was higher in Atherosclerotic cardiovascular disease with chronic periodontitis group than in Chronic periodontitis group and control groups with no statistical significant differences($P > 0.05$), except for Atherosclerotic cardiovascular disease with chronic periodontitis group when compared to control group ($P \leq 0.05$).

Concerning IgG anti-Phosphoryl choline, there was increase in median level of IgG Anti-Phosphoryl-Choline in Atherosclerotic cardiovascular disease with chronic periodontitis as compared to chronic periodontitis and control groups

respectively, Moreover, increase in median level in chronic periodontitis group as compared to control group and there was significant difference between them($P \leq 0.05$).

Concerning the correlation between serum levels of (high sensitivity C- reactive, IgM anti-cardiolipin antibody and IgG anti-phosphoryl choline) and clinical periodontal parameters in study groups, there were no significant correlation between immunological parameters and clinical periodontal parameters in all groups.

In addition, the correlation between serum levels of immunological parameters (high sensitivity C- reactive protein, IgM anti-cardiolipin antibody and IgG anti-phosphoryl choline) in (Atherosclerotic cardiovascular disease with Chronic periodontitis), were showed significant positive correlation between IgG Anti-phosphoryl choline with IgM anticardiolipin antibody. Whereas, no correlation was found between them in chronic periodontitis group.

Conclusion: This study demonstrated that serum levels of high sensitivity C-reactive protein and IgM anti-Cardiolipin antibody were significantly higher in chronic periodontitis with atherosclerosis cardiovascular disease than in the chronic periodontitis without. So increased risk for atherosclerosis might be associated with elevated levels of high sensitivity C-reactive protein and anti-cardiolipin antibody that are due to chronic periodontitis, furthermore, the gradual increase in serum level of IgG antiphosphoryl choline in chronic periodontitis and atherosclerosis cardiovascular disease with chronic periodontitis groups as compared to control group might indicate the effect of periodontal disease in atherosclerosis cardiovascular disease.

List of content

Title No.	*Subject*	*Page No.*
	Acknowledgement	VI
	Abstract	VI
	List of Contents	VI
	List of Tables	VI
	List of figures	VI
	List of Abbreviations	XVI
	Introduction	1
	Aims of the Study	4
Chapter one :Review of Literature		
1.1	Periodontal Diseases	5
1.1.1	Gingivitis	6
1.1.2	Periodontitis	6
1.1.3	Chronic periodontitis	7
1.2	Atherosclerotic cardiovascular disease (ASCVD)	9
1.2.1	Atherosclerosis	9
1.2.2	Mechanism of plaque formation and plaque rupture in atherosclerosis cardiovascular disease	9
1.2.3	Risk factors for ASCVD	13
1.2.3.1	Major risk factors	13
1.2.3.2	Emerging risk factors	15
1.2.4	Diagnosis of ASCVD	16
1.2.5	Inflammation in atherosclerosis	18
1.3	Associations of Chronic periodontitis and Atherosclerotic cardiovascular diseases	19
1.4	High sensitivity C- reactive protein	22

1.4.1	Role of High sensitivity C- reactive protein in patient with chronic periodontitis	24
1.4.2	Role of High sensitivity C- reactive protein in patient with ASCVD	25
1.5	Anti-Cardiolipin Antibody	27
1.5.1	Role of Anti-Cardiolipin Antibody in patient with chronic periodontitis	28
1.5.2	Role of Anti-Cardiolipin Antibody in patient with ASCVD	29
1.6	Anti-phosphoryl choline	30
1.6.1	Role of Anti-phosphoryl choline in patient with chronic periodontitis	32
1.6.2	Role of Anti-phosphoryl choline in patient with chronic periodontitis	34
	Chapter two :Material and methods	
2.1	Subjects	36
2.1.1	Inclusion criteria	36
2.1.2	Exclusion criteria	36
2.1.3	Study design	37
2.2	Instrument and materials	39
2.2.1	Materials and instrument used for clinical examination	39
2.2.2	Materials and instrument used for blood sample collection	39
2.2.3	Instrument, Equipment and kits used in laboratory	40
2.2.3.1	instrument	40
2.2.3.2	equipment	40
2.2.3.3	kits	40
2.3	methods	42
2.3.1	The clinical periodontal parameters	42
2.3.1.1	Plaque index	42
2.3.1.2	Gingival index	43

2.3.1.3	Bleeding on probing	43
2.3.1.4	Probing pocket depth	43
2.3.1.5	Clinical attachment level	44
2.3.2	Serum sample collection	45
2.3.3	Immunological assays	45
2.3.3.1	Principle of serum hs C-reactive protein	45
2.2.3.1.1	Content of the test kit	46
2.3.3.1.2	Assay procedure for serum hs C-reactive protein	46
2.3.3.2	Principle of serum anticardiolipin antibody ELISA	47
2.3.3.2.1	Content of the test kit	47
2.3.3.2.2	Assay procedure for serum anticardiolipin antibody	48
2.3.3.3	Principle of serum anti-phosphoryl choline	49
2.3.3.3.1	Content of the test kit	50
2.3.3.3.2	Assay procedure for serum anti-phosphoryl choline	51
2.4	examiner alignment	52
2.4.1	Inter examiner alignment	52
2.4.2	Intra examiner alignment	52
2.5	Statistical analysis	52
2.5.1	Descriptive statistics	52
2.5.2	Inferential statistics	53
Chapter three : Results		
3.1	Demographic characteristics of the study subject	54
3.2	Clinical periodontal parameters analysis	55
3.2.1	Plaque index and gingival indices	55
3.2.2	Bleeding on probing	56
3.2.3	Probing pocket depth and clinical attachment level	58
3.3	Immunological analysis	59
3.3.1	Serum hs-CRP analysis	59
3.3.2	Serum anti-cardiolipin (IgM) antibody level analysis	61

3.3.3	Serum anti-phosphoryl choline(IgG)level analysis	62
3.4	Correlation between Immunological parameters and clinical periodontal parameters	64
3.4.1	Correlation between serum hs-CRP level and clinical periodontal parameters	64
3.4.2	Correlation between serum levels of anti-cardiolipin (IgM) antibody and clinical periodontal parameters	65
3.4.3	Correlation between serum levels of anti-phosphoryl choline (IgG) and clinical periodontal parameters	66
3.5	Correlation between serum levels of Immunological parameters	68
3.5.1	Correlation between serum levels of Immunological parameters in CP+ATH group	68
3.5.2	Correlation between serum levels of Immunological parameters in CP group	69
Chapter four : Discussion		
4.1	Characteristics of study group	70
4.2	Periodontal Health Status	71
4.2.1	Plaque and gingival indices	71
4.2.2	Bleeding on probing	71
4.2.3	Probing pocket depth and clinical attachment level	72
4.3	Serum hs-CRP levels	73
4.4	Correlations between serum hs- CRP and clinical periodontal parameters	74
4.5	Serum anti-cardiolipin (IgM) antibody levels	76
4.6	Correlation between anti-cardiolpin (IgM) antibody and clinical periodontal parameters	77
4.7	Serum antiphosphoryl choline levels	78
4.8	Correlations between serum anti-phosphoryl choline and clinical periodontal parameters	79

4.9	Correlation between serum levels of immunological parameters in CP+ATH group	79
4.10	Correlation between serum levels of immunological parameters in CP group	80
Chapter five: Conclusions & Suggestions		
5.1	Conclusion	82
5.2	Suggestion	83
	References	
	References	84
	Appendix	
	Appendix I	105
	Appendix II	106
	Appendix III	109

List of tables

Table No.	*Title of Tables*	*Page No.*
1.1	Basic classification of periodontal diseases and conditions	7
3.1	Demographic characteristics of study group	54
3.2	The difference in mean values of plaque and gingival indices among study group	55
3.3	Inter group comparisons of the mean values of plaque index between all pairs of study group	56
3.4	Inter group comparisons of the mean values of gingival index between all pairs of study group	56
3.5	The distribution of the numbers and percentages of sites according to the presence or absence of bleeding on probing of the CP group and ATH with CP group.	57
3.6	The difference in mean values of PPD and CAL for CP group and ATH+CP group	59
3.7	The difference in mean values of serum hs- CRP (mg/dl) among study groups.	60
3.8	Inter group comparisons of the median values of serum hs- CRP (mg/dl) between all pairs of study groups	60
3.9	The difference in median values of serum IgM Anti-Cardiolipin (RU/ml) antibody among study groups	61
3.10	Intergroup comparisons for serum IgM Anti-Cardiolipin (RU/ml) antibody between all pairs of study groups	62
3.11	The difference in median values of serum IgG Anti-phosphoryl choline (U/ml) among study groups.	63

3.12	Intergroup comparisons for serum IgG Anti-phosphoryl choline (U/ml) between all pairs of study groups.	63
3.13	Spearman Correlation Coefficients among serum hs-CRP and clinical periodontal parameters in CP group and ATH+CP group	65
3.14	Spearman Correlation Coefficients among serum IgM Anti-cardiolipin antibody and clinical periodontal parameters in CP group and ATH+CP group	66
3.15	Spearman Correlation Coefficients among serum IgG Anti-phosphoryl choline and clinical periodontal parameters in CP group and ATH+CP group	67
3.16	Spearman rho correlation coefficient among immunological parameters in ATH+CP group	68
3.17	Spearman rho correlation coefficient among immunological parameters in CP group	69

List of Figures

figure No.	*Title of figures*	*Page No.*
1.1	Endothelial dysfunction	11
1.2	Fatty streak formation	12
1.3	Formation of an Advanced, Complicated Lesion of Atherosclerosis	12
1.4	Unstable Fibrous Plaques in Atherosclerosis	13
1.5	Schematic overview of potential inflammatory mechanisms linking periodontitis to cardiovascular diseases	22
1.6	C - reactive protein structure	23
1.7	Schematic picture showing the effect of CRP in atherosclerosis and periodontitis	26
1.8	Biosynthesis and remodeling of Cardiolipin in mitochondria	28
1.9	Anti-phosphoryl choline	31
1.10	Molecular mimicry between phosphoryl choline and platelet activating factor receptor	35
2.1	Diagram illustrate the study design	38
2.2	Microplate ELISA shaker (Human, Germany)	41
2.3	Microplate ELISA Reader device (Human, Germany)	41
2.4	ARCHITECT CRP VARIO Reader device and Kit	46
2.5	Human serum Anti-Cardiolipin Antibody ELISA Kit.	47
2.6	Human serum Anti-Phosphoryl Choline ELISA Kit	50

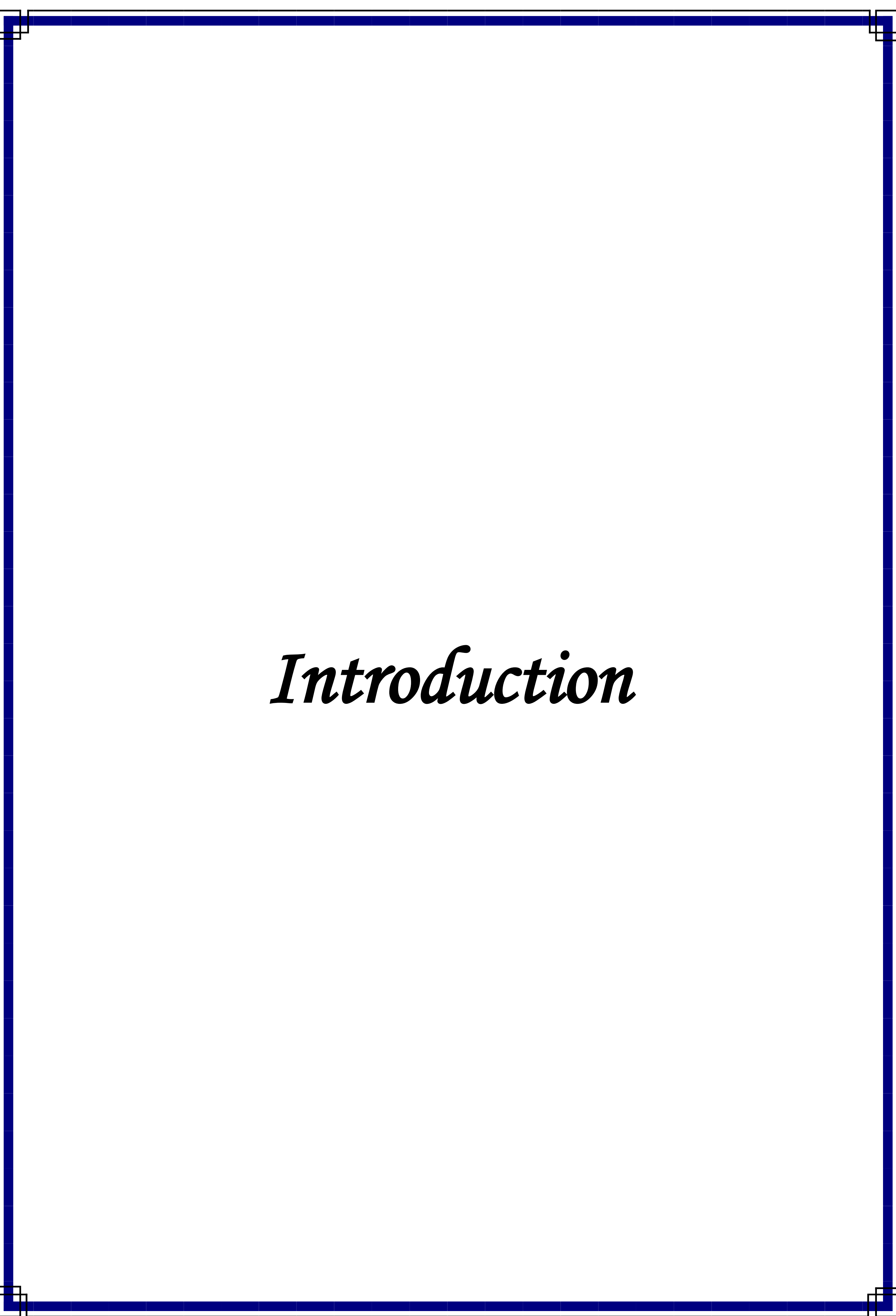
3.1	Pie chart for percentages of score 0 and score 1 of bleeding on probing for CP group and ATH+CP group.	58
3.2	Percentile distribution chart for serum Anti-PC (U/ml) in CP and CP with ATH group and control group.	64
3.3	Correlation between IgG anti-PC and IgM ACL in CP with ATH group.	69

List of Abbreviations

AAP	American academy of periodontology
AMI	Acute myocardial infarction
ANOVA	Analysis of Variance Test
Anti-CL	Anti-Cardiolipin antibody
Anti-PC	Anti-phosphoryl choline
APLS	Anti-Phospholipid Syndrome
ASCVD	Atherosclerotic cardiovascular disease
ATH	Atherosclerotic cardiovascular disease
b2GPI	β-2-glycoprotein-I-dependent phospholipid
baPWV	Brachial ankle pulse wave velocity
BOP	Bleeding on probing
BSA	Bovine serum albumin
C°	Degree Celsius
CAD	Coronary artery disease
CAL	Clinical attachment loss
CAVI	Cardio- ankle vascular index
CEJ	Cemento-enamel junction
CP	Chronic periodontitis
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular disease
e.g.	Exempli gratia
ECM	Extracellular matrix
ELAM-1	Endothelial leukocyte adhesion molecule-1
ELISA	Enzyme Linked Immuno- Sorbent Assay
F	Fahrenheit
G.I	Gingival index

Hs-CRP	High sensitivity C- reactive protein
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
IVUS	Intravascular Ultrasound
JE	Junctional epithelium
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein-cholesterol
LPS	Lipopolysaccharide
MDCT	Multidetector row computed tomography
Mg/dl	Milligrams per decilitre
Mg/l	Milligrams per litre
MHz	Mega hertz
MI	Myocardial infraction
MMP	Matrix metalloproteinases
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
N	Number
NHANES	National health and nutrition examination survey
nm	Nanometer
NO	Nitric oxide
NS	Non-significant
oxCL	Oxidized Cardiolipin
oxLDL	Oxidized low density lipoprotein
oxPLs	Oxidized Phospholipids
P.I	Plaque index
PAF	palate activating factor
PC	Positive control
PD	Periodontal disease
PGE2	Prostaglandin E2

PPD	Probing pocket depth
PRRs	pattern recognition receptor
RBCs	Red blood cells
Rgp	Arg-gingipain
RU/ml	Relative unit per mililitre
SD	Standard deviation
Sig	Significant
SLE	Systemic Lupus Erythematous
SMC	Smooth muscle cell
Std. Dev	Standard deviation
TMB	Tetramethy benzidine
TNF-α	Tumor necrosis factor alpha
U/ml	Unit per milliliter
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelium growth factor
VLDL	Very low density lipoprotein
VSMCs	Vascular smooth muscle cells
WHO	World health organization
α-PC	Alpha-phosphoryl choline
μl	Microliter



Introduction

Introduction

Periodontal diseases are chronic inflammatory conditions that disturb the tissues surrounding and supporting the teeth. Initially, periodontal disease presents as gingivitis, a reversible inflammation of the periodontal soft tissues resulting in gingival bleeding and swelling. In susceptible individuals with a compromised immune response, gingivitis might lead to periodontitis, which gradually destroys the periodontal tissue support, including the bone surrounding the teeth **(Peres et al., 2019).**

Atherosclerosis and its cardiovascular ischemic complications are the most common causes of death and disability worldwide **(Vilahur et al., 2014)**. Actually, in 2010 the World Health Organization (WHO) stated that cardiovascular disease account for around 30% of global deaths and estimated that more than 23.3 million of peoples will die from cardiovascular disease annually by 2030 **(Lozano et al., 2012, Mathers and Loncar, 2006).** It's a chronic lipid-driven inflammatory disease of the arterial wall characterized by the involvement of the innate and adaptive immune systems **(Libby and biology, 2012, Badimon et al., 2011).** Inflammation plays a critical role in the atherosclerotic process in various vascular beds, starting from endothelial dysfunction through all steps of plaque build-up until its harmful clinical ischemic complications **(Koenig, 2013).**

Associations between periodontitis and atherosclerosis would be predicted based on inflammatory mechanisms initiated locally or systemically by bacteria associated with periodontal lesions, that then effect the initiation or propagation of the atherosclerotic lesion **(Hajishengallis, 2015)**. Such lesions may be initiated by inflammatory stimuli including systemic and locally produced inflammatory

cytokines and chemotactic agents that cause changes in the endothelium such as up-regulation of adhesion molecules. These changes promote interactions with leucocytes, such as monocytes, that promote leucocyte migration into the intimal layer of the artery **(Schenkein and Loos, 2013).**

C-reactive protein is an acute-phase reactant that is produced mostly by the liver in response to a variety of inflammatory cytokines such as IL-6. It therefore serves in a variety of conditions as a marker for systemic inflammation **(Abd et al., 2011).**

There has been increasing documentation of Proatherogenic and prothrombotic effects of CRP. Studies have revealed that increased plasma concentrations of CRP lead to low-grade inflammation, a major pathogenic component of endothelial dysfunction. In addition, the dissociation of circulating pentameric CRP into its monomeric form has been shown to be associated with noticeable inflammatory properties in vivo (**Thiele et al., 2014**). Actually, the accumulation of monomeric CRP with inflammatory cells has been observed in human infarcted myocardial tissue and in atherosclerotic plaque, indicating that CRP dissociation may play a role in acute and chronic inflammatory processes (**Thiele et al., 2014, Stancel et al., 2016).** Furthermore, Chronic bacterial infections such as periodontitis, are one of the well-established risk factors for moderately elevated CRP level **(Leite et al., 2014).**

Cardiolipin is a phospholipid (diphosphatidylglycerol) found in inner mitochondrial membrane primarily, but in general it is also a minor constituent of mammalian membranes. Cardiolipin can evoke an antibody reaction in diseases with mitochondrial damage **(Rozee et al., 2003, Al-Ghurabei, 2012).**

Moreover, previous studies have suggested that the association between anti-CL and periodontal disease antibodies is caused by viral and bacterial infections that

induce production of these antibodies via molecular mimicry mechanism **(García-Carrasco et al., 2009, Mohamad et al., 2017).**

Phosphoryl choline (PC) is an immunogenic epitope in the capsular polysaccharide of *Streptococcus pneumoniae* and the presence of serum anti-PC has been suggested to be partly due to exposure to this organism **(Kikuchi et al., 2010)**. However, periodontitis patients have high levels of anti-PC and this antibody reacts with 30–40% of bacteria in dental plaque samples, including periodontitis-associated bacteria, so that the ability of periodontal pathogenic bacteria to produce a systemic response to phosphoryl choline is demonstrated by higher serum levels of phosphoryl choline associated antibodies (anti-phosphoryl choline IgG) in patients with loss of attachment , compared to patient with healthy gingiva **(Schenkein et al., 1999, Karnoutsos et al., 2008)**.

Additionally, other researchers suggest that both phosphoryl choline bearing strains of oral bacteria and oxidized low-density lipoproteins (oxLDL) react with human serum anti-phosphoryl choline IgG **(Schenkein et al., 2001)**. This suggests that antibodies produced against certain periodontal bacteria would also react to phosphoryl choline bearing oxLDL **(Shaw et al., 2003, Tew et al., 2012)** and, therefore, magnify the uptake of this lipid by foam cells, promoting further atherosclerosis progression.

Aims of the study

To assess and compare the serum levels of high sensitivity C-reactive protein, IgM anticardiolipin antibody and IgG antiphosphoryl choline in study and control groups, and to evaluate their correlations with clinical periodontal parameters in study groups.

Objectives:

1. Determine and compare the periodontal heath status in study and control groups by measuring the following clinical periodontal parameters: Plaque index (P .l), Gingival index (GI), Bleeding on probing (BOP), Probing pocket depth (PPD) and Clinical attachment loss (CAL).
2. Assessment and compare the serum levels of high sensitivity C-reactive protein, IgM anticardiolipin antibody and IgG antiphosphoryl choline in study and control groups by means of ELISA, and to evaluate their correlations with clinical periodontal parameters in study groups.
3. To determine the correlation between the serum level of the immunological parameters (IgM anticardiolipin antibody, IgG antiphoshporyl choline and hs-C reactive protein) for study groups.

Hypothesis:

This study hypothesized that there is a significant difference in periodontal health status and serum levels of (hs- CRP, IgM anticardiolipin antibody and IgG antiphosphoryl choline) among diseased groups compared to healthy control.

Chapter One

Review of literature

Review of literature

1.1 Periodontal Disease

Periodontal diseases are chronic diseases that occur as a consequence of interaction between bacteria and host, leading to inflammation and damage in the hard and soft supporting tissues of the tooth **(Kizildag et al., 2014).**

The prevalence and incidence statistics of periodontal diseases vary because of bias, case misclassification, and the number of teeth and the sites examined. As stated by Canadian Health Measures Survey 2007-2009, the amount of loss of periodontal ligament attachment is regarded as a criterion for recording periodontal disease incidence. National Health and Nutrition Examination Survey (NHANES) determined the attachment loss (AL) and probing depth (PD) at six sites of all teeth (excluding third molars) for the estimation of periodontal disease in the U.S **(Nazir, 2017).**

In 1999, a comprehensive classification of periodontal diseases and conditions organized by American academy of periodontology (AAP) given a detailed for classification of periodontal diseases and conditions. Plaque induced inflammatory periodontal diseases are broadly classified into two types depending on the extent of the inflammatory infiltrate, and the absence or presence of clinical attachment loss (CAL), these two types are gingivitis and periodontitis **(Armitage, 1999).**

According to the new classification scheme, periodontal disease and conditions can be classified into three major classes: Periodontal health and gingival diseases, Periodontitis, and Other conditions affecting the periodontium each with subcategories **(Caton et al., 2018).**

1.1.1 Gingivitis

Plaque induced gingivitis is the most prevalent form of periodontal disease **(Kotsakis, 2019).** In gingivitis the inflammation is limited to the gingiva with no loss of connective tissue attachment, and the junctional epithelium is still at the cemento-enamel junction (CEJ). This form is reversible because after removal of the dental plaque the condition returns to normal **(Albandar, 2005).**

1.1.2 Periodontitis

Periodontitis is the second most common oral infectious disease after dental caries, which affects the periodontium. It is a result of the inflammatory response caused by the accumulation of bacterial plaque at the gingival edge of the tooth. In its first stage, gingivitis, an inflammatory process occurs in the gingival tissue causing oral discomfort, but the subsequent stages of periodontal disease are characterized by progressive destruction of the supporting tissues of the tooth, impairment of dental function, increased dental mobility and finally tooth loss **(Hughes, 2015, Seciu et al., 2018).**

Similarly to other chronic diseases, periodontitis requires a susceptible host. Periodontitis susceptibility is determined by genetic factors that may predispose to hyper inflammatory responses or by environmental factors (*e.g*., diet and stress) and risk-related behavior (*e.g*., smoking) that can change the host immune response in a destructive direction **(Divaris et al., 2013, Laine et al., 2012).**The destructive form of periodontal disease, periodontitis, affects about half of adults and over 60% of over 65 year olds, with severe periodontitis affecting 10–15% of populations **(Chapple et al., 2013)**.

Periodontal health, gingival diseases and conditions:
Periodontal health
intact periodontium
reduced periodontium*
Gingivitis: dental biofilm-induced
intact periodontium
reduced periodontium*
Gingival diseases and conditions: non-dental biofilm-induced
Periodontitis
Necrotising periodontal diseases
Periodontitis**
Periodontitis as a manifestation of systemic disease
Other conditions affecting the periodontium
Systemic diseases or conditions affecting the periodontal supporting tissues
Periodontal abscesses and endodontic-periodontal lesions
Mucogingival deformities and conditions
Traumatic occlusal forces
Tooth and prosthesis related factors

Table (1-1) Basic classification of periodontal diseases and conditions (Dietrich et al., 2019).

1.1.3 Chronic periodontitis

Chronic periodontitis (CP) is a common complex disease of the oral cavity that is characterized by an inflammatory response to commensal and pathogenic oral bacteria and is found in approximately 20% of the adult population in United States. It manifests with gingival pocket formation and clinical attachment loss (CAL) that

leads to gradual destruction of periodontal tissues and tooth-supporting alveolar bone **(Divaris et al., 2013).**

Chronic periodontitis is associated with the accumulation of plaque and calculus. It usually has a slow to moderate rate of disease progression, but periods of more rapid destruction may also be observed **(Chauhan et al., 2012)**. Increases in the rate of disease progression may be caused by the influence of local, systemic, or environmental factors that may influence the normal host–bacteria interaction. Local factors may influence plaque accumulation, whereas systemic diseases (e.g., diabetes mellitus, acquired immune deficiency syndrome) may influence the host's defenses, and environmental factors (e.g., cigarette smoking, stress) may influence the response of the host to plaque accumulation **(Newman et al., 2012).**

A key feature of chronic periodontitis is site specificity, the characteristic periodontal pockets and the accompanying attachment loss and bone loss do not occur uniformly throughout the dentition. Consequently, the definition of a case of periodontitis relies strongly on the specific thresholds for both disease extent (the number of affected teeth) and disease severity (the magnitude of pocket depth, clinical attachment loss and alveolar bone loss at the affected teeth) **(Kinane et al., 2017).**

Depending upon the number of sites affected, chronic periodontitis may be either localized or generalized. Localized periodontitis is described as 30 % or less of sites affected and generalized periodontitis being more than 30% of sites affected **(Highfield, 2009).**

1.2 Atherosclerotic cardiovascular disease (ASCVD)

1.2.1 Atherosclerosis

Atherosclerosis, the major underlying cause of cardiovascular disease (CVD), is a chronic low grade inflammation in the artery wall, characterized by the accumulation of modified lipoproteins, dead cells and an abundance of activated immune cells that produce pro-inflammatory cytokines **(Fiskesund et al., 2012).**

Atherosclerotic lesions (atheroma) are asymmetric focal thickenings of the inner most layer of the artery (the intima). They are made up of cells, connective-tissue elements, lipids, and debris. Blood-borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being smooth-muscle and vascular endothelial cells. The atheroma is preceded by a fatty streak, an accumulation of lipid-laden cells beneath the endothelium. Most of these cells in the fatty streak are macrophages, together with some T cells **(Libby et al., 2009).**

1.2.2 Mechanism of plaque formation and plaque rupture in atherosclerosis cardiovascular disease

The vascular wall is composed of three layers, the tunica intima, the tunica media and the tunica adventitia **(Lusis, 2000)**. The normal vascular endothelium resists binding to blood cells.

Atherosclerosis is a silent chronic vascular pathology that is the cause of the majority of cardiovascular ischemic events. Development of vascular disease involves a combination of endothelial dysfunction, extensive lipid deposition in the intima, proliferation of vascular smooth muscle cells, exacerbated innate and

adaptive immune responses and remodeling of the extracellular matrix, resulting in atherosclerotic plaque formation **(Badimon and Vilahur, 2014).**

The formation of atherosclerotic plaques is precipitated by damage to vascular endothelium resulting in an inflammatory response in which circulating monocytes adhere to the vascular endothelium figure **(1.1) (Badimon and Vilahur, 2014, Ross, 1999**).

Numerous adhesion molecules on the endothelial cell surface, including endothelial leukocyte adhesion molecule-1(ELAM-1), vascular cell adhesion molecule-1(VCAM-1), and intercellular adhesion molecule-1(ICAM-1) that facilitate the attachment of monocytes to the damaged vascular endothelium **(Linton et al., 2019**). These adhesion molecules are up-regulated by a number of factors, including bacterial LPSs, prostaglandins, and proinflammatory cytokines **(Kinane, 1998).**

Monocytes penetrate the endothelium after binding to the endothelial cell lining and migrate under the arterial intima **figure (1.1)(Ross, 1999**). The monocytes ingest and absorb circulating low-density lipoprotein in their oxidized state, forming the foam cells characteristic of atheromatous plaque **(Schenkein and Loos, 2013**). After entering the arterial media, monocytes may also transform to macrophages.

A host of proinflammatory cytokines such as IL-1, TNF-α, and PGE2 are then produced, and they propagate the atheromatous lesion. Mitogenic factors such as fibroblast growth factor and platelet-derived growth factor stimulate smooth muscle and collagen proliferation within the media, thus thickening the arterial wall **(Lowe, 1998).**

It becomes more complex as the atherosclerotic plaque grows. Fatty streaks progress **figure (1.2)(Ross, 1999)** to fibro-fatty lesions that become advanced plaques with a necrotic core covered by a fibrous cap **figure(1.3) (Gelsomino et al., 2017, Ross, 1999)**. Accumulation of immune cells, such as T cells, occurs at the shoulder regions of the plaque. Symptoms of atherosclerosis typically arise when the cap fails to withstand the pulsatile force from the blood pressure and superficial fissures are formed, usually near the edges of the plaque. Plaques that are susceptible to rupture are characterized by a thin fibrous cap, a large lipid-filled necrotic core, and continuing inflammation. Rupture or ulceration of the fibrous plaque can rapidly lead to thrombosis and usually occurs at sites of thinning of the fibrous cap that covers the advanced lesion **figure (1.4) (Ross, 1999, Gisterå and Hansson, 2017).**

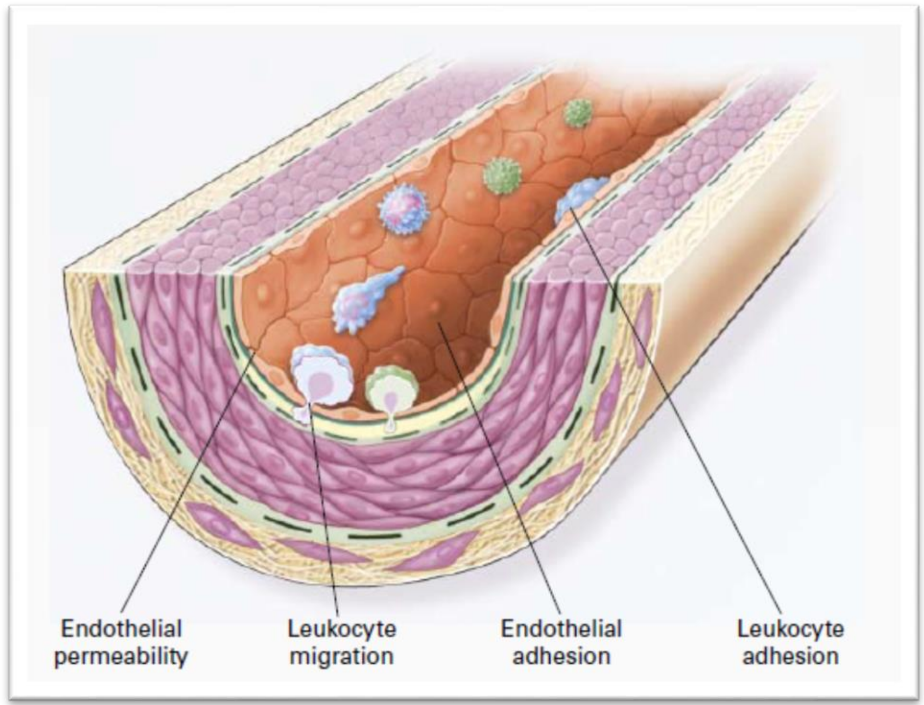


Figure (1.1): Endothelial Dysfunction in Atherosclerosis (Ross, 1999).

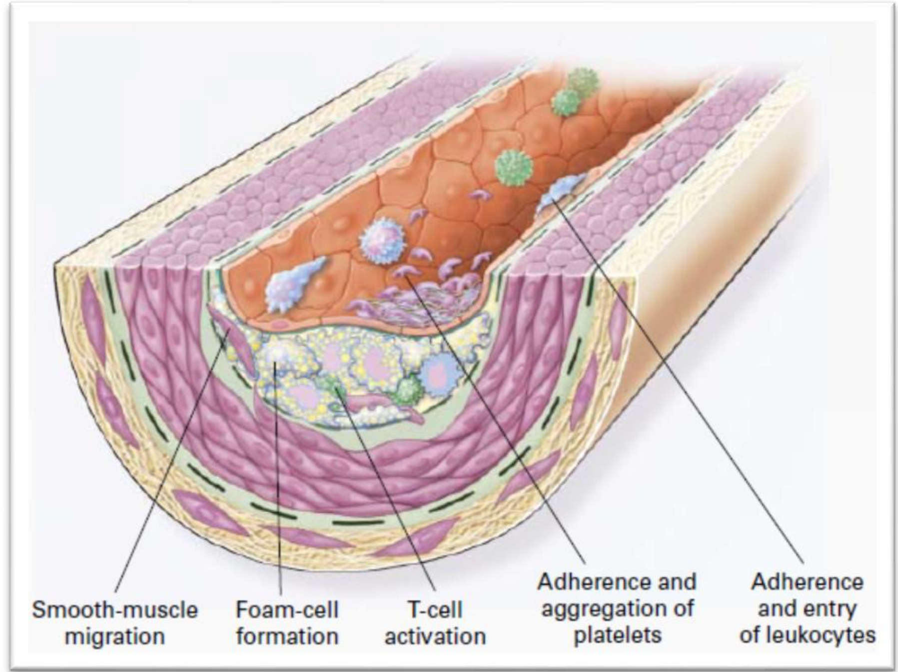


Figure (1.2): Fatty streak formation in atherosclerosis (Ross, 1999).

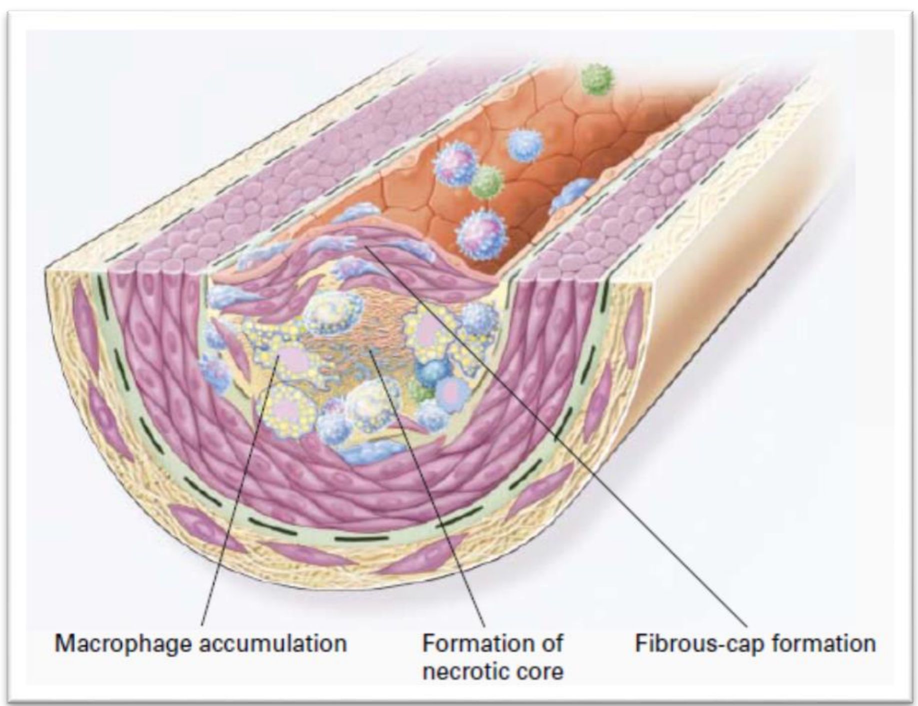


Figure (1.3): Formation of an Advanced, Complicated Lesion of Atherosclerosis (Ross, 1999).

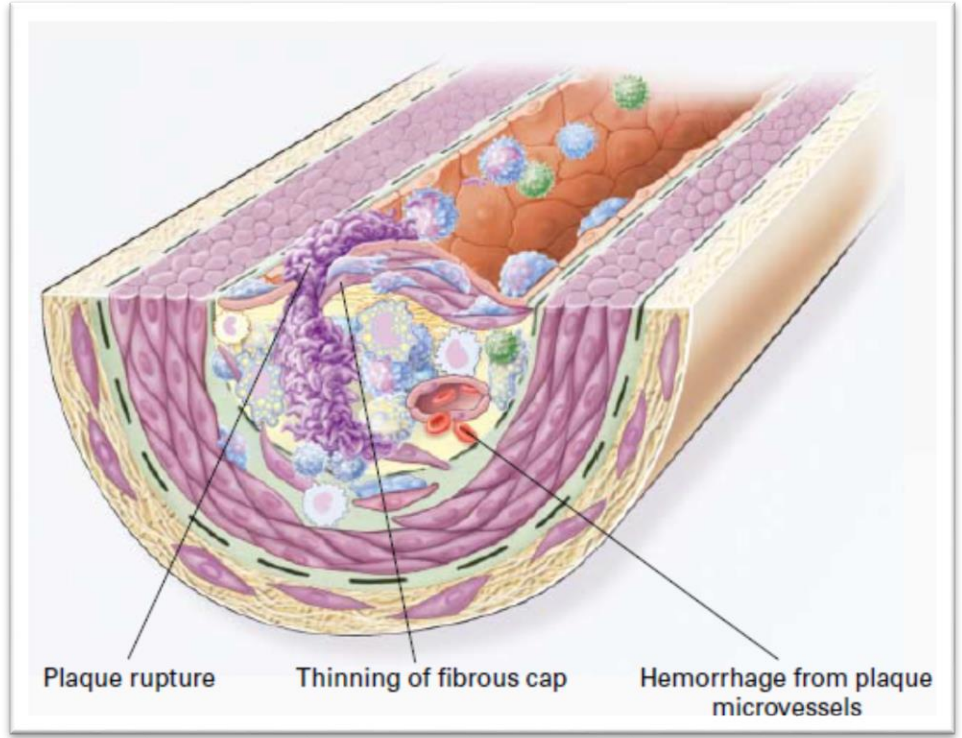


Figure (1.4): Unstable Fibrous Plaques in Atherosclerosis (Ross, 1999).

1.2.3 Risk factors for ASCVD

1.2.3.1 Major risk factors

- **Unhealthy blood Cholesterol**: Hypercholesterolemia increases superoxide free radicals production in the vessels and decreases synthesis and release of endothelium derived vasodilators. It also increases nitric oxide (NO) deactivation after its release from endothelial cells **(Ross, 1999)**.
- **Lipoproteins level**: High concentrations of some plasma lipoproteins are related to atherogenesis. Atheromagen lipoproteins include very low density

lipoprotein (VLDL), low density lipoprotein (LDL), and intermediate-density lipoproteins **(Saito et al., 2004)**.

- **Hypertension:** Hypertension is a risk factor in cardiovascular diseases and stroke. These complications are generally caused by high diastolic blood pressure. Hypertension damages endothelium by increasing the hemodynamic pressure on endothelium and may increase the permeability of arterial walls for lipoproteins. Elevated angiotensin II concentration stimulates SMC growth, increases inflammation and finally accelerates LDL oxidation in such patients **(Asgary et al., 2014, Asgary et al., 2013)**.
- **Smoking:** Smoking doesn't allow sufficient oxygen to reach the body's tissues. Smoking can also damage and tighten blood vessels, raise cholesterol levels, and raise blood pressure.
- **Insulin resistance:** This condition occurs if the body can't use its insulin properly. Insulin resistance may lead to diabetes.
- **Diabetes:** The body doesn't make enough insulin or doesn't use its insulin properly, hence the blood sugar is high.
- **Overweight or obesity:** The terms "overweight" and "obesity" refer to body weight that's greater than what is considered healthy for a certain height.
- **Lack of physical activity:** A lack of physical activity can worsen other risk factors for atherosclerosis, such as unhealthy blood cholesterol levels, high blood pressure, diabetes, overweight and obesity.
- **Unhealthy diet**: Foods that are high in saturated and transfats, cholesterol, sodium and sugar can worsen other atherosclerosis risk factors.
- **Old age:** Genetic or lifestyle factors cause plaque to build up in the arteries as with age. In men, the risk increases after age 45 and in women, the risk increases after age 55.

- **Family history of early heart disease:** The risk for atherosclerosis increases if the father or a brother was diagnosed with heart disease before 55 years of age, or if the mother or a sister was diagnosed with heart disease before 65 years of age.
- **Inflammation:** Inflammation is the body's response to injury or infection. Damage to the arteries inner walls seems to trigger inflammation and help plaque grow **(Rafieian-Kopaei et al., 2014).**

1.2.3.2 Emerging risk factors

According to **(Rafieian-Kopaei et al., 2014).**

- **High levels of CRP:** High levels of CRP are a sign of inflammation in the body and high level of CRP may develop atherosclerosis at a higher rate. Research is under way to find out whether reducing inflammation and lowering CRP levels also can reduce the risk for atherosclerosis.

- **Triglycerides**: High levels of Triglycerides in the blood also may raise the risk for atherosclerosis especially in woman.

- **Sleep apnea**: Untreated sleep apnea can raise the risk for high blood pressure and even a heart attack or stroke.

- **Stress**: The most commonly reported "trigger" for a heart attack is an emotionally upsetting event, especially the one involving anger.

- **Alcohol**: Heavy drinking can damage the heart muscle and worsen other risk factors for atherosclerosis.

1.2.4 Diagnosis of ASCVD

According to **(Teramoto et al., 2013).**

1. Ultrasonography

Noninvasive imaging tests include body surface ultrasonography (a high-frequency probe of ≥ 7 MHz), enabling observation of the stenosis degree and plaque formation (localized atherosclerotic lesions) in the peripheral arteries, such as the carotid arteries and arteries of the lower extremities.

2. Computed Tomography (CT)

Multidetector row CT (MDCT) proposals superior imaging speed and spatial resolution and permits the coronary arteries to be visualized after the injection of contrast medium into peripheral veins.

3. Magnetic Resonance Imaging (MRI) and MR Angiography (MRA).

Magnetic Resonance Angiography is used to visualize the cerebral/carotid arteries, aorta and renal arteries and to visualize of the stenosis of coronary lesions.

4. Angiography

Invasive diagnostic imaging techniques include angiographic evaluations of the degree of stenosis. That remains a central diagnostic technique for evaluating arterial stenosis.

5. Intravascular Ultrasound (IVUS)

Intravascular Ultrasound is a technique used to observe the arterial wall from the arterial lumen using an ultrasound device. It enable both the plaque volume and the plaque properties to be evaluated.

6. Angioscopy

Angioscopy is a technique used to observe the color of the plaque surface and estimate the properties of plaques.

7. Physiological Tests

Diagnostic techniques other than morphological tests include physiological tests, such as the brachial ankle pulse wave velocity (baPWV) and cardio-ankle vascular index (CAVI). Although these parameters are easily determined by measuring the pulse wave in the extremities using a dedicated device, it should be noted that the values act as indicator of artery stiffness and do not always represent atherosclerosis.

8. Exercise Electrocardiography

Exercise electrocardiography has been shown to have a sensitivity of approximately 70 percent and a specificity of approximately 75 percent for detecting significant coronary stenosis. Since the technique can be easily accomplished at a low cost, it is broadly used.

9. Myocardial Perfusion Scintigraphy

This technique is widely used in the diagnosis of coronary artery disease (CAD) to assess disease severity, myocardial viability and the prognosis and aids in decision making concerning therapeutic strategies. It is also used to screen for significant coronary stenosis, is relatively minimally invasive and can be a useful tool for monitoring atherosclerosis.

1.2.5 Inflammation in atherosclerosis

Inflammation is a key factor in the development of atherosclerosis at all phases. In the initial phase of atherosclerosis, oxidized low-density lipoproteins (oxLDL) accumulation in the aortic wall stimulates the expression of adhesion molecules that promote the monocytes migration into the aortic wall **(de Vries and Quax, 2016)**. Differentiation of monocytes into macrophages engulfing oxLDL and convert them into foam cells filled with lipid. Accumulation of modified LDL by macrophages activates cytokine production which sequentially promote the influx and activation of other inflammatory cells and their retention in the plaque. Most inflammatory cells in the plaque, and especially macrophages, are metabolic very active cells that exhibit high oxygen consumption which leads to oxygen deprivation in the plaque **(Marsch et al., 2013)**.

In addition, monocytes/macrophages release pro-angiogenic factors such as vascular endothelium growth factor (VEGF) and by interacting with vascular smooth muscle cells (VSMC), macrophages induce unbalanced synthesis of the extracellular matrix (ECM) leading to secretion of VEGF by VSMC **(Khurana et al., 2005).**

In advanced lesions, neovessel leakage constitutes the main entrance for inflammatory cells. Red blood cells (RBCs) can facilitate the extravasation of circulating inflammatory cells by increasing the numbers of rolling and adhering monocytes by increasing the force and/or the frequency of collision of monocytes with the endothelium **(Melder et al., 2000)**. Not only monocytes are increasingly found around neovessels, it has also been found that neutrophils and mast cells are associated with neovessels. These cells can release their granular content rich in serine proteases and matrix metalloproteases (MMP) that can digest components of

elastic fibers (elastin) and of the basement membrane (collagen, laminin and fibronectin) **(Michel et al., 2014)**. This high proteolytic activity can eventually lead to fibrous cap thinning and plaque erosion **(Leclercq et al., 2007, Dorweiler et al., 2008).** This malfunction increases inflammation and reduces cholesterol efflux which contributes to the expansion of necrotic core and ultimately increase the risk of plaque rupture **(Tabas and Bornfeldt, 2016).**

1.3 Associations of Chronic periodontitis and Atherosclerotic cardiovascular diseases

Clinical observations and animal models have established a clear correlation between periodontal disease and atherosclerosis. In particular polymicrobial infection with *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, and *Fusobacterium nucleatum* has been shown to promote progression of atherosclerosis **(Cortés-Vieyra et al., 2016).**

Associations between periodontitis and atherosclerosis would be predicted based on inflammatory mechanisms initiated locally or systemically by bacteria associated with periodontal lesions, that then effect the initiation or propagation of the atherosclerotic lesion as shown in figure(1.5) **(Bartold and Narayanan, 2006, Bresolin et al., 2013)**. Such lesions may be initiated by inflammatory stimuli including systemic and locally produced inflammatory cytokines and chemotactic agents that cause changes in the endothelium such as up-regulation of adhesion molecules. These changes promote interactions with leucocytes, such as monocytes, that promote leucocyte migration into the intimal layer of the artery **(Schenkein and Loos, 2013)**.

In a study on *P. gingivalis*, it was shown that *P. gingivalis* invaded and adhered to cardiac endothelial cells in fetal bovine, human umbilical vein endothelial cells and bovine aortic endothelial cells. The effects of invasion were 0.1%, 0.2% and 0.3% for bovine aortic endothelial cells, human umbilical vein endothelial cells and fetal bovine cardiac endothelial cells, respectively. It was reported that atherosclerotic lesions develop in the aorta by injecting *P. gingivalis* in mice **(Amar et al., 2009)**. In a study on *gingipain R.*, a proteolytic enzyme released by *P. gingivalis*, it was shown that *gingipain R*. can trigger Factor X, prothrombin and protein C and enhance thrombotic tendency, platelet aggregation, transformation of fibrinogen to fibrin and formation of an intravascular clot **(Kizildag et al., 2014).** *P. gingivalis* produces arginine-specific cysteine proteases, called gingipain Rs that can activate both the cellular and humoral coagulation systems. These gingipain Rs activate protease-activated receptors 1 and 4 (PAR-1 and PAR-4) on platelets to a degree sufficient to support ex *vivo* aggregation **(Pham et al., 2002)**.

In *vitro* studies have shown that gingipains R and K produced by *P gingivalis* degrade plaque constituents and that cultured monocytes exhibit foam cell phenotypes when exposed to *P gingivalis* FDC 381. The combination of intense oral and systemic inflammation and frequent bacteremias with an invasive prothrombotic organism suggests that periodontitis could contribute to atherosclerosis and its thrombotic complications by several mechanisms **(Brodala et al., 2005)**.

A number of inflammatory mediators and markers are present in higher concentrations in the systemic circulation of patients with periodontitis than in periodontal healthy individuals **(Schenkein and Loos, 2013).** There are a sufficient data indicating that inflammatory cytokines and other mediators are produced in the periodontal lesion **(Preshaw and Taylor, 2011)**.

It has been assumed that these mediators could “spill over” into the circulation. This would lead to inflammatory changes in the endothelium such as up-regulation of adhesion molecules and promotion of cytokine production, and thus initiation or acceleration of atheroma development **(Teles and Wang, 2011).**

In periodontal disease, the atherosclerotic plaque was reported to be associated with viable microorganisms entering the circulation and inducing thromboembolic events like ulceration, thrombosis, and apoptosis of vascular cells. Specific periodontal pathogens (*Aggregatibacteractinomycetemcomitans and Porphyromonas gingivalis*) were found to possess a peptide sequence similar to that of b-2-glycoprotein-I-dependent phospholipid (b2GPI). β2GPI in the vascular endothelium has been reported to play a direct role in mediating platelet destruction **(Gunupati et al., 2011).**

Finally**,** other study shown the presence of chronic periodontitis enhanced subclinical inflammation and eventually contributed to endothelial and vascular dysfunction **(Thomopoulos et al., 2010).**

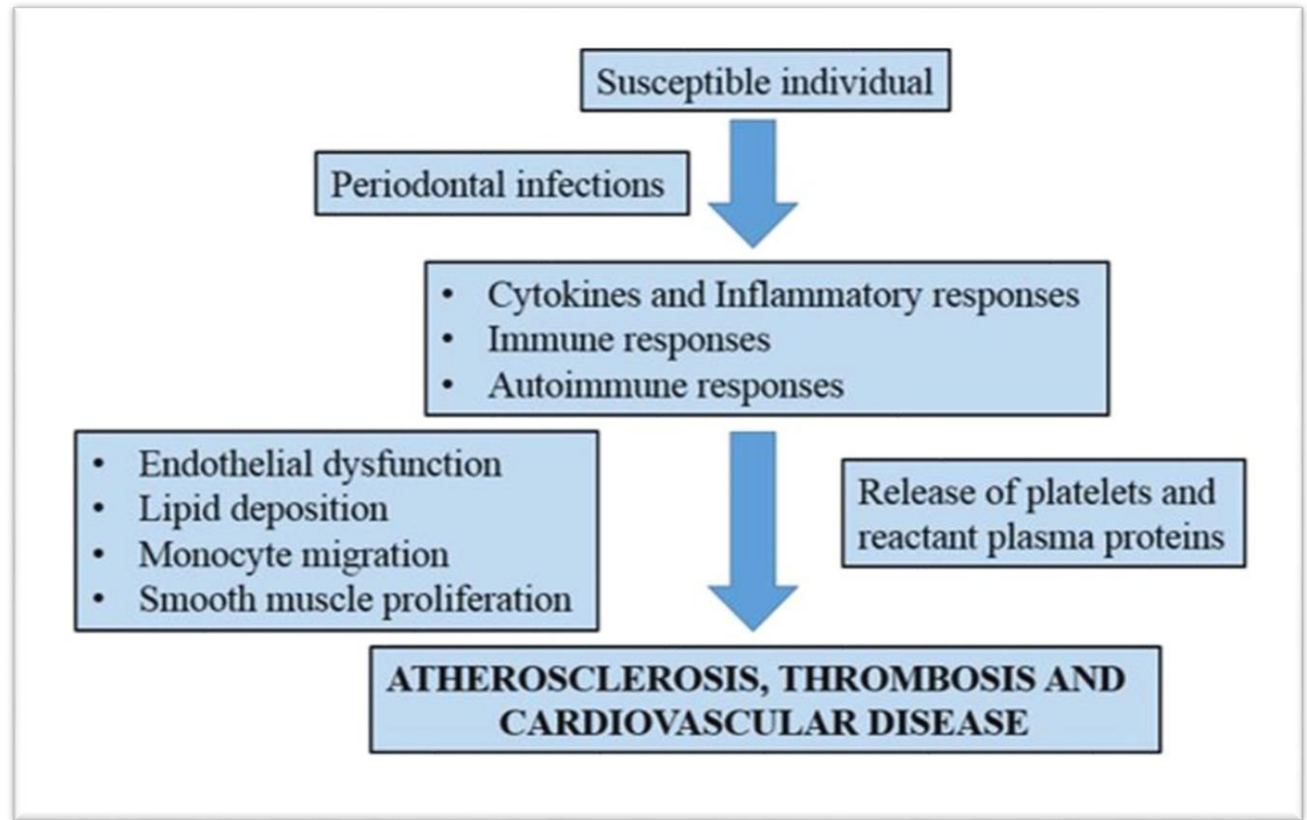


Figure (1.5): Schematic overview of potential inflammatory mechanisms linking periodontitis to cardiovascular diseases (Bartold and Narayanan, 2006)

1.4 High Sensitivity C- reactive protein

C-reactive protein was discovered by Tillet and Frances and its name is derived from its ability to precipitate the C-polysaccharide of Streptococcus pneumoniae **(Tillett and Francis, 1930).** It is an acute-phase reactant and nonspecific marker of inflammation, produced predominantly in hepatocytes as a pentamer of identical subunits in response to several cytokines, **Figure(1.9) (Norata et al., 2009, Lau et al., 2005**). Also, CRP is produced by neurons and its concentration in plasma is increased in Alzheimer disease **(Nakou et al., 2008).**

C-reactive protein regulated by cytokines like interleukin-6 (IL- 6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). These in turn cause systemic changes including hepatic release of a range of plasma proteins, activation of complement proteins and various metabolic changes **(Bansal et al., 2014)**. During bacterial infection, up to a 10,000-fold increase in circulating CRP levels due to de-novo hepatic synthesis can be seen. The rise is generally proportional to the degree of tissue damage **(Williams and Offenbacher, 2000, Gupta et al., 2017).**

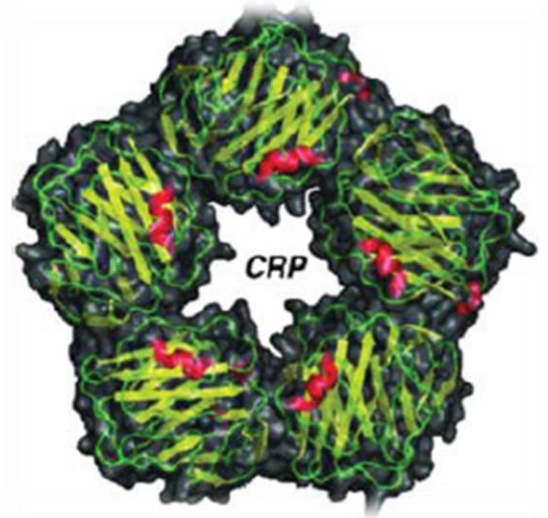


Figure. (1-6) : C - reactive protein structure (Lau et al., 2005)

CRP and other acute phase molecules are usually present in plasma at relatively low levels, but may be increased dramatically within 72hrs of tissue injury or with infection, the normal CRP levels vary between populations, with mean values between 1.0 to 3.0 mg/l. However, using ultrasensitive methods, it is possible to detect CRP levels as low as <1.0 mg/l **(Bansal et al., 2014).**

Although CRP is mostly produced by the liver, it has also been reported to be produced by adipocytes, vascular smooth cells and gingival tissues in response to an increase in interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). Thus, CRP

has been considered to be a possible mediator of the relationship between periodontitis and systemic conditions **(Mohan et al., 2014).**

1.4.1 Role of High Sensitivity C-reactive protein in patient with chronic periodontitis

Periodontal diseases involve chronic inflammatory processes resulting from interaction of selected gram negative bacterial species with the host defense in disease susceptible individuals. The host responds to the microbial challenge, with a high inflammatory response with increased levels of cytokines like IL-1, IL-6, TNF-α. These mediators promote activation of the acute phase reactants resulting in elevated serum levels of CRP, ceruloplasmin, α1-acid glycoprotein and serum amyloid A **(Gupta et al., 2017).**

Numerous studies have shown a positive association between the presence of chronic periodontitis and high serum CRP levels **(Noack et al., 2001, Slade et al., 2003, Gomes-Filho et al., 2011)** because it is biologically believable that inflammatory mediators (IL-1, IL-6 and TNF-α) are released under conditions of periodontitis and present the capacity to stimulate the hepatocytes to produce CRP. Similarly , in the presence of chronic periodontitis , higher serum CRP levels can be predictable **(Rutger Persson et al., 2003, Persson et al., 2005)**.

Other study reported that the presence of periodontal disease and high serum titer to *Porphyromonas gingivalis* independently related to high CRP levels **(Dye et al., 2005)**. In contrast, the titer of *A. actinomycetecomitans* was not related to the high CRP levels. Similar results for *P. gingivalis* were also observed by **(Pitiphat et al., 2008),** which has received considerable attention since they show the association

of the periodontal disease with the increased level of CRP **(Pitiphat et al., 2008, Bansal et al., 2014).**

Pervious study examined the effect of antimicrobial periodontal treatment on CRP, adiponectin and TNF-α levels, as they reported that periodontal treatment is effective in reducing CRP and TNF-α, while adiponectin does not seem to be influenced by periodontal treatment. Elevated levels of CRP and TNF-α may be associated with increased risk for further development of a thrombosclerosis in periodontitis patients **(Iwamoto et al., 2003)**.

1.4.2 Role of High Sensitivity C- reactive protein in patient with ASCVD

C-reactive protein, an inflammatory marker, is a novel and evolving biomarker for the extent and severity of atherosclerotic lesion and provides a useful predictive indicator for subsequent cardiovascular events **(Elgharib et al., 2003)**.

C-reactive protein may play a role in the atherosclerotic lesion, since it reduces the expression of nitric oxide (NO) synthase and prostacyclin synthase, and binds LDL-C and promotes its uptake by macrophages, a key step in atherogenesis. CRP also up-regulates the expression of adhesion molecules on endothelial cell (EC). All these phenomena are associated with atherogenesis (**Mehta et al., 2007, Shrivastava et al., 2015).**

Furthermore, When CRP binds to phospholipids expressed on the cell surface it can stimulate endothelial, smooth muscle and inflammatory cells. Proliferation of smooth muscle cells can cause blood vessel thickening and increased macrophage activity can lead to foam cells formation, which trigger the atherosclerotic process.

Stimulation of endothelial cells can provoke expression of inflammatory receptors **(Toregeani et al., 2014).**

C-reactive protein induces the coagulation pathway by stimulating tissue factor release from mononuclear, endothelial, and smooth muscle cells **(Anitha et al., 2015)**. Moreover, C-reactive protein increases plasminogen activator inhibitor-1 activity in human aortic endothelial cells with a concomitant reduction in tissue plasminogen activator activity, resulting in an overall impaired fibrinolytic capacity. In line, human volunteers also exhibit increased thrombin generation and impaired fibrinolysis upon C-reactive protein challenge **(Bisoendial et al., 2010).**

Both the proposed pro-atherogenetic role and the increment of circulating CRP in plasma are largely dependent on the ability of CRP to interact with vascular endothelial cells **(Ferri et al., 2007)**.

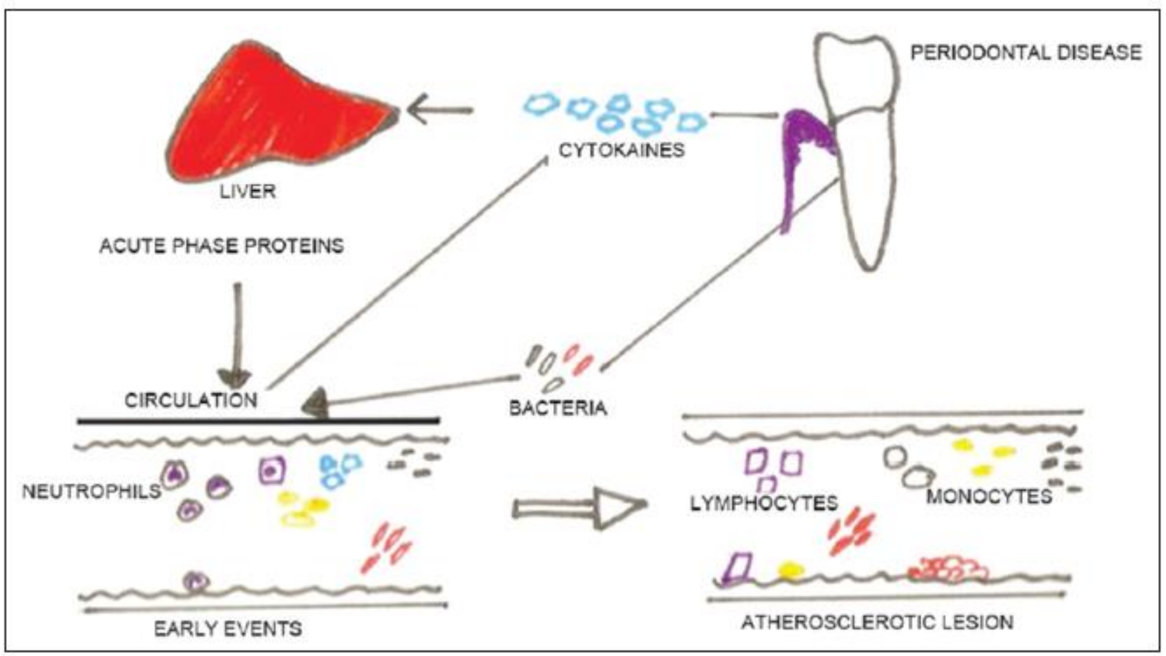


Figure (1.7): Schematic picture showing the effect of CRP in atherosclerosis and periodontitis (Thasleema and Don, 2019).

1.5 Anti-Cardiolipin Antibody

Cardiolipin is a phospholipid (diphosphatidylglycerol) that synthesized by cardiolipin synthase in the inner mitochondrial membrane of eukaryotic cells and some prokaryotic bacteria as shown in figure(1.6) **(Paradies et al., 2010, Dudek et al., 2018)** . This enzyme has a high activity in tissue with increased metabolic activity, including heart **(Frostegård et al., 2014)**. It plays as an essential role as normal electron transport and energy metabolism. Cardiolipin can start an antibody response in various diseases involving mitochondrial damage **(Rozee et al., 2003)**.

Cardiolipin antibodies are part of family of auto-antibodies directed against phospholipids of cell membrane which are known as anti-CL antibodies. They are found in 1% to 5% of people who are systemically healthy and can increase in several infectious diseases. These antibodies are found commonly in individuals with Systemic Lupus Erythematous (SLE) or Anti-Phospholipid Syndrome (APLS) **(Levine et al., 2002)**. These prothrombotic auto-antibodies also are associated with adverse pregnancy outcomes such as fetal involution, prematurity, low birth weight with cardiovascular sequelae such as atherosclerosis, stroke and myocardial infarction **(Schenkein et al., 2007)**.

Anti-cardiolipin antibodies can be classified in subclasses as IgM, IgG and IgA or as β2–glycoprotein dependent or independent. Anti-cardiolipin antibodies require presence of β2GPI to bind to Cardiolipin in β2–glycoprotein dependent group as in autoimmune diseases like SLE or APS. Meanwhile, in patient with syphilis or other infectious diseases the antibodies react directly; they are not only independent but also inhibited by β2GPI **(Hunt and Krilis, 1994, Mohamad et al., 2017).**

β-2-glycoprotein-I-dependent phospholipid is a plasma protein that binds to negatively charged phospholipid and is believed to have protective homeostasis mechanism by preventing platelets or endothelial cell pathological prothrombotic reaction **(Kumar, 2011).**

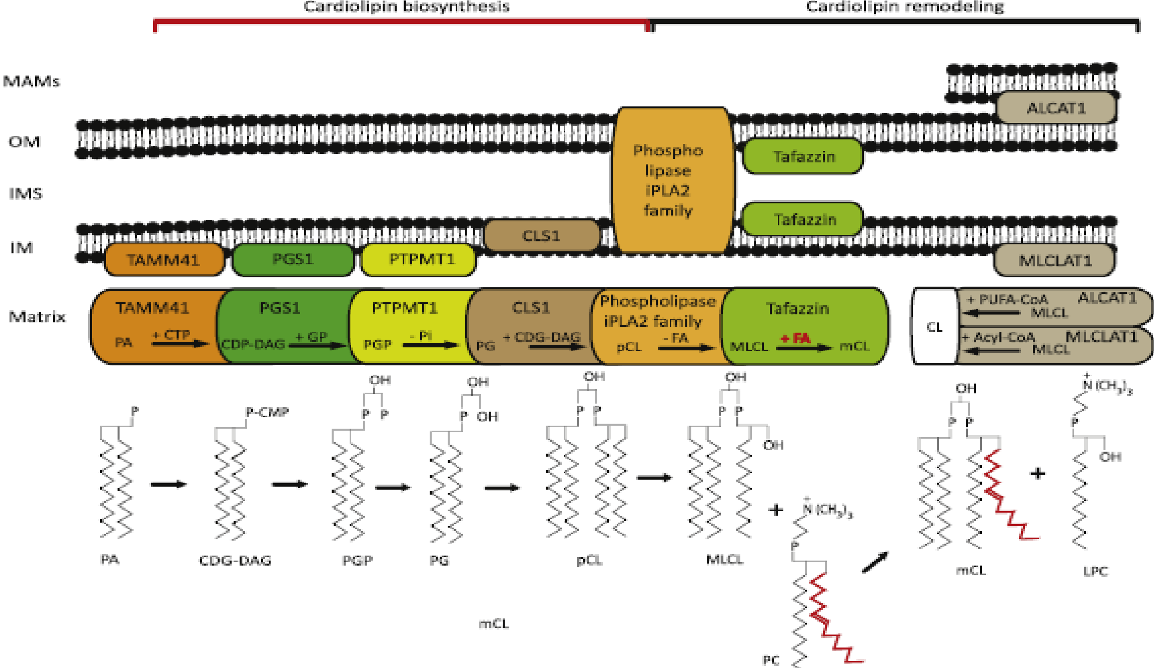


Figure. (1-8): Biosynthesis and remodeling of CL in mitochondria (Dudek et al., 2018).

1.5.1 Role of Anti-Cardiolipin Antibody in patient with chronic periodontitis

According to the Swiss Prot database, sequences homologous to the TLRVYK peptide of b2GPI are present in the arg-gingipain (Rgp) protease of *Porphyromonas gingivalis* and the phosphoglycerate kinase of *Treponema denticola*, So periodontal bacteria might stimulate anti-CL antibody production through molecular mimicry between bacterial peptides and TLRVYK peptide on b2GPI **(Chen et al., 2009).**

Other studies supported that homologous peptide on *Haemophilus influenza* or *Neisseria gonorrhea* might induce pathogenic anti-b2GPI antibodies via the

mechanism of molecular mimicry **(Blank and Shoenfeld, 2004).** Thus, bacterial infection could lead to production of pathogenic anti-CL and be responsible for a subset of cases of APS **(Schenkein et al., 2003).**

Another study found that the prevalence of b2GPI-dependent anti-Cardiolipin autoantibodies was greater in patients with chronic periodontitis and generalized aggressive periodontitis (16.2 and 19.3%, respectively) than in healthy controls and in patients with localized aggressive periodontitis (6.8 and 3.2%, respectively). Patients with these autoantibodies demonstrated increased pocket depth and attachment loss compared with patients lacking the antibodies **(Schenkein et al., 2003).**

1.5.2 Role of Anti-Cardiolipin Antibody in patient with ASCVD

Antiphospholipid Antibodies (aPLs) exert proinflammatory and procoagulant effects directly on endothelial cells, and the inflammatory and immune components of autoantibody mediated thrombosis may play an indirect role in atherogenesis **(Matsuura et al., 2006, Long and Leya, 2008).**

Anticardiolipin antibody was identified as an independent predictive factor for the increase in Intima-media thickness **(Ames et al., 2002)**. In an experimental model a clear link between aCL and atherosclerosis was also demonstrated since immunization of LDL cholesterol receptor deficient mice with cardiolipin determined high aCL titres accelerated Atherosclerosis **(Alves and Ferreira, 2008).**

The previous studies reported that increased anti-oxCL IgM antibodies are associated with atherosclerosis development **(Türkoğlu et al., 2008, Su et al.,**

2013). oxCL is recognized as a natural antigen that stimulates proinflammatory effects in the artery and promotes atherosclerotic plaques formation **(Bochkov et al., 2010)**.

Furthermore, Oxidized CL (oxCL) is found to accumulate both in rabbit and human atherosclerotic lesions **(Tuominen et al., 2006)** and in the aortic root of mice fed a high fat diet **(Zhong et al., 2014)**.

However, additional study purport that autoantibodies to oxCL may serve a protective role against the onset and development of atherosclerosis **(Frostegård et al., 2014)**. The discrepancies regarding the effects of anti-oxCL antibodies on atherosclerosis may reveal the influence of potential physiological modifiers, including gender ,age, and other existing diseases **(Shen et al., 2015).**

1.6 Anti-Phosphoryl choline

Phosphoryl choline was first detected in 1967 in the Gram-positive bacterium *Streptococcus pneumoniae,* where it was found to be associated with a polysaccharide component of the cell wall **(Tomasz, 1967)**, subsequently shown to be teichoic acid **(Brundish and Baddiley, 1968)**. In addition, it was later found in cell membrane lipoteichoic acid **(Briles and Tomasz, 1973)**. Phosphoryl choline is also a component of a variety of other Gram-positive *organisms, including Clostridium spp.***(Sanchez-Beato et al., 1995)***, Lactococcus spp*. **(Gillespie et al., 1996)**, and *Bacillus* spp.**(Gillespie 1996).**

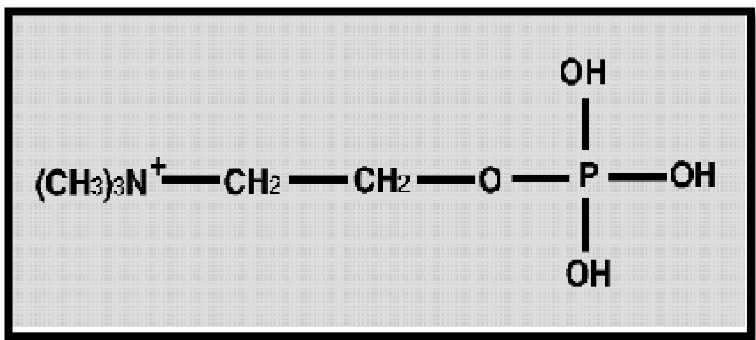


Figure (1-9): phosphoryl choline structure (Su, 2009).

Oxidative modification of phosphatidylcholine (PtC) in the membranes of LDL induce conformational changes to reveal Phosphoryl choline epitopes as shown in figure (1-7).

Phosphoryl choline is an antigenic component on the cell surface of many commensal and pathogenic bacteria that reside in the upper airway **(Goldenberg et al., 2004).**

Anti-Phosphoryl choline antibodies has been first found to protect mice against *Streptococcus pneumoniae* infection.

Anti-Phosphoryl choline antibodies are found in normal serum and they represent a significant component of natural immunity **(Harnett and Harnett, 1999)**.These antibodies are detectable in the serum of normal individuals at mean concentrations of 320µg/ml for the IgG class and 110µg/ml for the IgM class. These antibodies are present in most adults under the age of 60 years. However, they are absent in very young children and are found at low levels in the elderly and IgG2-deficient adults, which are known to respond poorly to carbohydrate antigens. The

fluctuation of anti-PC antibodies levels in healthy individuals over time is small **(Padilla et al., 2004).**

Phosphoryl choline-bearing dental plaque microorganisms can induce an antibody response to PC that can affect the inflammatory response of atherosclerosis **(Schenkein et al., 2001) .**

Finally, in *vivo* molecular mimicry between the PC moiety of the cell wall polysaccharide of bacterial pathogens and the PC moiety of OxPLs, present on OxLDL and on apoptotic cells **(Chang, Binder et al. 2004)**. So PC epitopes shared by oxidation, apoptosis and infection act as a PAMP recognized by PRRs **(Su, 2009).**

1.6.1 Role of Anti-phosphoryl choline in patient with chronic periodontitis

Periodontitis is regarded as a consequence of interaction between the host and the bacteria. Lipopolysaccharide located in the Gram-negative periodontal bacteria on the outer membrane is a vital component of the etiology of periodontitis. This molecule has 3 major component parts: the core, the external O-antigen and the lipid A which is embedded within the lipid portion of the outer membrane **(Chaston, 2006)** and is responsible for the endotoxin properties. Minor lipopolysaccharide antigenic components have also been identified. One such molecule is phosphoryl choline that is attached to cell wall polysaccharide and lipoteichoid acid, which has been identified in over 30% of the supragingival and subgingival flora, including *Streptococcus oralis*, *Sterptococcus sanguis, Actinomyces israelii, Actinomyces naeslundii, Fussobacterium mucleatum, Haemophilus aphrophilus and Actinobacillus actinomycetemcomitans* ***(Karnoutsos et al., 2008).***

The possible function of phosphoryl choline is that certain bacteria may utilize it to gain access to endothelial cells **(Schenkein et al., 2000)** or the circulation. Bacterial adherence, colonization and invasion are dependent on surface phosphoryl choline. It has been shown that *Streptococcus pneumoniae* and *Actinobacillus actinomycetemconimans* invasion into endothelial cells is based on the interaction between surface phosphoryl choline and endothelial surface receptors for platelet activating factor**(Clark et al., 2013).**

Phosphoryl choline, as a component of the lipopolysaccharide (LPS) motive of many bacteria, plays a role in provoking a host immune response. Studies have shown that Phosphoryl choline influences polyclonal B-cell differentiation and activation. Additionally, there is host production of IgG and IgM antibodies directed against Phosphoryl choline, which can assist to monocyte recognition and phagocytosis of the pathogenic bacteria **(Purkall et al., 2002).**

Signs of such host-periodontal pathogen interplay are not only detected locally but also systemically. The ability of periodontal pathogen bacteria of producing a systemic response to phosphoryl choline is demonstrated by higher serum levels of antibodies (anti phosphoryl choline IgG) directed toward phosphoryl choline in patients with attachment loss, in comparison with those with healthy gingiva **(Schenkein et al., 1999) .** Additional studies demonstrated that gingival crevicular fluid contains higher concentrations of IgG α-PC than serum from the same individuals, indicating that there is local production of α-PC that is likely induced by oral bacteria **(Schenkein et al., 2004).**

Other study found that serum levels of IgG anti-PC, IgG anti-CL, and IgM anti-CL decreased after periodontal scaling and root planning but this change was only

significant for IgG anti-PC **(Chaston, 2006).** Finally, many oral bacteria carry PC groups on the surface and induce robust anti-PC responses which are almost exclusively of the IgG2 isotype **(Schenkein et al., 1999).**

1.6.2 Role of Anti-phosphoryl choline in patient with ASCVD

Atherosclerosis is characterized by the presence of large numbers of macrophages and T cells and production of inflammatory cytokines in the atherosclerotic lesions. One important antigen in atherosclerosis is OxLDL, which is immune-stimulatory, pro-inflammatory and is also taken up by macrophages in the artery wall. Many researcher revealed that many pro-inflammatory effects of oxLDL are caused by inflammatory phospholipids generated during LDL oxidation. These have palate activating factor (PAF) like properties since a major ligand for the PAF receptor, phosphoryl choline (PC), is exposed on OxLDL **(Su et al., 2008)**. Oxidation of these lipids is therefore associated with an inflammatory response and a conformational change that expose the PC epitope, which is exposed on the surface of modified and oxidized LDL (ox-LDL) **(Gigante et al., 2014).**

Phosphoryl choline mediates the uptake of OxLDL by macrophages thus inducing foam cells formation, which is the hallmark of the early fatty streak **(Su, 2009).**

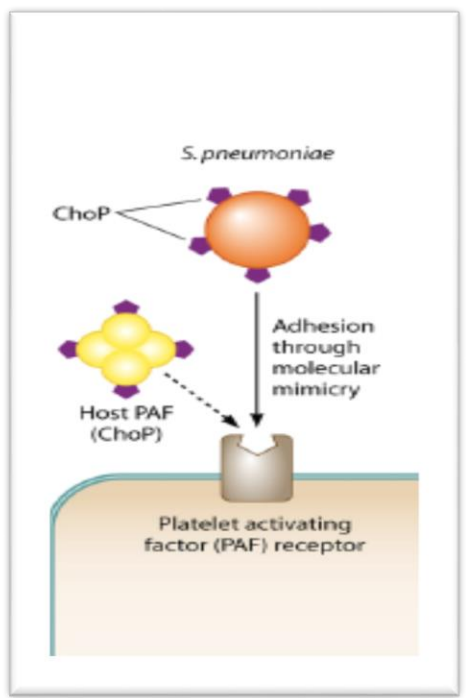


Figure (1-10):molecular mimicry between phosphoryl choline and platelet activating factor (PAF) receptor (Clark et al., 2013).

One possible mechanism was that PC-bearing oral bacteria could access to the immune system, and possibly the general circulation through interaction with the PAF receptor on endothelial cells **(Schenkein et al., 2000).**

Some researchers suggest that both phosphoryl choline bearing strains of oral bacteria and oxidized low-density lipoproteins (oxLDL) react with human serum anti-phosphoryl choline IgG **(Schenkein et al., 2001)**. This proposes that antibodies produced against certain periodontal bacteria would also react to phosphoryl choline-bearing oxLDL **(Shaw et al., 2003)** and, therefore, increase the absorption of this lipid by foam cells, promoting further progress of atherosclerosis. Furthermore, Elevated titers of antibody to oxLDL (which include anti-PC) have been shown to occur in angiographically verified coronary artery disease and coronary stenosis **(Lehtimäki et al., 1999).**

Chapter Two

Materials and Methods

Materials and methods

2.1 Subjects

Eighty five (85) subjects, males and females aged (35-64) years old were recruited in this study. They were from attendants seeking treatment in the cardiology clinic in Al Sadr Teaching Hospital, patients were seeking periodontal treatment in department of periodontics at College of dentistry, University of Basra and people from the Iraqi national blood bank.

2.1.1 Inclusion criteria

Selective criteria for patient with chronic periodontitis require the presence of:

- At least 20 teeth.
- At least 4 sites with PPD of ≥4mm and at least 4 sites with CAL of (1-2) mm **(Lang et al., 1999) .**

While for patient with Atherosclerotic cardiovascular disease

- Patients diagnosed with Atherosclerotic cardiovascular disease by Angiography.

- ### 2.1.2 Exclusion criteria
    - Any patient had history of other chronic, systemic diseases with known associations with PD as diabetes mellitus, Rheumatoid Arthritis, etc.
    - Smoker.
    - Medication (anti- inflammatory or antimicrobial therapy) within previous 3 months.

- Pregnancy.
- Contraceptive pills.
- Previous periodontal treatment within previous 3 months.

2.1.3 Study design

All the individuals were informed about the purpose of this investigations. The study was carried out in period from November 2018 to April 2019.

The selection was performed according to inclusion and exclusion criteria. Informed consent **(Appendix Ⅰ)** was obtained from each participant, and a questionnaire was used to record the background information, dental and medical histories of the participants, then full examination of clinical periodontal parameters (P.I, GI, BOP, PPD and CAL) and 5ml of venous blood was harvested from each subject as shown in figure (2.1) **(a copy of data sheet is provided in appendix II)**.

The study protocol was approved by the ethical committee of the college of Dentistry/ University of Baghdad **(Appendix III).**

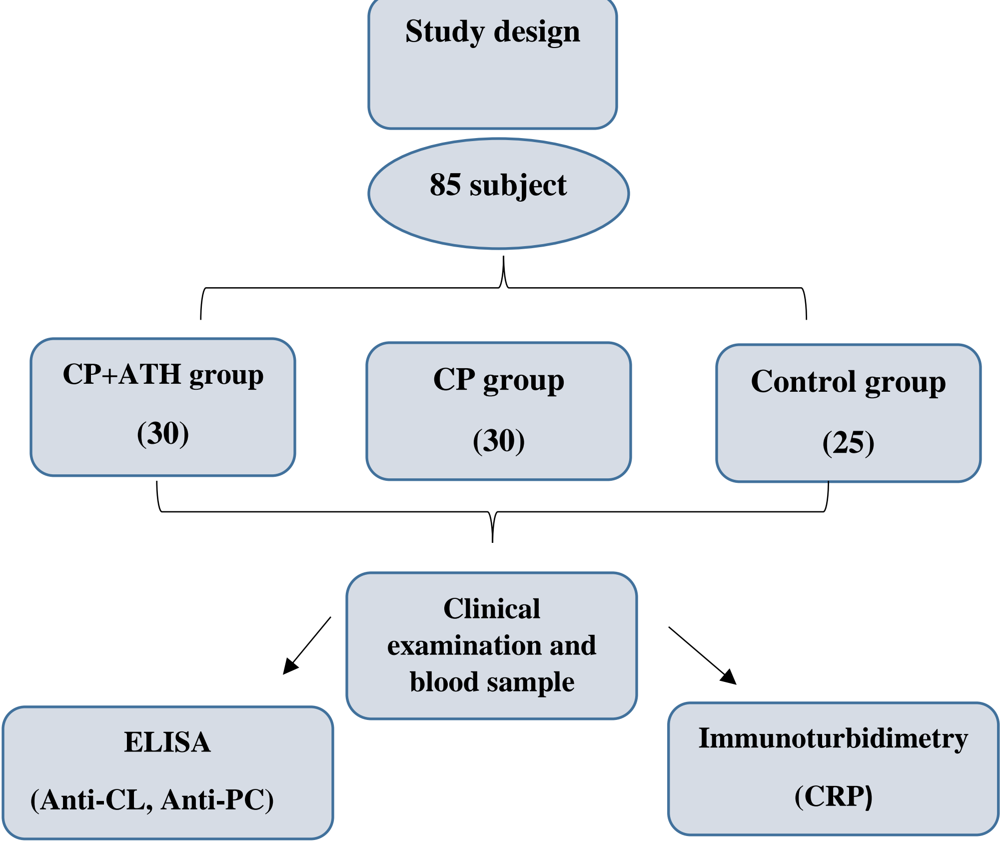


Figure (2.1): Diagram illustrate the study design

The subjects were divided into three main groups:

1. Chronic periodontitis (study group):

Thirty patients diagnosed to have chronic periodontitis and didn't have any systemic diseases. Chronic periodontitis in patients was defined as the presence of at least four sites with PPD of ≥ (4) mm and clinical attachment loss of (1-2) mm, this made according to the international classification system for PD (***Lang et al, 1999***).

2. Atherosclerosis and chronic periodontitis (study group):

Thirty patients diagnosed to have chronic periodontitis and Atherosclerosis cardiovascular disease according to catheterization.

3. Systemically healthy with clinically healthy periodontium (Control group):

Twenty five subjects with clinically healthy periodontium as determined via GI scores < 0.5 **(Löe and Silness, 1963)** and without periodontal pocket or clinical attachment loss and apparently without any systemic disease. This group represents as a base line data for the level of serum IgM anti-cardiolipin, serum IgG anti-phosphoryl choline and serum hs C - reactive protein.

2.2 Instruments& materials

2.2.1 Materials and instruments used for clinical examination

1. Disposable dental mouth mirrors.
2. Periodontal probes (the University of Michigan O probe, with William's markings at (1, 2, 3, 5, 7, 8, 9 and 10 mm).
3. Autoclave / Delma for sterilized instruments.
4. Cotton wool, gauze.
5. Gloves and masks.
6. Case sheet form.

2.2.2. Materials and Instruments used for blood sample collection

1. 5ml disposable plastic syringes.
2. 70% Isopropyl Alcohol pad.
3. Gel separating tube
4. Tourniquet

5. Indelible marker pen
6. Cooling box and ice pack.
7. Test tube racks.

2.2.3 Instruments, Equipment and kits used in the laboratory

2.2.3.1 Instrument

1. Eppendorf tubes.
2. Adjustable micro pipettes (SLAMED 100/1000)
3. Disposable test tubes

2.2.3.2 Equipment

1. Freezer(-20C)
2. Centrifuge (HETTICH –GERMANY).
3. Microplate ELISA Shaker (Human, Germany), **figure (2.2).**
4. Microplate ELISA reader device (Human, Germany), **figure (2.3).**

2.2.3.3 Kits

1. Test kit, quantitative immunoturbidimetric determination of serum hs-CRP of ARCHITECT LOT. NO.80517Y600. EXP.31-1-2020, **figure (2.4).**
2. Test kit, ELISA Kit (96-wells) for quantitative determination of serum Anti-Cardiolipin (IgM) of EUROIMMUM LOT.NO.E 180708AK. EXP.7.Jul.2019, **Figure (2.5).**
3. Test kit, ELISA Kit (96-wells) for quantitative determination of Serum Anti-Phosphoryl choline of AESKULISA LOT.NO.17230. EXP.2-2020, **figure (2.6).**

Figure (2.2): Microplate ELISA shaker (Human, Germany)

Figure (2.3): Microplate ELISA Reader device (Human, Germany)

2.3 Methods

2.3.1 The clinical periodontal parameters

Assessment of the periodontal status was carried out for all participants by using Michigan O periodontal probe, Four areas of each tooth (buccal/labial, lingual/palatal, mesial and distal) were examined to assess plaque deposits, gingival condition and the loss of periodontal support. Scores were given according to the criteria of the following indices:

2.3.1.1 Plaque Index (P.I):

The four surfaces of each tooth except 3rd molar were examined and scored according to Plaque Index by **(Silness and Löe, 1964).**

- **Score 0:** Absence of plaque in the gingival area.
- **Score 1:** A film of plaque adhering to the free gingival margin &adjacent area of the tooth surface, the plaque may be recognized only by running a probe across the tooth surface.
- **Score 2:** Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/ or adjacent tooth surface, which can be seen by naked eye.
- **Score 3:** Abundance of soft matter within the gingival pocket and /or the gingival margin and adjacent tooth surface.

2.3.1.2 Gingival Index (GI):

The gingival inflammation at the 4 surfaces of each tooth except 3rd molars was assessed using the criteria of the gingival index system **(Löe, 1967).**

- **Score 0:** Normal gingiva.
- **Score 1:** Mild inflammation, slight change in color, slight edema, no bleeding on probing.
- **Score 2:** Moderate inflammation, redness and glazing, edema, bleeding on probing.
- **Score 3:** Severe inflammation, marked redness and edema, ulceration tendency to spontaneous bleeding.

2.3.1.3 Bleeding on probing (BOP):

The periodontal probe was inserted to the bottom of gingival crevice, (periodontal pocket) , if there is bleeding within 15-30 seconds, the site was given score 1 and score 0 for non-bleeding site **(Muhlemann, 1971).**

2.3.1.4 Probing Pocket Depth (PPD):

Probing pocket depth was determined by inserting a periodontal probe into the periodontal pocket without pressure until resistance is felt when the probe tip stops at the base of the pocket, and then measuring the distance from the gingival margin to the base of the pocket **(Papapanou and Lindhe, 2015)**. The sites of measurement were mid-buccal line, midpalatal/ lingual line, mesio-buccal and disto-buccal line angles. The PPD measurement has been performed using Michigan O periodontal

probe. The selective criteria for patient with CP required the presence of at least 4 sites with probing pocket depth ≥ 4mm **(Lang et al., 1999).**

2.3.1.5 Clinical Attachment Loss (CAL):

Clinical attachment level was determined by inserting a periodontal probe into the periodontal pocket without pressure until resistance is felt when the probe tip stops at the base of the pocket, and then measuring the distance from cemento-enamel junction (CEJ) to the base of the pocket. If there is no gingival recession, CEJ can be detected by feeling with the probe **(Papapanou and Lindhe, 2015)**. CAL was measured at 4 sites for each tooth including: disto-buccal, mid-buccal, mesio-buccal and mid-lingual sites. If the gingival margin is located at the CEJ, the CAL equals to the PPD, if the gingival margin is located apical to the CEJ, the CAL is greater than the PPD and lastly if the gingival margin is located coronal to the CEJ, the CAL is less than PPD and determined by subtracting the distance between the gingival margin and CEJ from the PPD.

In some situations where the CEJ was totally obliterated, these teeth must be excluded from examinations:

1. Full crown coverage.
2. Badly carious tooth, were extending mesially, distally, labially/ buccally or lingually / palatally below the CEJ.
3. Disto- occlusal, mesio-occlusal, labially/buccally, palataly/lingual or MOD fillings were extended below CEJ.
4. Heavy calculus covers the teeth.

2.3.2 Serum samples collection

Five ml of venous blood was collected from each participant by venipuncture from the cubital fossa using 5ml plastic disposable syringe, then transferred into jel separating tubes, centrifuged for 15 minutes at (1000x g) and then sera were separated, then the tubes were labelled and stored at (-20°C) for later analysis by Enzyme Linked Immuno-Sorbent Assay (ELISA) for quantitative determination of serum IgM Anti-Cardiolipin antibody and serum IgG anti-Phosphoryl choline, while for hs-C reactive protein serum level was estimated by immunoturbidimetry.

2.3.3 Immunological assays

2.3.3.1 Principle of serum high sensitivity C- reactive protein

MULTICENT CRP Vario is a latex immunoassay developed to accurately and reproducibly measure blood CPP in serum and plasma. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination results. This agglutination was detected as an absorbance change (572 nm), with the rate of change being proportional to the quantity of CRP in the sample.

Figure (2.4): ARCHITECT CRP VARIO Reader device and Kit

2.3.3.1.1 Content of test kit

MULTIGENT CRP vario is supplied as a two-reagent kit which contains:

R1: Glycerin buffer (ph7.0)

R2: Anti-CRP polyclonal antibodies
Adsorbed on latex particles

2.3.3.1.2 Assay procedure for serum hs-C reactive protein

The test performance were carried out fully automatically using an analysis device (ARCHITECT plus 4000).

2.3.3.2 Principle of serum IgM Anti-Cardiolipin Antibody ELISA

The test kit contains microtiter strips each with 8 break-off reagent wells coated with Cardiolipin. In the first reaction step, diluted patient sample were incubated in the wells. In many cases, Antibodies to Cardiolipin rely on a plasma protein (B2-glycoprotien I) as a cofactor for antigen recognition. In the case of positive samples, the specific (IgM) antibody will have bound to the antigen. To detect the bound antibody, a second incubation was carried out using an enzyme-labelled anti-human IgM (enzyme conjugate) catalyzing a color reaction.

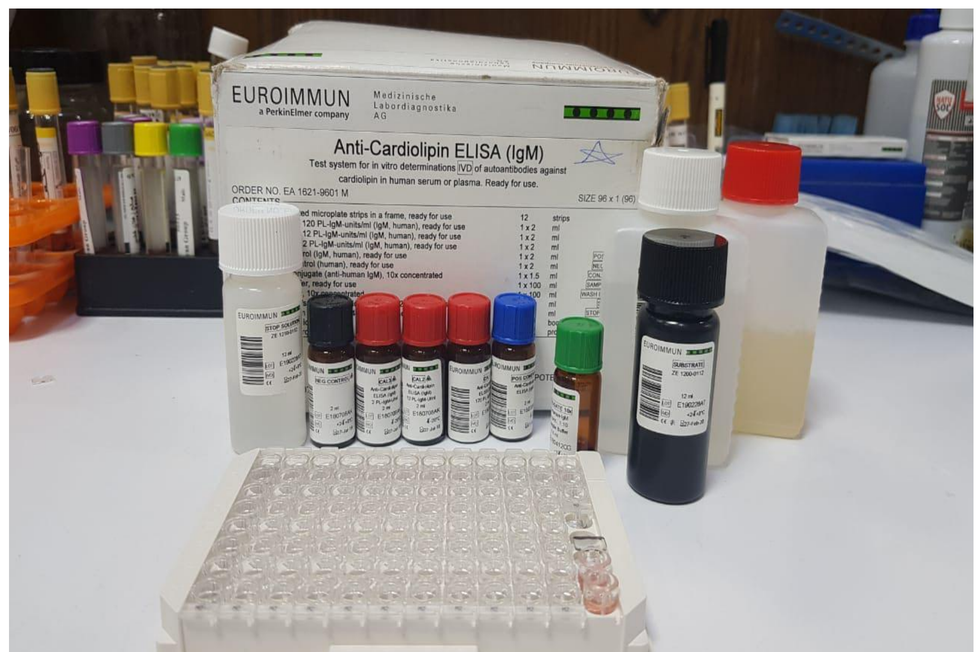


Figure (2.5): Human serum Anti-Cardiolipin ELISA Kit.

2.3.3.2.1 Content of the test kit

1. **Microplate wells coated with antigens:** 12 microplate strips each containing 8 individual break-off wells in a frame
2. **Calibrator 1:**120 RU/ml (IgM, human)
3. **Calibrator 2:**12RU/ml (IgM, human)
4. **Calibrator 3:** 2RU/ml (IgM, human)
5. **Positive control:** (IgM, human)

6. **Negative control:** (IgM, human)
7. **Enzyme conjugate:** peroxidase-labelled anti-human (rabbit, goat), 10x concentrate
8. **Sample buffer:** contain b2-glycoprotien I
9. **Wash buffer:** 10x concentrate
10. **Chromogen/substrate:** TMB/H2O2
11. **Stop solution:** 0.5M sulphuric acid
12. **Test instruction**
13. **Quality control certificate**

2.3.3.2.2 Assay procedure for serum IgM Anti-Cardiolipin

1. The patient samples were diluted 1:20 in sample buffer.
2. 100 µl of the calibrators, positive and negative controls or diluted patient samples were transferred into individual microplate wells according to the pipetting protocol.
3. Then the sample were Incubated for 30 minutes at room temperature(+18°C to +25 C)
4. The wells were emptied and subsequently washed 3 times using 300 µl f working strength wash buffer for each wash
5. 100 µl of working strength enzyme conjugate (peroxidase-labelled microplate anti-human IgM) was added into each of the microplate wells. Incubated for 30 minutes at room temperature (+18°C to +25 C), then the wells were emptied. Washed as described above
6. 100 µl of chromogen/substrate solution was added into each of the wells. Incubated for 15 minutes at room temperature (+18°C to +25°C) protect from direct sunlight.

7. 100 ul of stop solution was added into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.
8. Photometric measurement of the color intensity was made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring the microplate was slightly shake to ensure homogeneous distribution of the solution.

2.3.3.3 Principle of serum IgG Anti-Phosphoryl choline ELISA

Serum samples diluted 1:101 were incubated in the microplates coated with the specific antigen. Patient´s antibodies, if present in the specimen, bind to the antigen. The unbound fraction was washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) were incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate was washed off in the following step. Added of TMB-substrate generated an enzymatic colorimetric (blue) reaction, which was stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen was a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

Figure (2.6): Human serum Anti-Phosphoryl Choline ELISA Kit.

2.3.3.3.1 Content of the kit

1. **Sample Buffer (5x):** 5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).
2. **Wash Buffer (50x):** 50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative).
3. **Negative Control:** Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).
4. **Positive Control:** Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).
5. **Cut-off Calibrator:** Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).
6. **Calibrators:** Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).
7. **Conjugate, IgG:** Containing Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA).
8. **TMB Substrate**: Stabilized tetramethybenzidine and hydrogen peroxide (TMB/H2O2).
9. **Stop Solution:** 1M Hydrochloric Acid
10. **Microtiter plate:** 12 x 8 well strips N/A with breakaway microwells.

2.3.3.3.2 Assay procedure for serum Anti-Phosphoryl choline

1. The concentrated sample buffer was diluted 1:5 with distilled water.
2. 100 µl of Calibrators (CAL.A to CAL.F) was added into the designated wells for QUANTITATIVE and 100 µl of each of the following: Negative control (NC) and Positive control (PC), and Patients diluted serum (P1, P2...).
3. Then the sample was incubated for 30 minutes at 20-32°C/68-89.6°F.
4. The sample was Washed 3x with 300 µl washing buffer (diluted 1:50).
5. 100 µl conjugate was added into each well
6. Then, the sample was Incubate for 30 minutes at 20-32°C/68-89.6°F and Washed 3x with 300 µl washing buffer (diluted 1:50)
7. 100 µl TMB substrate was added into each well.
8. Then, Incubated for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
9. 100 µl stop solution was added into each well, using the same order as pipetting the substrate.
10. Incubated 5 minutes minimum
11. Then, the plate was Agitated carefully for 5 sec
12. Absorbance at 450 nm (recommended 450/620 nm) was read within 30 minutes.

2.3 Examiner alignment

Inter and intra examiner calibrations were performed to ensure validity and reproducibility of the clinical periodontal parameters.

2.4.1. Inter examiner alignment

For inter examiner calibration, the periodontal parameters for 5 subjects was measured by the researcher and the supervisor at the same time.

2.4.2 Intra examiner alignment

For intra examiner calibration, the periodontal parameters for 5 subjects were measured twice by the researcher with two days interval between the two measurements.

Measurements were compared, and the results of calibration revealed a non-significant difference.

2.5 Statistical analysis

Data were processed and analyzed using SPSS 21 (statistical package for social science). Both descriptive and inferential analyses were used.

2.5.1 Descriptive Statistics

- Tables, Figures, Ratio and Percentage.
- Arithmetic Mean and Median
- Standard Deviation (Std. Dev.).
- Mean Difference.

2.5.2 Inferential Statistics

These were used to accept or reject the statistical hypotheses, which included non-parametric test for non-normally distributed variables and parametric test for normally distributed variables as below:

- Analysis of Variance Test (ANOVA) One Way.
- Gabriel test
- Student t- Test for equality of means of two independent groups.
- Chi – Square Test.
- Kruskal Wallis test
- Mann-Whitney *U*-test.
- spearman correlations coefficient was used for testing the correlation between the two independent variables; the clinical and immunological parameters.
- Spearman rho Correlations Coefficient was used for testing the correlation between the two independent variables; between the immunological parameters

The level of significance (Sig.) was accepted at $p < 0.05$, highly significance when $p < 0.01$and non-significant at $p > 0.05$.

Chapter Three

Results

Results

3.1 Demographic characteristics of the study subjects

The demographic characteristic of (85) subjects included in the present study were shown in table (3.1) including 3 group: 30 Patients with chronic periodontitis (CP study group) with mean age ±SD (44.5±5.67), the 2nd group composed of 30 patients with Atherosclerosis and chronic periodontitis (ATH+CP study group) with mean ag ±SD (52.9±5.61), while 3rd group composed of (25) apparently healthy volunteers (Control group) with mean age ±SD (42.16±5.65). Furthermore male: female numbers of the study groups, which were 19:11 for CP group, 27:3 for ATH +CP group and 12:13 for the control group.

Table 3.1: Demographic characteristics of study group

Gender group		CP cases N=30	CP+ATH cases N=30	Controls N=25
Male	NO.	19	27	12
	%	63%	90%	48%
Female	NO.	11	3	13
	%	37%	10%	52%
age	**Range**	35-64	35-64	35-64
	Mean ±SD	44.5±5.78	52.9±5.61	42.16±5.65

3.2 Clinical periodontal parameters analysis

3.2.1 Plaque index (P.I) and gingival index (G.I):

The analytic statistics for P.I and G.I among study groups were shown in table (3-2).

The current work revealed there was a significant difference among study groups regarding P.I at (p=0.0001) and G.I at (p=0.0001) as the mean P.I value (±SD) in the CP+ATH cases was 2.04 (±0.29), CP cases was 1.69((±0.36) and in the controls was 0.5 (±0.20), Furthermore, the mean G.I value (±SD) in the CP+ATH cases was 1.37 (±0.23), CP cases was 1.43((±0.27) and in the control group was 0.47(±0.17).

However, inter groups comparisons of mean values of plaque index revealed there were highly significant differences between the CP group and ATH+CP group, CP group and control group, as well as between the ATH+CP group and control group at p-value ≤ 0.01 as shown in table (3-3). Furthermore inter groups comparisons of mean values of gingival index revealed there were highly significant differences between the ATH+CP group and control group, CP group and control group at p-value ≤0.01. With no statistical difference was observed between the ATH+CP and CP groups as shown in table (3-4).

Table (3-2): The difference in mean values of plaque and gingival indices among groups.

Clinical periodontal parameter	CP+ATH group	CP group	Control group	P value (ANOVA test)
Mean P.I (±SD)	2.04(±0.29)	1.69(±0.36)	0.50(±0.20)	0.0001**
Mean G.I (±SD)	1.37(±0.23)	1.43(±0.27)	0.47(±0.17)	0.0001**

**** = Highly significant difference (p<0.01)**

Table (3-3): Inter groups comparisons of the mean values of plaque index between all pairs of groups

Grouping	Mean difference	P value(Gabriel test)
CP+ATH vs CP	0.342	0.0001**
CP+ATH vs Control	1.531	0.0001**
CP vs Control	1.188	0.0001**

**** = Highly significant difference (p≤0.01)**

Table (3-4): Inter groups comparisons of the mean values of gingival index between all pairs of groups

Grouping	Mean difference	P value (Gabriel test)
CP+ATH vs CP	-0.060	0.683^{NS}
CP+ATH vs Control	0.898	0.0001**
CP vs Control	0.958	0.0001**

NS= non-significant (p>0.05); ** = highly significant difference (p≤0.01)

3.2.2 Bleeding on probing (BOP)

The current study showed analytics statistics for BOP in the study groups (CP+ATH and CP groups) as illustrated in table (3-5).The number of examined sites for the CP group was (2662) and ATH+CP group was (2310). The sites that bleed were described as score (1) while the non-bleeding sites were described as score (0).

The percentage of sites with bleeding on probing (BOP score 1) was (32.91%) in the CP+ATH group and (40.54%) in the CP group. while the percentage of sites

with no bleeding on probing (BOP score 0) was (67.01%) in CP+ATH group and (59.46%) in CP group.

BOP score 1 was higher in the CP group compared to the CP with ATH group and by using Chi-square test the result revealed a highly significant difference between the two study groups at p-value ≤0.01.

Table (3-5): The distribution of the numbers and percentages of sites according to the presence or absence of bleeding on probing of the CP group and ATH with CP group.

group	Statistical analysis	BOP		Chi-square	p-value
		Score 1	Score 0	40.817	0.0001**
ATH+CP	NO.	735	1575		
	percentage	32.91%	67.01%		
CP	NO.	1081	1584		
	percentage	40.54%	59.46%		

****=highly significant p≤0.01**

Furthermore, in figure (3-1) the descriptive statistics for BOP was demonstrated graphically for both study groups.

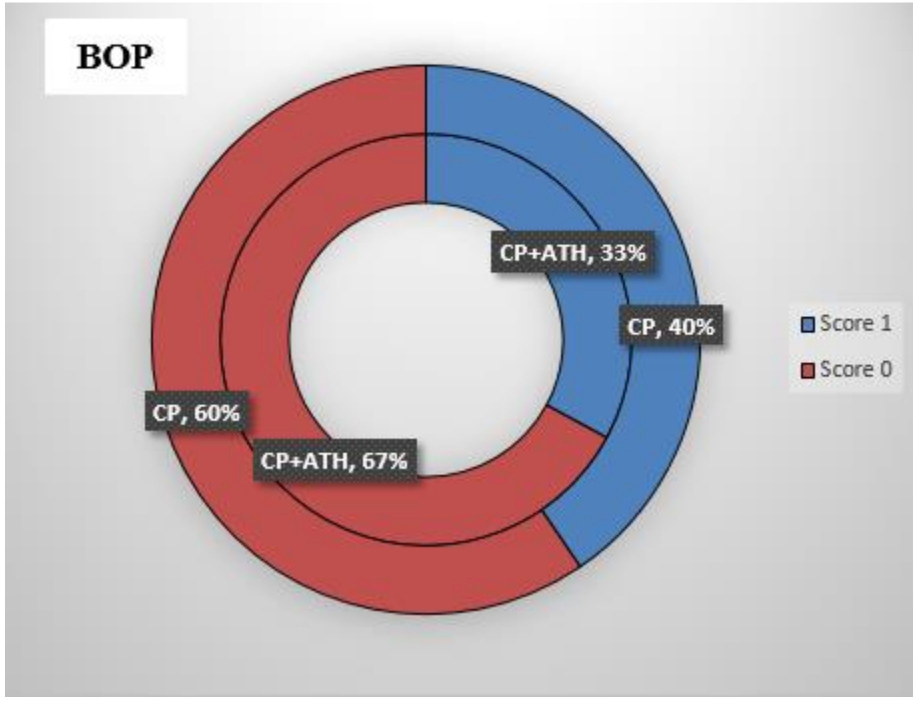


Figure (3.1): Pie chart for percentages of score 0 and score 1 of bleeding on probing for CP group and ATH+CP group

3.2.3 Probing pocket depth (PPD) and clinical attachment loss (CAL)

The CP group showed the higher mean value (±SD) of PPD was (4.91± 0.59) when compared with CP+ATH group, the mean value (±SD) of PPD was (4.61± 0.60), significant difference was observed between the ATH with CP and CP groups at P-value ≤ 0.05.

Furthermore, the CP with ATH group illustrated the higher mean value((±SD) of CAL was (5.09±1.115) when compared with CP, the mean value (±SD) of CAL was (4.51 ±0.74) and statistical analysis comparing between the study groups, using t-test, revealed a significant difference at p-value ≤ 0.05 , as shown in table (3-6).

Table (3-6): The difference in mean values of PPD and CAL for CP group and ATH+CP group

Clinical periodontal parameters	CP+ATH group	CP group	P-value (t- test)
PPD Mean (±SD)	4.61(±0.601)	4.91(±0.590)	0.05*
CAL Mean (±SD)	5.09(±1.115)	4.51(±0.741)	0.022*

***=Significant at P≤0 .05**

3.3 Immunological analysis

Immunological analysis for serum high sensitivity C- reactive protein, IgM anticardiolipin antibody and IgG antiphosphoryl choline were done for 85 sample (30 CP+ATH, 30 CP and 25 control).

3.3.1 Serum hs-CRP level analysis

The present study showed that the ATH+CP group with higher median value of (0.575mg/dl) followed by the CP group the median value of (0.210mg/dl) while the control group presented the lowest median value of (0.180mg/dl). Furthermore a highly significant statistical difference was observed among the study & control as illustrated in table (3-7). Intergroup comparisons of serum hs-CRP levels by using Mann Whitney test, there was highly significant difference between the ATH+CP group and control group (p≤0.01), and significant difference between CP group and CP+ATH group (P≤0.05), while there was no statistical difference between CP group as compared to control group, at p-value >0.05 as shown in table (3-8).

Table (3-7): The difference in mean values of serum CRP (mg/dl) among study groups.

parameter	Group	Mean rank	Median	P-value (Kruskal Wallis test)
CRP	CP+ATH	53.45	0.575	0.008**
	CP	40.65	0.210	
	Control	33.28	0.180	

****= Highly Significant at P≤0.01**

Table (3-8): Inter groups comparisons of the median values of serum CRP (mg/dl) between all pairs of study groups

Group	Mean rank	P value (Mann-Whitney)
CP+ATH	35.27	0.034*
CP	25.73	
CP+ATH	33.68	0.004**
Control	21.18	
CP	30.42	0.220
Control	25.10	

***= Significant at P≤0.05; **= Highly Significant at P≤0.01**

3.3.2 Serum Anti-Cardiolipin (IgM) antibody level analysis

Concerning the serum anticardiolipin (IgM) antibody, the present study show that the median of IgM Anti-Cardiolipin antibodies levels in CP+ATH, CP and control groups were 0.0715 RU/ml, 0.057 RU/ml and 0.048 RU/ml respectively with no statistically significant differences at ($p>0.05$)between them as shown in table (3-9). Moreover, the comparison for all pairs of study groups by using Mann Whitney test showed there was significant difference in CP+ATH when compared to control group, while there were no difference between CP and CP+ATH, CP and control respectively as shown in table (3-10).

Table (3-9): The difference in median values of serum IgM Anti-cardiolipin antibody (RU/ml) among groups.

Parameter	Group	Mean rank	Median	P value (Kruskal Wallis test)
Anti-Cardiolipin (IgM)	CP+ATH	48.40	0.0715	0.084^{NS}
	CP	45.07	0.0570	
	Control	34.04	0.0480	

NS=non-significant (P>0.05)

Table (3-10): intergroup comparison for serum IgM Anti-Cardiolipin (RU/ml) between all pairs of groups

Group	Mean rank	P value (Mann-Whitney)
CP+ATH	31.75	0.579
CP	29.25	
CP+ATH	32.15	0.035*
Control	23.02	
CP	31.32	0.092
Control	24.02	

*** = Significant at P≤0 .05**

3.3.3 Serum IgG Anti-Phosphoryl choline level analysis.

Regarding IgG anti-phosphoryl choline the present study revealed that there was increase in median level of anti-phosphoryl choline (IgG) in CP+ATH (1.7U/ml) as compared to CP(1.525U/ml) and control groups (1.25U/ml) respectively, with no statistically significant differences at ($p>0.05$) in Serum anti-phosphoryl choline(IgG) level existed among study groups as shown in table (3-11),while intergroup comparisons was showed significant difference between CP and control groups at P-value≤ 0.05 as shown in table (3-12).

Table (3-11): The difference in median values of serum IgG Anti-phosphoryl- choline (U/ml) among groups.

parameter	Group	Mean rank	Median	P value (Kruskal Wallis test)
Anti- phosphoryl-choline (IgG)	CP+ATH	46.83	1.700	0.134^{NS}
	CP	46.08	1.525	
	Control	34.79	1.250	

NS=non-significant (p>0.05)

Table (3-12): intergroup comparison for serum IgG Anti-phosphoryl-choline (U/ml) between all pairs of groups

Group	Mean rank	P (Mann-Whitney)
CP+ATH	31.15	0.773
CP	29.85	
CP+ATH	31.18	0.106
Control	24.18	
CP	31.73	0.05*
Control	23.52	

***=significant at P≤0 .05**

In addition, the descriptive statistics for Anti-PC was shown graphically in figure (3.2).

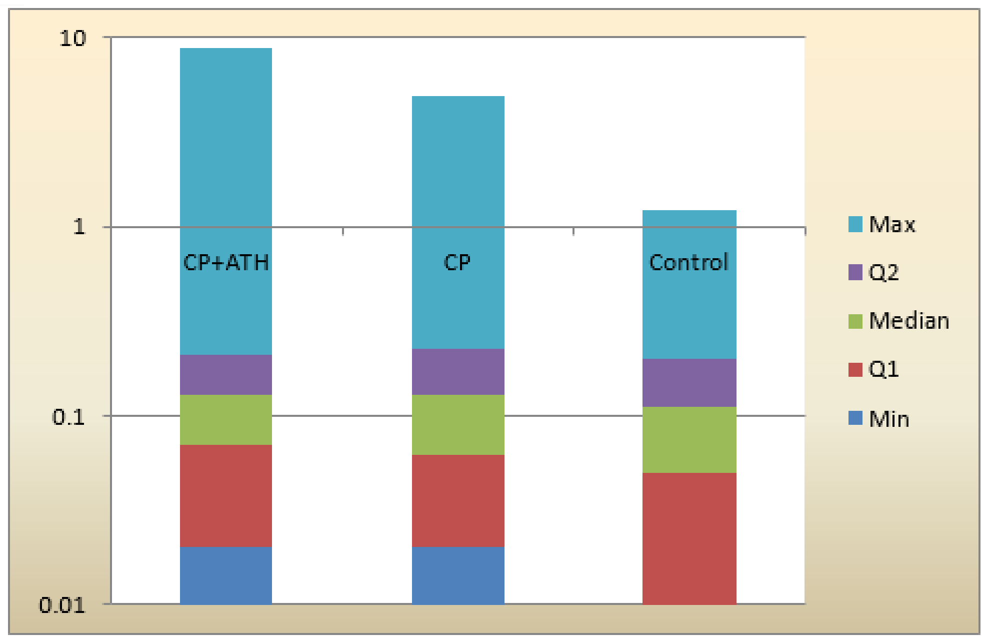


Figure (3.2): percentile distribution chart for serum Anti-PC (U/ml) in CP and CP with ATH group and control group

3.4 Correlation between Immunological parameters and clinical periodontal parameters:

3.4.1 Correlation between serum CRP levels and clinical periodontal parameters:

Concerning the correlation of CRP and clinical periodontal parameters in the present study, for CP group, there was non-significant correlation between serum level of CRP and GI, BOP and CAL (r=0.152; p=424),(r =256; p=0.17), (r=0.327; p=0.57) respectively, however; there was weak positive approached significance

correlation between serum level of CRP and P.I, CAL (r=0.33; p= 0.075), (r=0.327; p=0.078) respectively.

For ATH with CP group, there was non-significant correlation between serum level of CRP and P.I, G.I, BOP, PPD and CAL (r=.084; p=.659), (r=.068; p=.720) (r=.108; p=.571), (r=.141; p=.458) (r =.176; p=.352) respectively, as shown in table (3-13).

Table (3-13): Spearman Correlation Coefficients among serum CRP and clinical periodontal parameters in CP group and ATH+CP group.

Group	Statistical analysis	PI	GI	BOP	PPD	CAL
ATH+CP	Correlation coefficient (r)	.084	.068	.108	.141	.176
	P value	.659^{NS}	.720^{NS}	.571^{NS}	.458^{NS}	.352^{NS}
CP	Correlation coefficient(r)	.330	.152	.256	.108	.327
	P value	.075^{NS}	.424^{NS}	.171^{NS}	.570^{NS}	.078^{NS}

NS = Non- Significant at P>0.05

3.4.2 Correlation between serum levels of Anti-Cardiolipin (IgM) antibody and clinical periodontal parameters:

Regarding the correlation of IgM anticardiolipin antibody levels and clinical periodontal parameters. The current study did not observe significant correlation between serum level of IgM anticardiolipin antibody and clinical periodontal parameters (p>0.05) in both study groups as shown in table (3-14).

For ATH with CP group, there were non-significant positive correlation between serum level of IgM anticardiolipin antibody and G.I, BOP and CAL (r=.039; p=.838), (r=0.085; p=.656) (r=0.083; p=0.644) respectively, on the other hand, negative correlation between serum level of IgM anticardiolipin antibody and P.I, and PPD (r=_.213; p=.258), (r=_0.075; p=.694).

For CP group, there was non-significant positive correlation between serum level of IgM anticardiolipin antibody and P.I, G.I, BOP, PPD and CAL (r=.139; p=.464), (r=0.06; p=.753), (r=0.173; p=0.36), (r=0.217; p=.25), (r=0.24; p=0.202) respectively.

Table (3-14): Spearman Correlation Coefficients among serum IgM Anti-Cardiolipin antibody and clinical periodontal parameters in CP group and ATH+CP group.

group	Statistical analysis	PI	GI	BOP	PPD	CAL
ATH+CP	Correlation coefficient	-.213	.039	.085	-.075	.083
	P value	.258^NS	.838^NS	.656^NS	.694^NS	.664^NS
CP	Correlation coefficient	.139	.060	.173	.217	.240
	P value	.464^NS	.753^NS	.360^NS	.250^NS	.202^NS

NS=non-significant (p>0.05)

3.4.3 Correlation between serum levels of IgG Anti-phosphoryl-choline and clinical periodontal parameters

Regarding the correlations of serum IgG Antiphosphoryl choline levels and clinical periodontal parameters. The ATH with CP group was showed negative significant correlations between Serum IgG Antiphosphoryl choline levels and P.I,

PPD and CAL(r=_0.186; r=_0.02; r=_0.073) respectively, on the other hand, positive non- significant correlations were observed between serum IgG Antiphosphoryl choline levels with G.I and BOP (r=0.099; p=0.605); (r=0.09; p=0.636) respectively at p>0.05.

For CP group, there was negative correlations between Serum IgG Anti-phosphoryl choline levels were observed with G.I, BOP and PPD (r=_0.102; r=_0.128; r=_0.113) respectively, in addition, positive non-significant correlations were observed between serum IgG Anti-phosphoryl choline levels with P.I and CAL (r=0.088; r=0.644) ;(r=0.215; r=0.181) respectively at p>0.05as shown in table (3-15).

Table (3-15): Spearman Correlation Coefficients among serum IgG Anti-phosphoryl-choline and clinical periodontal parameters in CP group and ATH+CP group.

group	Statistical analysis	PI	GI	BOP	PPD	CAL
ATH+CP	Correlation coefficient	-.186	.099	.090	-.020	-.073
	P value	.325 NS	.605 NS	.636 NS	.916 NS	.702 NS
CP	Correlation coefficient	.088	-.102	-.128	-.113	.251
	P value	.644 NS	.593 NS	.502 NS	.551 NS	.181 NS

NS=non- significant (p>0.05)

3.5 Correlation between serum levels of immunological parameters

3.5.1 Correlation between serum levels of immunological parameters in CP+ATH group.

Regarding the correlation between CRP, IgM ACL and IgG anti-PC are clearly shown in table (3-16).

IgG anti-PC was showed significant positive correlation with IgM ACL (r=0.415, p=0.023) at p-value<0.05 in CP+ATH group.

Table (3-16): Spearman rho Correlation Coefficients among immunological parameters in ATH+CP group

Spearman rho Correlation		CRP	IgM ACL	IgG Anti-PC
CRP	Correlation Coefficient	.	.303	-.092
	Sig. (2-tailed)	.	.104 NS	.628 NS
IgM ACL	Correlation Coefficient	.303	.	.415
	Sig. (2-tailed)	.104 NS	.	.023*
IgG anti-PC	Correlation Coefficient	-.092	.415	.
	Sig. (2-tailed)	.628 NS	.023*	.

*** = Correlation is significant at p-value< 0.05 (2-tailed). NS: non-significant at p-value>0.05**

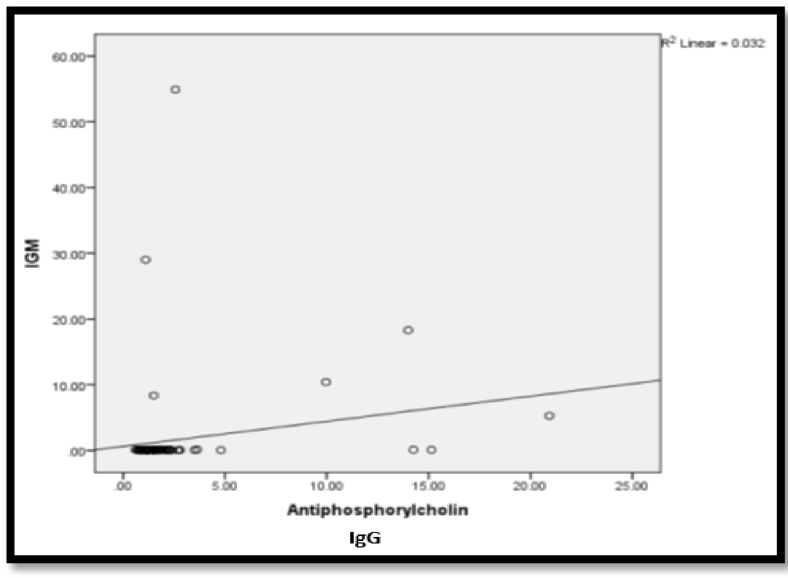


Figure (3.3): Correlation between IgG anti-PC and IgM ACL in CP with ATH group

3.5.2 Correlation between serum levels of immunological parameters in CP group.

Concerning the correlation between CRP, IgM ACL and IgG Anti-PC are clearly shown in table (3-17).There was non-significant correlation between these parameters in chronic periodontitis group at p- value >0.05.

Table (3-17): Spearman rho Correlation Coefficients among immunological parameters in CP group

Spearman's rho Correlation		CRP	IgM ACL	IgG Anti-PC
CRP	Correlation Coefficient	.	.231	.249
	Sig. (2-tailed)	.	.219NS	.184NS
IgM ACL	Correlation Coefficient	.231	.	.197
	Sig. (2-tailed)	.219NS	.	.297NS
IgG Anti-phosphoryl-choline	Correlation Coefficient	.249	.197	.
	Sig. (2-tailed)	.184NS	.297NS	.

NS: non-significant at P-Value >0.05

Chapter Four

Discussion

Discussion

The relationship between poor dental health and atherosclerosis has become a topic of interest in health care. Clinical measures of periodontitis have been found to show a positive association with coronary heart disease and importance is now being placed on understanding the mechanism of interaction between periodontal disease and atherosclerosis. As such, this study has focused on the systemic influence of periodontal bacteria by measuring serum levels of anti-PC, anti-CL antibodies and hs-CRP.

4.1 Characteristics of the study subjects

The age range of CP and CP with ATH cases was 35-64 years, because chronic periodontitis and CP with ATH are more common in adults. Controls were selected from nearly the same age group to ensure good match.

Atherosclerosis, is more prevalent in males than female, as the Estrogen is protect female as it has multiple effects including effects on lipids, nitric oxide, vascular tone and antioxidant properties, however, the gender difference disappears rapidly after the menopause **(Lansky et al., 2012).**

4.2 Periodontal Health Status

4.2.1 Plaque and Gingival indices

The present study revealed a highly statistical difference of mean values of P.I and G.I between the cases (ATH+ CP group and CP group) and control.

The current result was consistent with other result reported by **(Persson and Persson, 2008, Willershausen et al., 2009, Nesarhoseini, 2010, Androsz-Kowalska et al., 2013, Kumar et al., 2014)**, as they found significant increase in mean of (PI, GI) in ATH with CP patient as compared to CP and control.

This result could be explained by presence of dental plaque which is the main clinical finding for CP and it is coincide with the severity of the disease and it is expected to be accumulating more in CP, another possible explanation of such results as the hospitalized ASCVD patients neglect the oral hygiene measures and didn't brush their teeth regularly.

Furthermore, Bacteria of dental biofilm present in periodontal pockets, which occur in the majority of the adult population, are a potential source of infection resulting in inflammation of various organs.

4.2.2 Bleeding on probing

Results of the BOP in the present study showed a highly statistical differences between the study groups (ATH+CP group and CP group). Higher percentage of bleeding on probing sites demonstrated by CP group when compared with ATH+CP group.

This result agreed with **(Androsz-Kowalska et al., 2013, Raheem and Ahmed, 2014)** they found mean bleeding index in chronic periodontitis was significantly higher than in CAD with chronic periodontitis and control. While disagreed with **(Cueto et al., 2005, Oe et al., 2009, Willershausen et al., 2009)** they found that the BOP was more in ATH+CP patients than in CP patient.

The possible explanation for that percentage of BOP sites demonstrated by CP group was higher when compared with ATH+CP group, may be related to disease severity and periodontal instability because bleeding on probing is a more precise and objective indicator of the inflammation at the base of the periodontal pocket (critical area for disease progression).

4.2.3 Probing Pocket Depth and Clinical Attachment Loss

Regarding the CAL and PPD, the present study clarified that the mean value of PPD was higher in CP group than in CP + ATH group, while the mean value of CAL was higher in CP + ATH group than in CP group and results illustrated significant differences between them.

The current result agreed with **(Gotsman et al., 2007, Thakare et al., 2010, Vražić et al., 2015, Deepa et al., 2016)** for CAL only and disagreed with them for PPD**,** as they found that the PPD and CAL was more in ATH with CP as compared to CP group, also the current results disagreed with **(Ehlers et al., 2011)** whom found there were no statistical differences regarding PPD and CAL between AMI+CP and CP patients. However the AMI patients demonstrated poorer dental hygiene than the CP patients.

Loss of clinical attachment and increased pocket depth result from the destruction of the connective tissue apical to the junctional epithelium by the inflammatory infiltrate in response to bacterial dental plaque and apical migration of the junctional epithelium **(CARRANZA, 2015)**. However, pocket depth (from gingival margin to the pocket base) by itself is not as accurate as clinical attachment level (from cemento-enamel junction to the pocket base) in assessing disease severity, because in cases of recession the probing depth may be less than 3mm while the CAL may be much more. CAL indicates the amount of the root surface denuded of periodontal attachment, thus it was used as an indicator of disease severity**(Armitage, 1999).**

The mean value of CAL was higher in ASCVD patients attributed to poor oral hygiene that high mean values of PL.I were showed in ASCVD patients. Bacterial dental plaque elaborate various compounds, that elicit an inflammatory response that is protective but also is responsible for loss of periodontal tissue, pocket formation, and loosening of teeth **(Sorsa et al., 2011).**

4.3 Serum High sensitivity C- reactive protein levels

The current results illustrated that highly significant statistical differences of median values of serum hs- CRP were observed between the study (ATH with CP group & CP group) and control groups. The present results agreed with **(Radafshar et al., 2006, Thakare et al., 2010, Chrysanthakopoulos and Chrysanthakopoulos, 2013, Kumar et al., 2014)** who found that serum levels of hs CRP were higher in ASVCD with CP patients than CP patients and control individuals. These findings point to an association between periodontal infection and cardiovascular disease.

The present result found that there was increase in median serum level in CP group compared to control but statistically non- significant that was partially agreement with (**Gomes-Filho et al., 2011, Koppolu et al., 2013, Kizildag et al., 2014, Chandy et al., 2017, Ansari Moghadam et al., 2017**) who found higher serum CRP concentrations in periodontitis patients than in controls.

The explanation for increased CRP in chronic periodontitis because it is biologically believable that inflammatory mediators, especially IL-1 and -6 and TNF-a are released under conditions of periodontitis and present the capacity to stimulate hepatocytes to produce CRP **(Gomes-Filho et al., 2011)**. In this manner, it can be expected that, in the presence of chronic periodontitis, higher serum CRP levels would be found **(Rutger Persson et al., 2003, Persson et al., 2005).** In addition direct actions of CRP which contribute to the induction of a prothrombotic state may be the enhancement of the procoagulant activity **(Libby and Simon, 2001)** or the reduction of fibrinolysis **(Cushman et al., 1999)**. CRP has been suggested to induce a prothrombotic state via induction of tissue factor expression in human monocytes , but only in the presence of and through direct interaction with other blood cells as T-lymphocytes, B-lymphocytes and natural killer cells **(Paffen et al., 2004).**

4.4 Correlations between serum hs-CRP and clinical periodontal parameters.

The current study did not observe significant correlation between serum level of CRP and clinical periodontal parameters in CP group, however, Pl.I and CAL approached significant correlation with serum level of CRP. In ATH with CP group

showed non- significant correlation between serum level of hs CRP and all clinical periodontal parameters.

Results of present study are inconsistent with other results reported by **(Linden et al., 2008, Thakare et al., 2010, Haba et al., 2011, Malhotra and Parkash, 2013)** who found that there was significant positive correlation between serum CRP levels and clinical periodontal parameters (PI, GI, PPD, CAL and BOP) in ATH with CP and CP group. While agreed with Ide and co-workers reported that there were no correlation between serum hs CRP and clinical periodontal parameters (PL.I,GI, BOP,PPD & CAL) and they concluded that improvement in periodontal health status by non-surgical periodontal treatment did not influence the serum levels of hs CRP **(Ide et al., 2003).**

In addition CRP revealed a weak positive approached significant correlation with PL.I and CAL that was partially agreed with **(Pejcic et al., 2011)** as they reported that the percentage of subjects with elevated CRP levels of >5 mol/L was greater in the higher CAL group compared to the group with less attachment loss.

The possible explanation is that the levels of circulating CRP and cytokines are induced not only by periodontal diseases but by other ongoing, possibly occult, atherosclerotic or other disease **(Armitage, 2000, Yasojima et al., 2001),** Furthermore, it is possible that the baseline levels of disease seen in this population **(Ide et al., 2003)**.

4.5 Serum Anti-Cardiolipin (IgM) Antibody levels

Regarding IgM anticardiolipin antibodies the present result revealed that the median level of serum IgM anticardiolipin antibodies in ATH with CP patient higher than median level of CP and control groups and there was a significant difference between ATH with CP group when compared to control group. Furthermore, the median level of serum IgM anti-Cardiolipin antibodies in CP group higher than median level of control groups.

The current finding agreed with Sumanth et al. **(Gunupati et al., 2011)** who denoted that serum ACLA-IgM level was significantly higher in patients with acute myocardial infarction associated with CP than in patients with acute myocardial infarction. In addition, they showed significant alterations in concentrations of serum ACLA-IgM after phase I periodontal therapy. Moreover, in agreement with **(Türkoğlu et al., 2008)** who reported an elevated concentrations of serum ACL to be considered a risk factor for cardiovascular disease. Furthermore, partially agreed with **(Faghihi et al., 2009, Kumar, 2011, Kiany and Hedayati, 2015)** who found significant increase in serum ACLA-IgM level in CP patients when compared to healthy individuals.

While disagreed with **(Al-Ghurabei, 2012)** who found there was no significant differences in serum ACLA-IgM levels between patients and control groups.

The possible explanation for the higher level of serum IgM anti-CL antibodies among ATH with CP patients in this study could be attributed to different reasons. First, gingival inflammation upon continuous exposure to Gram-negative bacteria and lipopolysaccharide causes the release of cytokines such as tumor necrosis

factor - alpha, interleukin-1b, and prostaglandin E2, which possibly contributes to an increase in the net rate of aCL expression by the activation of endothelial cells and the induction of oxidant-mediated injury. Upon cross-reactivity, this release of cytokines may interfere with the natural anticoagulant function of b2GPI from the gingival tissue and cause the elevation of serum aCL levels, which, in turn, could be a source of circulating aCL in patients with periodontitis **(Karnoutsos et al., 2008).** Second, the body defense mechanism, itself, may counteract periodontal inflammation because aCLs are a part of the immune response and host defense mechanism **(Schenkein et al., 2003, Türkoğlu et al., 2008).**

4.6 Correlations between serum Anti-cardiolipin (IgM) antibody and clinical periodontal parameters.

The current study did not observe significant correlation between serum levels of IgM anticardiolipin and clinical periodontal parameters in both groups.

The present result agreed with study in 2014 who found that there was no significant association between the serum IgM anticardiolipin antibodies levels and periodontal parameters (GI, PI and PPD) **(Mohamad et al., 2014).**

The present result was partially consistent with other result reported by **(Schenkein et al., 2003, Türkoğlu et al., 2008)** as they did not report an association between BOP, supragingival plaque, and serum anti-CL levels, on the other hand, found that patients with elevated anti-CL have greater mean attachment loss and increased pocket depth. Thus, it appears that the presence, in some patients, of forms of periodontitis demonstrating greater extent and severity of disease and inflammation may lead to the production of anti-CL in those patients.

Furthermore, disagreed with **(Faghihi et al., 2009)** who found a positive correlation between ACLA levels and supragingival plaque, GI, PD and CAL.

However, this result could be attributed to indirect effect of anticardiolipin (IgM) in initiation and development of periodontal disease, yet, the available references to support the present result are few although it was apparently elevated in chronic periodontist group with cardiovascular diseases.

4.7 Serum Anti-phosphoryl choline levels

Regarding IgG anti-phosphoryl choline revealed that the median level of serum anti-phosphoryl choline antibodies in ATH with CP patient higher than median level of CP and control groups with no statistical difference between them and there was a significant difference between CP group when compared to control group and these result in agreement with **(Schenkein et al., 1999, Schenkein et al., 2001)** as they found that patients with periodontal attachment loss have higher concentrations of anti-PC IgG than do individuals who demonstrate no attachment loss.

While disagreed with **(Lehtimäki et al., 1999)** who found elevated titers of antibody to oxLDL (which include anti-PC) have been shown to occur in angiographically verified coronary artery disease and coronary stenosis.

The possible explanation for the higher level of serum IgG anti-phosphoryl-choline antibodies among CP patients in present study that inflamed periodontal tissues permit ingress of antigens from oral bacteria which leads to increased systemic production of anti-PC **(Schenkein et al., 2001) .**

4.8 Correlations between serum Anti- phosphoryl choline and clinical periodontal parameters.

The current study did not observe significant correlation between serum level of IgG anti-PC and clinical periodontal parameters in CP and CP with ATH groups.

The present result disagreed with **(Schenkein et al., 1999, Schenkein et al., 2004)** as they reported higher serum levels of antibodies (anti-phosphoryl choline IgG) directed toward phosphoryl choline in patients with attachment loss, in comparison with those with healthy gingiva. Furthermore disagreed with **(Moore and Moore, 1994)** who found high levels of anti-PC in subgingival plaque.

The discrepancies observed between various studies could be caused, in part, to the differences in the sample size of each study, differences in types of samples used for each study and differences in sampling methods.

4.9 Correlation between serum levels of immunological parameters in CP+ATH group

The results of correlation among CRP, IgM ACL, and IgG anti-PC in ATH with CP group was showed significant positive correlation between IgG anti-PC with IgM ACL at $p\text{-value} < 0.05$.

The current result agreed with **(Schenkein et al., 1999, Karnoutsos et al., 2008, Tew et al., 2012)** as have been demonstrated that IgG-antibodies to PC have been associated with both atherosclerosis and periodontitis.

Phosphoryl choline (PC) bearing dental plaque microorganisms may induce an antibody response to PC that could influence the inflammatory response associated with atherosclerosis **(Schenkein et al., 2001) .**

Previous study was reported that Increased anti-oxCL IgM antibodies are associated with atherosclerosis development **(Lopez et al., 2003, Türkoğlu et al., 2008)**. oxCL is recognized as a natural antigen that stimulates proinflammatory effects in the artery and promotes formation of atherosclerotic plaques **(Bochkov et al., 2010).** Furthermore, other study observed the relationship among the elevation of concentrations of serum IgM anticardiolipin, chronic periodontitis and AMI **(Gunupati et al., 2011).**

However, some studies purport that autoantibodies to oxCL may serve a protective role against the onset and development of atherosclerosis **(Su et al., 2013, Frostegård et al., 2014).** The discrepancies regarding the effects of anti-oxCL antibodies on atherosclerosis may reflect the influence of potential physiological modifiers, including age, gender, and other existing diseases **(Shen et al., 2015).**

4.10 Correlation between serum levels of immunological parameters in CP group.

The results of correlation among CRP, IgM ACL, and IgG anti-PC illustrated non-significant correlation among the immunological parameters in chronic periodontitis group.

The previous studies did not mention the direct correlation between hsCRP, IgM ACL and IgG anti-PC in chronic periodontitis. However in patients with acute coronary syndrome the correlation between hs-CRP and ACL was studies and did

not found significant correlation between them in determining short term outcome in patients with acute coronary syndrome **(Malhotra et al., 2011).**

Chapter Five

Conclusions and Suggestions

Conclusions and Suggestions

5.1 conclusion

- There is a significant increase in periodontal destruction among ATH+CP group than CP group.

- The level of serum CRP in ATH+ CP was higher than CP group, so chronic periodontitis may be considered a significant risk factor for developing atherosclerotic lesions.

- The elevated levels of serum IgM anticardiolipin antibodies in ATH+CP when compared with CP group and control group, suggesting to have a role in development of atherogenesis and CVD.

- The increased level of anti-PC in ATH+CP and CP group when compared to control group possibly will indicate that oral flora may contribute significantly to serum IgG antibody reactive with PC.

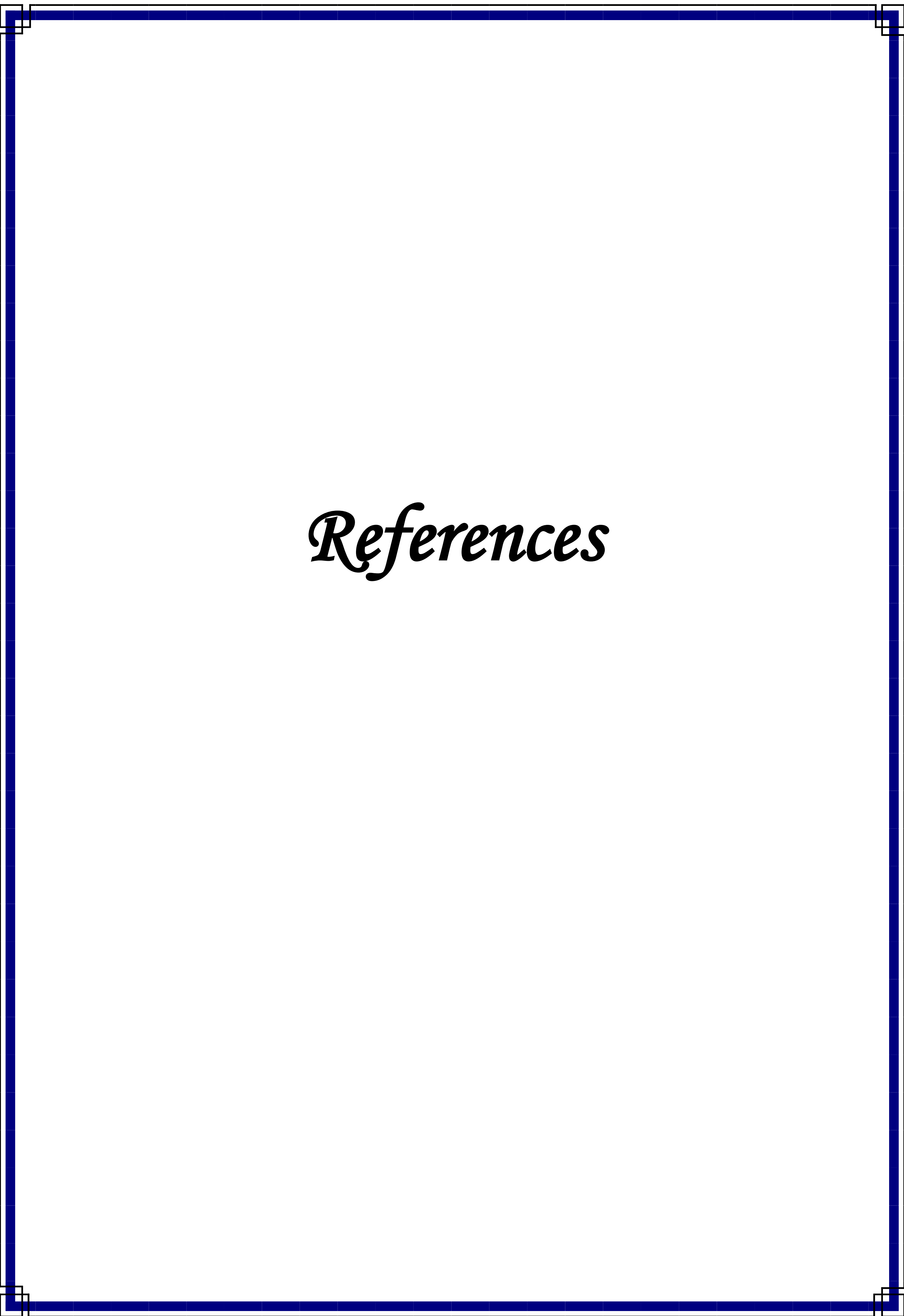
- The correlations between immunological parameters and clinical periodontal parameters were non-significant. So, they could not be used as an indicator of disease activity.

- The correlation between serum levels of IgM anti-CL and IgG anti-PC was significant positive correlation in ATH with CP group. Which may be predict there role in chronic periodontitis and atherosclerosis cardiovascular diseases.

- The correlation between serum levels of CRP, IgM ACL and IgG anti-PC in chronic periodontitis group was non- significant that might suggest no direct correlation between them in CP disease.

5.2 Suggestions

- Performed the same research design with a large sample size.
- Carry out the same research design in patient with other systemic disease (SLE and adverse pregnancy outcome).
- Proceed the same research design and measure IgM anti-phosphoryl choline in serum and study its correlation with chronic periodontitis and atherosclerosis cardiovascular disease.
- Longitudinal trials need to be performed in order to obtain more conclusive result regarding the effect of periodontal disease on cardiovascular disease and vice versa.
- Measuring the immunological markers in GCF and its association with clinical periodontal parameters



References

References

ABD, T. T., EAPEN, D. J., BAJPAI, A., GOYAL, A., DOLLAR, A. & SPERLING, L. J. C. A. R. 2011. The role of C-reactive protein as a risk predictor of coronary atherosclerosis: implications from the JUPITER trial. *J Current atherosclerosis reports* 13**,** 154-161.

AL-GHURABEI, B. H. J. J. O. B. C. O. D. 2012. Evaluation of serum anti-cardiolipin antibody, hs-CRP and IL-6 levels in chronic periodontitis as possible risk factors for cardiovascular diseases. *J Journal of baghdad college of dentistry* 24**,** 161-165.

ALBANDAR, J. M. J. D. C. 2005. Epidemiology and risk factors of periodontal diseases. *The Dental Clinics of North America,* 49**,** 517-532.

ALVES, P. & FERREIRA, B. J. S. M. W. 2008. The role of inflammation, humoral and cell mediated autoimmunity in the pathogenesis of atherosclerosis. *Swiss medical weekly*138.

AMAR, S., WU, S.-C. & MADAN, M. J. T. J. O. I. 2009. Is Porphyromonas gingivalis cell invasion required for atherogenesis? Pharmacotherapeutic implications. *The Journal of Immunology*182**,** 1584-1592.

AMES, P., MARGARITA, A., ALVES, J. D., TOMMASINO, C., IANNACCONE, L. & BRANCACCIO, V. J. L. 2002. Anticardiolipin antibody titre and plasma homocysteine level independently predict intima media thickness of carotid arteries in subjects with idiopathic antiphospholipid antibodies. *Lupus* 11**,** 208-214.

ANDROSZ-KOWALSKA, O., JANKOWSKI, K., RYMARCZYK, Z., KOWALSKI, J., PRUSZCZYK, P. & GÓRSKA, R. J. K. P. 2013. Correlation between clinical parameters of periodontal disease and mean platelet volume in patients with coronary artery disease: a pilot study. *J Kardiologia Polska* 71**,** 600-605.

ANITHA, V., NAIR, S., SHIVAKUMAR, V., SHANMUGAM, M., PRIYA, B. M. & RAJESH, P. J. I. J. O. D. R. 2015. Estimation of high sensitivity C-reactive protein in patients with periodontal disease and without coronary artery disease. *Indian Journal of Dental Reseach* 26**,** 500.

ANSARI MOGHADAM, S., ZADFATTAH, S., RISBAF FAKOUR, S., ANSARI MOGHADDAM, A., NAEBI, M. J. J. O. D. M. & TECHNIQUES 2017. Comparison of C-reactive protein levels in chronic periodontitis patients with normal subjects. *Journal of Dental Materials Techniques* 6**,** 181-185.

ARMITAGE, G. C. J. A. O. P. 1999. Development of a classification system for periodontal diseases and conditions. *J Annals of periodontology* 4**,** 1-6.

ARMITAGE, G. J. O. D. 2000. Periodontology/Oral Medicine: Periodontal infections and cardiovascular disease—how strong is the association? *Oral diseases* 6**,** 335-350.

ASGARY, S., KELISHADI, R., RAFIEIAN-KOPAEI, M., NAJAFI, S., NAJAFI, M. & SAHEBKAR, A. J. P. C. 2013. Investigation of the lipid-modifying and antiinflammatory effects of Cornus mas L. supplementation on dyslipidemic children and adolescents. *J Pediatric cardiology* 34**,** 1729-1735.

ASGARY, S., SAHEBKAR, A., AFSHANI, M. R., KESHVARI, M., HAGHJOOYJAVANMARD, S. & RAFIEIAN-KOPAEI, M. J. P. R. 2014. Clinical evaluation of blood pressure lowering, endothelial function improving, hypolipidemic and anti-inflammatory effects of pomegranate juice in hypertensive subjects. *J Phytotherapy Research* 28**,** 193-199.

(B)

BADIMON, L., STOREY, R. F., VILAHUR, G. J. T. & HAEMOSTASIS 2011. Update on lipids, inflammation and atherothrombosis. *J Thrombosishaemostasis* 105**,** S34-S42.

BADIMON, L. & VILAHUR, G. J. J. O. I. M. 2014. Thrombosis formation on atherosclerotic lesions and plaque rupture. *Journal of Internal Medicine,* 276**,** 618-632.

BANSAL, T., PANDEY, A., DEEPA, D., ASTHANA, A. K. J. J. O. C. & JCDR, D. R. 2014. C-reactive protein (CRP) and its association with periodontal disease: a brief review. *Journal of clinicaldiagnostic research: JCDR*8**,** ZE21.

BARTOLD, P. M. & NARAYANAN, A. S. J. P. 2006. Molecular and cell biology of healthy and diseased periodontal tissues. *Periodontology,* 40**,** 29-49.

BISOENDIAL, R. J., BOEKHOLDT, S. M., VERGEER, M., STROES, E. S. & KASTELEIN, J. J. J. E. H. J. 2010. C-reactive protein is a mediator of cardiovascular disease. *European Heart Journal,* 31**,** 2087-2091.

BLANK, M. & SHOENFELD, Y. J. C. I. 2004. Beta-2-glycoprotein-I, infections, antiphospholipid syndrome and therapeutic considerations. *J Clinical Immunology*112**,** 190-199.

BOCHKOV, V. N., OSKOLKOVA, O. V., BIRUKOV, K. G., LEVONEN, A.-L., BINDER, C. J., STÖCKL, J. J. A. & SIGNALING, R. 2010. Generation and biological activities of oxidized phospholipids. *J Antioxidants redox signaling*12**,** 1009-1059.

BRESOLIN, A. C., PRONSATTI, M. M., PASQUALOTTO, L. N., NASSAR, P. O., JORGE, A. S., DA SILVA, E. A. A., NASSAR, C. A. J. V. H. & MANAGEMENT, R. 2013. Lipid profiles and inflammatory markers after periodontal treatment in children with congenital heart disease and at risk for atherosclerosis. *Vascular health risk management* 9**,** 703.

BRILES, E. B. & TOMASZ, A. J. J. O. B. C. 1973. Pneumococcal Forssman Antigen A CHOLINE-CONTAINING LIPOTEICHOIC ACID. *Journal of Biological Chemistry,* 248**,** 6394-6397.

BRODALA, N., MERRICKS, E. P., BELLINGER, D. A., DAMRONGSRI, D., OFFENBACHER, S., BECK, J., MADIANOS, P., SOTRES, D., CHANG, Y.-L., KOCH, G. J. A., THROMBOSIS, & BIOLOGY, V. 2005. Porphyromonas gingivalis bacteremia induces coronary and aortic atherosclerosis in normocholesterolemic and hypercholesterolemic pigs. *Arteriosclerosis, thrombosis, vascular biology,* 25**,** 1446-1451.

BRUNDISH, D. & BADDILEY, J. J. B. J. 1968. Pneumococcal C-substance, a ribitol teichoic acid containing choline phosphate. *Biochemical Journal,* 110**,** 573-582.

(C)

CARRANZA, F. A. C., P. M. 2015. The periodontal pocket. *Carranza's clinical periodontology* 12th ed.: Elsevier/Saunders.

CATON, J. G., ARMITAGE, G., BERGLUNDH, T., CHAPPLE, I. L., JEPSEN, S., KORNMAN, K. S., MEALEY, B. L., PAPAPANOU, P. N., SANZ, M. & TONETTI, M. S. J. J. O. P. 2018. A new classification scheme for periodontal and peri-implant diseases and conditions–Introduction and key changes from the 1999 classification. *J Am Dent Assoc,* 89**,** S1-S8.

CHANDY, S., JOSEPH, K., SANKARANARAYANAN, A., ISSAC, A., BABU, G., WILSON, B., JOSEPH, J. J. J. O. C. & JCDR, D. R. 2017. Evaluation of C-reactive protein and fibrinogen in patients with chronic and aggressive periodontitis: a clinico-biochemical study. *J Journal of clinical diagnostic research,* 11**,** ZC41.

CHAPPLE, I. L., GENCO, R. & PERIODONTOLOGY, W. G. O. T. J. E. A. W. J. J. O. C. 2013. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *Journal of Clinical Periodontology,* 40**,** S106-S112.

CHASTON, R. W. 2006. Serum Anti-phosphorylcholine and Anti-cardiolipin Concentrations Following Periodontal Scaling and Root Planing.

CHAUHAN, V. S., CHAUHAN, R. S., DEVKAR, N., VIBHUTE, A. & MORE, S. J. J. D. A. S. 2012. Gingival and periodontal diseases in children and adolescents. *Dent Allied Sci* 1**,** 26-9.

CHEN, Y. W., NAGASAWA, T., WARA-ASWAPATI, N., USHIDA, Y., WANG, D., TAKEUCHI, Y., KOBAYASHI, H., UMEDA, M., INOUE, Y. & IWAI, T. J. J. O. C. P. 2009. Association between periodontitis and anti-cardiolipin antibodies in Buerger disease. *Journal of clinical periodontology*36**,** 830-835.

CHRYSANTHAKOPOULOS, N. A. & CHRYSANTHAKOPOULOS, P. A. J. J. O. O. D. 2013. Clinically classified periodontitis and its association in patients with preexisting coronary heart disease. *J Journal of Oral Diseases* 2013.

CLARK, S. E., WEISER, J. N. J. I. & IMMUNITY 2013. Microbial modulation of host immunity with the small molecule phosphorylcholine. *J Infectionimmunity*81**,** 392-401.

CORTÉS-VIEYRA, R., ROSALES, C. & URIBE-QUEROL, E. J. J. O. I. R. 2016. Neutrophil functions in periodontal homeostasis. *Journal of immunology,* 2016.

CUETO, A., MESA, F., BRAVO, M. & OCAÑA-RIOLA, R. J. J. O. P. R. 2005. Periodontitis as risk factor for acute myocardial infarction. A case control study of Spanish adults. *J Journal of periodontal research* 40**,** 36-42.

CUSHMAN, M., LEMAITRE, R. N., KULLER, L. H., PSATY, B. M., MACY, E. M., SHARRETT, A. R., TRACY, R. P. J. A., THROMBOSIS, & BIOLOGY, V. 1999. Fibrinolytic activation markers predict myocardial infarction in the elderly: the Cardiovascular Health Study. *J Arteriosclerosis, thrombosis, vascular biology* 19**,** 493-498.

(D)

DE VRIES, M. R. & QUAX, P. H. J. C. O. I. L. 2016. Plaque angiogenesis and its relation to inflammation and atherosclerotic plaque destabilization. *Clin Rheumatol,* 27**,** 499-506.

DEEPA, D., GUPTA, C., GUPTA, A. J. J. O. C. & CARDIOLOGY, P. 2016. Assessment of high-sensitivity C-reactive protein values in chronic periodontitis patients with and without cardiovascular disease: A cross-sectional study. *J Journal of Clinical Preventive Cardiology* 5**,** 108.

DIETRICH, T., OWER, P., TANK, M., WEST, N., WALTER, C., NEEDLEMAN, I., HUGHES, F., WADIA, R., MILWARD, M. & HODGE, P. J. B. D. J. 2019. Periodontal diagnosis in the context of the 2017 classification system of periodontal diseases and conditions–implementation in clinical practice. *J British dental journal* 226**,** 16.

DIVARIS, K., MONDA, K. L., NORTH, K. E., OLSHAN, A. F., REYNOLDS, L. M., HSUEH, W.-C., LANGE, E. M., MOSS, K., BARROS, S. P. & WEYANT, R. J. J. H. M. G. 2013. Exploring the genetic basis of chronic periodontitis: a genome-wide association study. *J Human molecular genetics* 22**,** 2312-2324.

DORWEILER, B., TORZEWSKI, M., DAHM, M., KIRKPATRICK, C. J., LACKNER, K. J., VAHL, C.-F. J. T. & HAEMOSTASIS 2008. Subendothelial infiltration of neutrophil granulocytes and liberation of matrix-destabilizing enzymes in an experimental model of human neo-intima. *Thromb Haemost,* 99**,** 373-381.

DUDEK, J., HARTMANN, M. & REHLING, P. J. B. E. B. A.-M. B. O. D. 2018. The role of mitochondrial cardiolipin in heart function and its implication in cardiac disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*.

DYE, B. A., CHOUDHARY, K., SHEA, S. & PAPAPANOU, P. N. J. J. O. C. P. 2005. Serum antibodies to periodontal pathogens and markers of systemic inflammation. *J Clin Periodontol,* 32**,** 1189-1199.

(E)

EHLERS, V., WILLERSHAUSEN, I., KRAFT, J., MÜNZEL, T., WILLERSHAUSEN, B. J. H. & MEDICINE, F. 2011. Gingival crevicular fluid MMP-8-concentrations in patients after acute myocardial infarction. *J Headface medicine*7**,** 1.

ELGHARIB, N., CHI, D. S., YOUNIS, W., WEHBE, S. & KRISHNASWAMY, G. J. P. M. 2003. C-reactive protein as a novel biomarker: reactant can flag atherosclerosis and help predict cardiac events. *J Postgraduate medicine* 114**,** 39-44.

(F)

FAGHIHI, S., ROKN, A. & EBRAHIMI, R. J. J. O. D. O. T. U. O. M. S. 2009. Evaluation of serum anti-cardiolipin antibody titer of patients with chronic periodontitis in comparison with periodontally healthy population. *J Journal of Dentistry of Tehran University of Medical Sciences* 6-11.

FERRI, C., CROCE, G., COFINI, V., DE BERARDINIS, G., GRASSI, D., CASALE, R., PROPERZI, G. & DESIDERI, G. J. C. P. D. 2007. C-reactive protein: interaction with the vascular endothelium and possible role in human atherosclerosis. *Current pharmaceutical design* 13**,** 1631-1645.

FISKESUND, R., SU, J., BULATOVIC, I., VIKSTRÖM, M., DE FAIRE, U. & FROSTEGÅRD, J. J. R. I. I. 2012. IgM phosphorylcholine antibodies inhibit cell death and constitute a strong protection marker for atherosclerosis development, particularly in combination with other auto-antibodies against modified LDL. *Results in Immunology,* 2**,** 13-18.

FROSTEGÅRD, A. G., SU, J., HUA, X., VIKSTRÖM, M., DE FAIRE, U. & FROSTEGÅRD, J. J. P. O. 2014. Antibodies against native and oxidized cardiolipin and phosphatidylserine and phosphorylcholine in atherosclerosis development. *J PloS one,* 9**,** e111764.

(G)

GARCÍA-CARRASCO, M., GALARZA-MALDONADO, C., MENDOZA-PINTO, C., ESCARCEGA, R. O., CERVERA, R. J. C. R. I. A. & IMMUNOLOGY 2009. Infections and the antiphospholipid syndrome. *J Clinical reviews in allergyimmunology* 36**,** 104-108.

GELSOMINO, F., FIORENTINO, M., ZOMPATORI, M., POERIO, A., MELOTTI, B., SPERANDI, F., GARGIULO, M., BORGHI, C. & ARDIZZONI, A. J. A. O. O. 2017. Programmed death-1 inhibition and atherosclerosis: can nivolumab vanish complicated atheromatous plaques? *Annals of Oncology* 29**,** 284-286.

GIGANTE, B., LEANDER, K., VIKSTRÖM, M., BALDASSARRE, D., VEGLIA, F., STRAWBRIDGE, R. J., MCLEOD, O., GERTOW, K., SENNBLAD, B. & SHAH, S. J. A. 2014. Low levels of IgM antibodies against phosphorylcholine are associated with fast carotid intima media thickness progression and cardiovascular risk in men. *Atherosclerosis,* 236**,** 394-399.

GILLESPIE, S., AINSCOUGH, S., DICKENS, A. & LEWIN, J. J. J. O. M. M. 1996. Phosphorylcholine-containing antigens in bacteria from the mouth and respiratory tract. *J. Med Microbiol,* 44**,** 35-40.

GISTERÅ, A. & HANSSON, G. K. J. N. R. N. 2017. The immunology of atherosclerosis. 13**,** 368.

GOLDENBERG, H. B., MCCOOL, T. L. & WEISER, J. N. J. T. J. O. I. D. 2004. Cross-reactivity of human immunoglobulin G2 recognizing phosphorylcholine and evidence for protection against major bacterial pathogens of the human respiratory tract. *The Journal of infectious diseases* 190**,** 1254-1263.

GOMES-FILHO, I. S., FREITAS COELHO, J. M., DA CRUZ, S. S., PASSOS, J. S., TEIXEIRA DE FREITAS, C. O., ARAGÃO FARIAS, N. S., AMORIM DA SILVA, R., SILVA PEREIRA, M. N., LIMA, T. L. & BARRETO, M. L. J. J. O. P. 2011. Chronic periodontitis and C-reactive protein levels. *J Journal of periodontology* 82**,** 969-978.

GOTSMAN, I., LOTAN, C., SOSKOLNE, W. A., RASSOVSKY, S., PUGATSCH, T., LAPIDUS, L., NOVIKOV, Y., MASRAWA, S. & STABHOLZ, A. J. J. O. P. 2007. Periodontal destruction is associated with coronary artery disease and periodontal infection with acute coronary syndrome. *Journal of periodontology* 78**,** 849-858.

GUNUPATI, S., CHAVA, V. K. & KRISHNA, B. P. 2011. Effect of phase I periodontal therapy on anti-cardiolipin antibodies in patients with acute myocardial infarction associated with chronic periodontitis. *J Periodontol,* 82**,** 1657-64.

GUPTA, S., GUPTA, I., GUPTA, R. & GUPTA, P. J. I. J. C. M. R. 2017. Role of C-reactive protein in periodontal disease–a review. *International Journal of Contemporary Medical Research,* 4**,** 980-985.

(H)

HABA, D., TESLARU, S., UNGUREANU, D., HODOROG, D., ALECU, C., BENGHIAC, A.-G., ZETU, L., ANCUŢA, C., ANCUŢA, E. & NEMŢOI, A. J. R. J. M. E. 2011. Evaluation of serum and gingival crevicular fluid C-reactive protein and IL-6 levels in patients with periodontitis and transient ischemic attacks. *J Rom J Morphol Embryol* 52**,** 1243-7.

HAJISHENGALLIS, G. J. N. R. I. 2015. Periodontitis: from microbial immune subversion to systemic inflammation. *Nature Reviews Immunology* 15**,** 30.

HARNETT, W. & HARNETT, M. M. J. I. T. 1999. Phosphorylcholine: friend or foe of the immune system? *Immunology today,* 20**,** 125-129.

HIGHFIELD, J. J. A. D. J. 2009. Diagnosis and classification of periodontal disease. *Aust Dent J,* 54**,** S11-S26.

HUGHES, F. J. 2015. Periodontium and periodontal disease. *Stem Cell Biology and Tissue Engineering in Dental Sciences.* Elsevier.

HUNT, J. & KRILIS, S. J. T. J. O. I. 1994. The fifth domain of beta 2-glycoprotein I contains a phospholipid binding site (Cys281-Cys288) and a region recognized by anticardiolipin antibodies. *The Journal of Immunology* 152**,** 653-659.

(I)

IDE, M., MCPARTLIN, D., COWARD, P., CROOK, M., LUMB, P. & WILSON, R. J. J. O. C. P. 2003. Effect of treatment of chronic periodontitis on levels of serum markers of acute-phase inflammatory and vascular responses. *J Journal of clinical periodontology,* 30**,** 334-340.

IWAMOTO, Y., NISHIMURA, F., SOGA, Y., TAKEUCHI, K., KURIHARA, M., TAKASHIBA, S. & MURAYAMA, Y. J. J. O. P. 2003. Antimicrobial periodontal treatment decreases serum C-reactive protein, tumor necrosis factor-alpha, but not adiponectin levels in patients with chronic periodontitis. *J Periodontol,* 74**,** 1231-1236.

(K)

KARNOUTSOS, K., PAPASTERGIOU, P., STEFANIDIS, S. & VAKALOUDI, A. J. H. 2008. Periodontitis as a risk factor for cardiovascular disease: the role of anti-phosphorylcholine and anti-cardiolipin antibodies. *J Hippokratia* 12**,** 144.

KHURANA, R., SIMONS, M., MARTIN, J. F. & ZACHARY, I. C. J. C. 2005. Role of angiogenesis in cardiovascular disease: a critical appraisal. *Circulation,* 112**,** 1813-1824.

KIANY, F. & HEDAYATI, A. J. O. 2015. Evaluation of serum anti-cardiolipin antibodies after non-surgical periodontal treatment in chronic periodontitis patients. *Odontology* 103**,** 203-209.

KIKUCHI, T., EL SHIKH, M., EL SAYED, R., PURKALL, D., ELAASSER, M., SARRAF, A., BARBOUR, S., SCHENKEIN, H. & TEW, J. J. J. O. P. R. 2010. Anti-phosphorylcholine-opsonized low-density lipoprotein promotes rapid production of proinflammatory cytokines by dendritic cells and natural killer cells. *Journal of periodontal research,* 45**,** 720-730.

KINANE, D. F., STATHOPOULOU, P. G. & PAPAPANOU, P. N. J. N. R. D. P. 2017. Periodontal diseases. *DISEASE PRIMERS,* 3**,** 17038.

KINANE, D. J. A. O. P. 1998. Periodontal diseases' contributions to cardiovascular disease: an overview of potential mechanisms. *Annals of Periodontology,* 3**,** 142-150.

KIZILDAG, A., ARABACI, T. & DOGAN, G. E. J. W. J. O. S. 2014. Relationship between periodontitis and cardiovascular diseases: A literature review. *World Journal of Stomatology,* 3**,** 1-9.

KOENIG, W. J. I. J. O. C. 2013. High-sensitivity C-reactive protein and atherosclerotic disease: from improved risk prediction to risk-guided therapy. *International journal of cardiology* 168**,** 5126-5134.

KOPPOLU, P., DURVASULA, S., PALAPARTHY, R., RAO, M., SAGAR, V., REDDY, S. K. & LINGAM, S. J. P. A. M. J. 2013. Estimate of CRP and TNF-alpha level before and after periodontal therapy in cardiovascular disease patients. *J Pan African Medical Journal* 15.

KOTSAKIS, J. E. H. G. A. 2019. Classification of Diseases and Conditions Affecting the Periodontium. *Newman and carranza clinical periodontology.*

KUMAR, K., RANGANATH, V., NAIK, R., BANU, S. & NICHANI, A. J. J. O. P. R. 2014. Assessment of high-sensitivity C-reactive protein and lipid levels in healthy adults and patients with coronary artery disease, with and without periodontitis–a cross-sectional study. *J Journal of periodontal research* 49**,** 836-844.

KUMAR, P. J. S. J. O. R. I. D. S. 2011. Quantitative analysis of IgG, IgM Anticardiolipin antibodies in healthy and chronic Periodontitis patients. *J SRM Journal of Research in Dental Sciences* 2**,** 28.

(L)

LAINE, M. L., CRIELAARD, W. & LOOS, B. G. J. P. 2012. Genetic susceptibility to periodontitis. *J Periodontology* 58**,** 37-68.

LANG, N., BARTOLD, P. M., CULLINAN, M., JEFFCOAT, M., MOMBELLI, A., MURAKAMI, S., PAGE, R., PAPAPANOU, P., TONETTI, M. & VAN DYKE, T. J. A. O. P. 1999. Consensus report: aggressive periodontitis. *J Annals of periodontology* 4**,** 53-53.

LANSKY, A. J., NG, V. G., MAEHARA, A., WEISZ, G., LERMAN, A., MINTZ, G. S., DE BRUYNE, B., FARHAT, N., NIESS, G. & JANKOVIC, I. J. J. C. I. 2012. Gender and the extent of coronary atherosclerosis, plaque composition, and clinical outcomes in acute coronary syndromes. *J JACC: Cardiovascular Imaging* 5**,** S62-S72.

LAU, D. C., DHILLON, B., YAN, H., SZMITKO, P. E., VERMA, S. J. A. J. O. P.-H. & PHYSIOLOGY, C. 2005. Adipokines: molecular links between obesity and atheroslcerosis. *American Journal of Physiology-Heart Circulatory Physiology* 288**,** H2031-H2041.

LECLERCQ, A., HOUARD, X., PHILIPPE, M., OLLIVIER, V., SEBBAG, U., MEILHAC, O. & MICHEL, J. B. J. J. O. L. B. 2007. Involvement of intraplaque hemorrhage in atherothrombosis evolution via neutrophil protease enrichment. *Journal of Leukocyte Biology,* 82**,** 1420-1429.

LEHTIMÄKI, T., LEHTINEN, S., SOLAKIVI, T., NIKKILÄ, M., JAAKKOLA, O., JOKELA, H., YLÄ-HERTTUALA, S., LUOMA, J. S., KOIVULA, T., NIKKARI, T. J. A., THROMBOSIS, & BIOLOGY, V. 1999. Autoantibodies against oxidized low density lipoprotein in patients with angiographically verified coronary artery disease. *J Arteriosclerosis, thrombosis, vascular biology* 19**,** 23-27.

LEITE, A. C. E., CARNEIRO, V. M. D. A. & GUIMARÃES, M. D. C. M. J. B. J. O. C. S. 2014. Effects of periodontal therapy on C-reactive protein and HDL in serum of subjects with periodontitis. *J Brazilian Journal of Cardiovascular Surgery* 29**,** 69-77.

LEVINE, J. S., BRANCH, D. W. & RAUCH, J. J. N. E. J. O. M. 2002. The antiphospholipid syndrome. *J New England Journal of Medicine* 346**,** 752-763.

LIBBY, P., RIDKER, P. M. & HANSSON, G. K. J. J. O. T. A. C. O. C. 2009. Inflammation in atherosclerosis: from pathophysiology to practice. *Journal of the American college,* 54**,** 2129-2138.

LIBBY, P. & SIMON, D. I. 2001. Inflammation and thrombosis: the clot thickens. Am Heart Assoc.

LIBBY, P. J. A., THROMBOSIS, & BIOLOGY, V. 2012. Inflammation in atherosclerosis. *J Arteriosclerosis, thrombosis, vascular biology,* 32**,** 2045-2051.

LINDEN, G. J., MCCLEAN, K., YOUNG, I., EVANS, A. & KEE, F. J. J. O. C. P. 2008. Persistently raised C-reactive protein levels are associated with advanced periodontal disease. *J Journal of clinical periodontology* 35**,** 741-747.

LINTON, M. F., YANCEY, P. G., DAVIES, S. S., JEROME, W. G., LINTON, E. F., SONG, W. L., DORAN, A. C. & VICKERS, K. C. 2019. The role of lipids and lipoproteins in atherosclerosis. *Endotext [Internet].* MDText. com, Inc.

LÖE, H. & SILNESS, J. J. A. O. S. 1963. Periodontal disease in pregnancy I. Prevalence and severity. *Acta odontologica scandinavica,* 21**,** 533-551.

LÖE, H. J. T. J. O. P. 1967. The gingival index, the plaque index and the retention index systems. 38**,** 610-616.

LONG, B. R. & LEYA, F. J. H. O. C. O. N. A. 2008. The role of antiphospholipid syndrome in cardiovascular disease. *Hematology/oncology clinics of North America,* 22**,** 79-94.

LOPEZ, D., KOBAYASHI, K., MERRILL, J. T., MATSUURA, E. & LOPEZ, L. R. J. J. O. I. R. 2003. IgG Autoantibodies against β2-glycoprotein I complexed with a lipid ligand derived from oxidized low-density lipoprotein are associated with arterial thrombosis in antiphospholipid syndrome. *J Journal of Immunology Research* 10**,** 203-211.

LOWE, G. D. 1998. Etiopathogenesis of cardiovascular disease: hemostasis, thrombosis, and vascular medicine. *Ann Periodontol,* 3**,** 121-6.

LOZANO, R., NAGHAVI, M., FOREMAN, K., LIM, S., SHIBUYA, K., ABOYANS, V., ABRAHAM, J., ADAIR, T., AGGARWAL, R. & AHN, S. Y. J. T. L. 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *J The lancet* 380**,** 2095-2128.

LUSIS, A. J. 2000. Atherosclerosis. *Nature,* 407**,** 233-241.

(M)

MALHOTRA, A. S., SHARMA, R., MEHTA, J., KASHYAP, J. R., GUPTA, V., KAPOOR, N., SACHDEV, A. J. O. J. O. H. & SCIENCES, A. 2011. Determination and Correlation of Anticardiolipin Antibody with High Sensitivity C-reactive Proteins and its Role in Predicting Short Term Outcome in Patients with Acute Coronary Syndrome. *J Online Journal of Health Allied Sciences* 10.

MALHOTRA, S. & PARKASH, H. J. J. 2013. Coronary artery disease and periodontitis-A prospective study. *JIMSA* 26**,** 93-7.

MARSCH, E., SLUIMER, J. C. & DAEMEN, M. J. J. C. O. I. L. 2013. Hypoxia in atherosclerosis and inflammation. *current opinion in lipidology,* 24**,** 393-400.

MATHERS, C. D. & LONCAR, D. J. P. M. 2006. Projections of global mortality and burden of disease from 2002 to 2030. *J PLoS medicine* 3**,** e442.

MATSUURA, E., KOBAYASHI, K., TABUCHI, M. & LOPEZ, L. R. J. R. D. C. 2006. Accelerated atheroma in the antiphospholipid syndrome. *Rheumatic Disease Clinics* 32**,** 537-551.

MEHTA, J., SUKHIJA, R., ROMEO, F. & SEPULVEDA, J. J. I. H. J. 2007. Value of CRP in coronary risk determination. *Indian Heart Journal* 59**,** 173-177.

MELDER, R. J., YUAN, J., MUNN, L. L. & JAIN, R. K. J. M. R. 2000. Erythrocytes enhance lymphocyte rolling and arrest in vivo. *Microvascular research,* 59**,** 316-322.

MICHEL, J.-B., MARTIN-VENTURA, J. L., NICOLETTI, A. & HO-TIN-NOÉ, B. J. A. 2014. Pathology of human plaque vulnerability: mechanisms and consequences of intraplaque haemorrhages. *atherosclerosis,* 234**,** 311-319.

MOHAMAD, W. M. W., NING, C. K., ZAINUDDIN, S. L. A. & TAIB, H. J. I. M. J. 2014. Evaluation of Serum Anti-Cardiolipin Antibodies in Patients with Chronic Periodontitis. *J International Medical Journal* 21**,** 1-4.

MOHAMAD, W. M. W., SAAD, N. R., TAIB, H., ZAINUDDIN, S. L. A. J. J. O. I. D. & RESEARCH, M. 2017. Anti-Cardiolipin Antibodies in Chronic Periodontitis Patients in Kelantan, Malaysia. *Journal of International DentalMedical Research* 10**,** 278.

MOHAN, M., JHINGRAN, R., BAINS, V. K., GUPTA, V., MADAN, R., RIZVI, I., MANI, K. J. J. O. P. & SCIENCE, I. 2014. Impact of scaling and root planing on C-reactive protein levels in gingival crevicular fluid and serum in chronic periodontitis patients with or without diabetes mellitus. *Journal of Periodontal & Implant Science,* 44**,** 158-168.

MOORE, W. & MOORE, L. V. J. P. 1994. The bacteria of periodontal diseases. *J Periodontology* 5**,** 66-77.

MUHLEMANN, H. J. H. O. A. 1971. Gingival sulcus bleeding-a leading symptom in initial gingivitis. *Helv Odontol Acta,* 15**,** 107-113.

NAKOU, E. S., LIBEROPOULOS, E. N., MILIONIS, H. J. & ELISAF, M. S. J. C. V. P. 2008. The role of C-reactive protein in atherosclerotic cardiovascular disease: an overview. *Current Vascular Pharmacology,* 6**,** 258-270.

NAZIR, M. A. J. I. J. O. H. S. 2017. Prevalence of periodontal disease, its association with systemic diseases and prevention. *J International journal of health sciences* 11**,** 72.

NESARHOSEINI, V. J. A. A. 2010. Periodontitis as a risk factor in non-diabetic patients with coronary artery disease. *ARYA atherosclerosis* 6**,** 106.

NEWMAN, M., TAKEI, H., KLOKKEVOLD, P. & CARRANZA, F. J. C. S. C. P. 2012. Classification of diseases and conditions affecting the periodontium. *J Carranza's Clinical Periodontology* 10**,** 104.

NOACK, B., GENCO, R. J., TREVISAN, M., GROSSI, S., ZAMBON, J. J. & DE NARDIN, E. J. J. O. P. 2001. Periodontal infections contribute to elevated systemic C-reactive protein level. *Journal of periodontology* 72**,** 1221-1227.

NORATA, G. D., MARCHESI, P., PULAKAZHI VENU, V. K., PASQUALINI, F., ANSELMO, A., MOALLI, F., PIZZITOLA, I., GARLANDA, C., MANTOVANI, A. & CATAPANO, A. L. J. C. 2009. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. *Circulation* 120**,** 699.

(O)

OE, Y., SOEJIMA, H., NAKAYAMA, H., FUKUNAGA, T., SUGAMURA, K., KAWANO, H., SUGIYAMA, S., MATSUO, K., SHINOHARA, M., IZUMI, Y. J. H. & VESSELS 2009. Significant association between score of periodontal disease and coronary artery disease. *Heart vessels*24**,** 103-107.

(P)

PADILLA, N. D., CIURANA, C., VAN OERS, J., OGILVIE, A. C. & HACK, C. E. J. J. O. I. M. 2004. Levels of natural IgM antibodies against phosphorylcholine in healthy individuals and in patients undergoing isolated limb perfusion. *J Immunol Methods,* 293**,** 1-11.

PAFFEN, E., VOS, H. L., BERTINA, R. M. J. A., THROMBOSIS, & BIOLOGY, V. 2004. C-reactive protein does not directly induce tissue factor in human monocytes. *J Arteriosclerosis, thrombosis,vascular biology* 24**,** 975-981.

PARADIES, G., PETROSILLO, G., PARADIES, V., RUGGIERO, F. M. J. F. R. B. & MEDICINE 2010. Oxidative stress, mitochondrial bioenergetics, and cardiolipin in aging. *Free Radical Biology Medicine* 48**,** 1286-1295.

PEJCIC, A., KESIC, L. & MILASIN, J. J. J. O. D. R., DENTAL CLINICS, DENTAL PROSPECTS 2011. Association between periodontopathogens and CRP levels in patients with periodontitis in Serbia. *J Journal of dental research, dental clinics, dental prospects* 5**,** 10.

PERES, M. A., MACPHERSON, L. M., WEYANT, R. J., DALY, B., VENTURELLI, R., MATHUR, M. R., LISTL, S., CELESTE, R. K., GUARNIZO-HERREÑO, C. C. & KEARNS, C. J. T. L. 2019. Oral diseases: a global public health challenge. *J The Lancet* 394**,** 249-260.

PERSSON, G. R. & PERSSON, R. E. J. J. O. C. P. 2008. Cardiovascular disease and periodontitis: an update on the associations and risk. *Journal of clinical periodontology* 35**,** 362-379.

PERSSON, G. R., PETTERSSON, T., OHLSSON, O. & RENVERT, S. J. J. O. C. P. 2005. High-sensitivity serum C-reactive protein levels in subjects with or without myocardial infarction or periodontitis. *J Journal of clinical periodontology* 32**,** 219-224.

PHAM, K., FEIK, D., HAMMOND, B., RAMS, T. & WHITAKER, E. J. P. 2002. Aggregation of human platelets by gingipain-R from Porphyromonas gingivalis cells and membrane vesicles. *Platelets* 13**,** 21-30.

PITIPHAT, W., SAVETSILP, W. & WARA-ASWAPATI, N. J. J. O. C. P. 2008. C-reactive protein associated with periodontitis in a Thai population. 35**,** 120-125.

PRESHAW, P. M. & TAYLOR, J. J. J. J. O. C. P. 2011. How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? *Journal of clinical periodontology,* 38**,** 60-84.

PURKALL, D., TEW, J. G. & SCHENKEIN, H. A. 2002. Opsonization of Actinobacillus actinomycetemcomitans by immunoglobulin G antibody reactive with phosphorylcholine. *Infect Immun,* 70**,** 6485-8.

(R)

RADAFSHAR, G., SHAD, B. & MIRFEIZI, M. J. J. O. D. O. T. U. O. M. S. 2006. Association between periodontal disease and elevated C-reactive protein in acute myocardial infarction patients. *J Journal of Dentistry of Tehran University of Medical Sciences* 126-134.

RAFIEIAN-KOPAEI, M., SETORKI, M., DOUDI, M., BARADARAN, A. & NASRI, H. J. I. J. O. P. M. 2014. Atherosclerosis: process, indicators, risk factors and new hopes. *Int J Prev Med.,* 5**,** 927.

RAHEEM, Z. J. & AHMED, M. A. J. J. O. B. C. O. D. 2014. Assessment of serum levels of MMP-8 and hs CRP in chronic periodontitis patients in relation to atherosclerotic cardiovascular disease. *J Journal of Baghdad College of Dentistry* 325**,** 1-12.

ROSS, R. J. N. E. J. O. M. 1999. Atherosclerosis—an inflammatory disease. *J New England journal of medicine* 340**,** 115-126.

ROZEE, K., ACOTT, P., LEE, S., CROCKER, J., GOUGH, D., MACDONALD, J., EVANS, J. & MURPHY, M. J. B. E. B. A.-M. B. O. D. 2003. Elevated anticardiolipin antibodies in acute liver failure. *J Biochimica et Biophysica Acta -Molecular Basis of Disease* 1637**,** 183-186.

RUTGER PERSSON, G., OHLSSON, O., PETTERSSON, T. & RENVERT, S. J. E. H. J. 2003. Chronic periodontitis, a significant relationship with acute myocardial infarction. *J European heart journal* 24**,** 2108-2115.

(S)

SAITO, H., LUND-KATZ, S. & PHILLIPS, M. C. J. P. I. L. R. 2004. Contributions of domain structure and lipid interaction to the functionality of exchangeable human apolipoproteins. 43**,** 350-380.

SANCHEZ-BEATO, A. R., RONDA, C. & GARCIA, J. L. J. J. O. B. 1995. Tracking the evolution of the bacterial choline-binding domain: molecular characterization of the Clostridium acetobutylicum NCIB 8052 cspA gene. *Journal of bacteriology,* 177**,** 1098-1103.

SCHENKEIN, H., BERRY, C., BURMEISTER, J., BROOKS, C., BARBOUR, S., BEST, A. & TEW, J. J. J. O. D. R. 2003. Anti-cardiolipin antibodies in sera from patients with periodontitis. *journal of dental research,* 82**,** 919-922.

SCHENKEIN, H. A., BARBOUR, S. E., BERRY, C., KIPPS, B., TEW, J. G. J. I. & IMMUNITY 2000. Invasion of human vascular endothelial cells byActinobacillus actinomycetemcomitans via the receptor for platelet-activating factor. *J Infection immunity* 68**,** 5416-5419.

SCHENKEIN, H. A., BERRY, C. R., BURMEISTER, J. A., BROOKS, C. N., BEST, A. M. & TEW, J. G. 2004. Locally produced anti-phosphorylcholine and anti-oxidized low-density lipoprotein antibodies in gingival crevicular fluid from aggressive periodontitis patients. *J Periodontol,* 75**,** 146-53.

SCHENKEIN, H. A., BERRY, C. R., PURKALL, D., BURMEISTER, J. A., BROOKS, C. N., TEW, J. G. J. I. & IMMUNITY 2001. Phosphorylcholine-dependent cross-reactivity between dental plaque bacteria and oxidized low-density lipoproteins. *Infection immunity* 69**,** 6612-6617.

SCHENKEIN, H. A., BEST, A. M., BROOKS, C. N., BURMEISTER, J. A., ARROWOOD, J. A., KONTOS, M. C. & TEW, J. G. J. J. O. P. 2007. Anti-cardiolipin and increased serum adhesion molecule levels in patients with aggressive periodontitis. *J Journal of periodontology* 78**,** 459-466.

SCHENKEIN, H. A., GUNSOLLEY, J. C., BEST, A. M., HARRISON, M. T., HAHN, C.-L., WU, J., TEW, J. G. J. I. & IMMUNITY 1999. Antiphosphorylcholine antibody levels are elevated in humans with periodontal diseases. *Infection* 67**,** 4814-4818.

SCHENKEIN, H. A. & LOOS, B. G. 2013. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. *J Clin Periodontol,* 40 Suppl 14**,** S51-69.

SECIU, A.-M., CRACIUNESCU, O. & ZARNESCU, O. 2018. Advanced regenerative techniques based on dental pulp stem cells for the treatment of periodontal disease. *Periodontology.* IntechOpen.

SHAW, P. X., GOODYEAR, C. S., CHANG, M.-K., WITZTUM, J. L. & SILVERMAN, G. J. J. T. J. O. I. 2003. The autoreactivity of anti-phosphorylcholine antibodies for atherosclerosis-associated neo-antigens and apoptotic cells. *J The Journal of Immunology* 170**,** 6151-6157.

SHEN, Z., YE, C., MCCAIN, K. & GREENBERG, M. L. J. B. R. I. 2015. The role of cardiolipin in cardiovascular health. *J BioMed research international* 2015.

SHRIVASTAVA, A. K., SINGH, H. V., RAIZADA, A. & SINGH, S. K. J. T. E. H. J. 2015. C-reactive protein, inflammation and coronary heart disease. *The Egyptian Heart Journal* 67**,** 89-97.

SILNESS, J. & LÖE, H. J. A. O. S. 1964. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. 22**,** 121-135.

SLADE, G. D., GHEZZI, E. M., HEISS, G., BECK, J. D., RICHE, E. & OFFENBACHER, S. J. A. O. I. M. 2003. Relationship between periodontal disease and C-reactive protein among adults in the Atherosclerosis Risk in Communities study. *J Archives of internal medicine* 163**,** 1172-1179.

SORSA, T., TERVAHARTIALA, T., LEPPILAHTI, J., HERNANDEZ, M., GAMONAL, J., TUOMAINEN, A. M., LAUHIO, A., PUSSINEN, P. J. & MÄNTYLÄ, P. J. P. R. 2011. Collagenase-2 (MMP-8) as a point-of-care biomarker in periodontitis and cardiovascular diseases. Therapeutic response to non-antimicrobial properties of tetracyclines. 63**,** 108-113.

STANCEL, N., CHEN, C.-C., KE, L.-Y., CHU, C.-S., LU, J., SAWAMURA, T. & CHEN, C.-H. J. C. C. 2016. Interplay between CRP, atherogenic LDL, and LOX-1 and its potential role in the pathogenesis of atherosclerosis. *J Clinical chemistry* 62**,** 320-327.

SU, J. 2009. *Natural antibodies against phosphorylcholine as potential protective factors in atherosclerosis, cardiovascular disease and systemic lupus erythematosus*, Institutionen för medicin, Huddinge Sjukhus/Department of Medicine at ….

SU, J., HUA, X., CONCHA, H., SVENUNGSSON, E., CEDERHOLM, A. & FROSTEGÅRD, J. J. R. 2008. Natural antibodies against phosphorylcholine as potential protective factors in SLE. *J Rheumatology* 47**,** 1144-1150.

SU, J., HUA, X., VIKSTRÖM, M., LEANDER, K., GIGANTE, B., HELLENIUS, M.-L., DE FAIRE, U. & FROSTEGÅRD, J. J. B. C. D. 2013. Low levels of IgM antibodies to oxidized cardiolipin increase and high levels decrease risk of cardiovascular disease among 60-year olds: a prospective study. *J BMC cardiovascular disorders,* 13**,** 1.

(T)

TABAS, I. & BORNFELDT, K. E. J. C. R. 2016. Macrophage phenotype and function in different stages of atherosclerosis. *J Circulation research*118**,** 653-667.

TELES, R. & WANG, C. Y. J. O. D. 2011. Mechanisms involved in the association between peridontal diseases and cardiovascular disease. *J Oral diseases,* 17**,** 450-461.

TERAMOTO, T., SASAKI, J., ISHIBASHI, S., BIROU, S., DAIDA, H., DOHI, S., EGUSA, G., HIRO, T., HIROBE, K., IIDA, M. J. J. O. A. & THROMBOSIS 2013. Executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan—2012 version. *J Journal of atherosclerosis thrombosis* 15792.

TEW, J., EL SHIKH, M., EL SAYED, R. & SCHENKEIN, H. J. J. O. D. R. 2012. Dendritic cells, antibodies reactive with oxLDL, and inflammation. *J Journal of dental research* 91**,** 8-16.

THAKARE, K. S., DEO, V. & BHONGADE, M. L. J. I. J. O. D. R. 2010. Evaluation of the C-reactive protein serum levels in periodontitis patients with or without atherosclerosis. *J Indian Journal of Dental Research* 21**,** 326.

THASLEEMA, S. A. & DON, K. J. D. I. T. 2019. Association between atherosclerosis and periodontitis-A brief review. *Drug Invention Today* 11.

THIELE, J. R., HABERSBERGER, J., BRAIG, D., SCHMIDT, Y., GOERENDT, K., MAURER, V., BANNASCH, H., SCHEICHL, A., WOOLLARD, K. J. & VON DOBSCHÜTZ, E. J. C. 2014. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation: in vivo proof of a powerful proinflammatory mechanism and a new anti-inflammatory strategy. *J Circulation* 130**,** 35-50.

THOMOPOULOS, C., TSIOUFIS, C., SOLDATOS, N., GIAMARELOS, G., DENDRINOS, C., SELIMA, M., KASIAKOGIAS, A., STEFANADI, E., TOUSOULIS, D. & STEFANADIS, C. J. J. O. H. 2010. Chronic periodontal disease and systemic inflammation on endothelial dysfunction in untreated hypertensive patients: Pp. 2.53. *J Journal of Hypertension* 28**,** e63.

TILLETT, W. S. & FRANCIS, T. J. J. O. E. M. 1930. Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *Journal of Experimental Medicine* 52**,** 561-571.

TOMASZ, A. J. S. 1967. Choline in the cell wall of a bacterium: novel type of polymer-linked choline in Pneumococcus. *J Science,* 157**,** 694-697.

TOREGEANI, J. F., NASSAR, C. A., TOREGEANI, K. A. M. & NASSAR, P. O. J. J. V. B. 2014. Periodontal disease and atherosclerosis. *Journal Vascular Brasileiro* 13**,** 208-216.

TUOMINEN, A., MILLER, Y. I., HANSEN, L. F., KESÄNIEMI, Y. A., WITZTUM, J. L., HÖRKKÖ, S. J. A., THROMBOSIS, & BIOLOGY, V. 2006. A natural antibody to oxidized cardiolipin binds to oxidized low-density lipoprotein, apoptotic cells, and atherosclerotic lesions. *Arteriosclerosis, thrombosis, vascular biology* 26**,** 2096-2102.

TÜRKOĞLU, O., BARıŞ, N., KÜTÜKÇÜLER, N., ŞENARSLAN, Ö., GÜNERI, S. & ATILLA, G. J. J. O. P. 2008. Evaluation of serum anti-cardiolipin and oxidized low-density lipoprotein levels in chronic periodontitis patients with essential hypertension. *J Journal of periodontology* 79**,** 332-340.

VILAHUR, G., BADIMON, J. J., BUGIARDINI, R. & BADIMON, L. J. E. H. J. S. 2014. Perspectives: The burden of cardiovascular risk factors and coronary heart disease in Europe and worldwide. *J European Heart Journal Supplements* 16**,** A7-A11.

VRAŽIĆ, D., MIOVSKI, Z., STROZZI, M., PUHAR, I., BADOVINAC, A., BOŽIĆ, D. & PLANČAK, D. J. A. S. C. 2015. Periodontal disease and its association with angiographically verified coronary artery disease. *Acta stomatologica Croatica* 49**,** 14.

(W)

WILLERSHAUSEN, B., KASAJ, A., WILLERSHAUSEN, I., ZAHORKA, D., BRISENO, B., BLETTNER, M., GENTH-ZOTZ, S. & MÜNZEL, T. J. J. O. E. 2009. Association between chronic dental infection and acute myocardial infarction. *J Journal of endodontics* 35**,** 626-630.

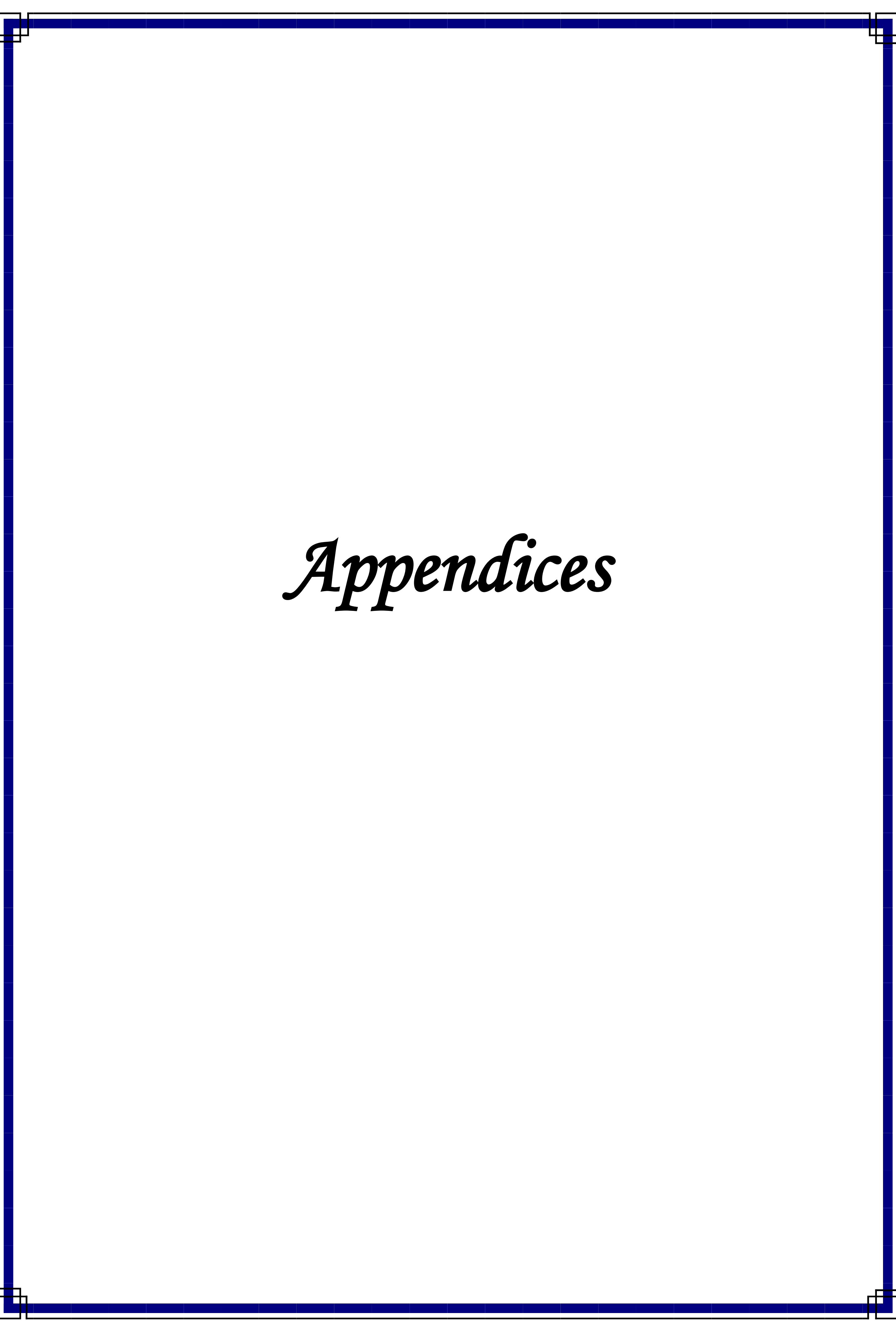
WILLIAMS, R. C. & OFFENBACHER, S. J. P. 2000. Periodontal medicine: the emergence of a new branch of periodontology. *Periodontology* 23**,** 9-12.

(Y)

YASOJIMA, K., SCHWAB, C., MCGEER, E. G. & MCGEER, P. L. J. T. A. J. O. P. 2001. Generation of C-reactive protein and complement components in atherosclerotic plaques. *The American journal of pathology* 158**,** 1039-1051.

(Z)

ZHONG, H., LU, J., XIA, L., ZHU, M. & YIN, H. J. R. B. 2014. Formation of electrophilic oxidation products from mitochondrial cardiolipin in vitro and in vivo in the context of apoptosis and atherosclerosis. *Redox biology,* 2**,** 878-883.



Appendices

Appendix Ⅰ

Appendix Ⅰ

أنت مدعو/مدعوة للمشاركة طوعيا في هذه الدراسة التي تجرى في جامعة بغداد/ كلية طب الاسنان/ فرع أمراض و جراحة ما حول الأسنان من قبل طالبة الماجستير رهام عدنان راضي وبأشراف الأستاذة الأء عمران علي

في هذه الدراسة تقوم الباحثة بسحب عينات دم من المشاركين لدراسة تأثير مرض التصلب العصيدي على مرض النساغ المزمن ويتم سحب5 مل من الوريد لأغراض بحثيه وعلمية فقط.

أذا كنت موافقا على هذه الأجراء نرجو تقديم معلوماتك:

أسم المريض..

توقيع المريض...

العنوان...

التاريخ...

أسم الباحثة..

توقيع الباحثة..

Appendix Ⅱ

Case sheet

Participant’s Name: ----------------------------

Participant’s age: ------------ Participant’s gender: -----------

Participant’s number: --------------------------

Control: ------------ patient: ------------

Periodontal disease type: -----------------------

Dental history:

Previous periodontal treatment: ----------------------------------

Tooth brushing: --

Medical history:

History of systemic diseases: -------------------------------------

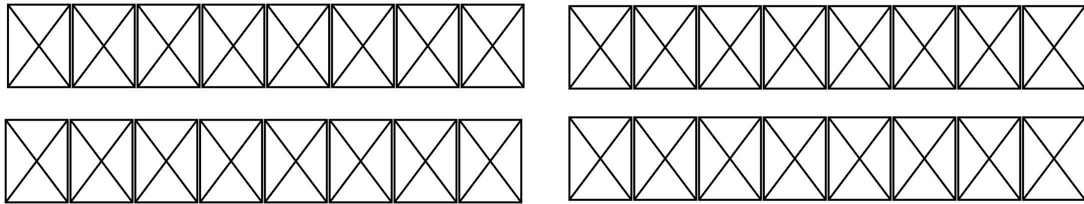
Smoking: ---

Medications: ---

Others: --

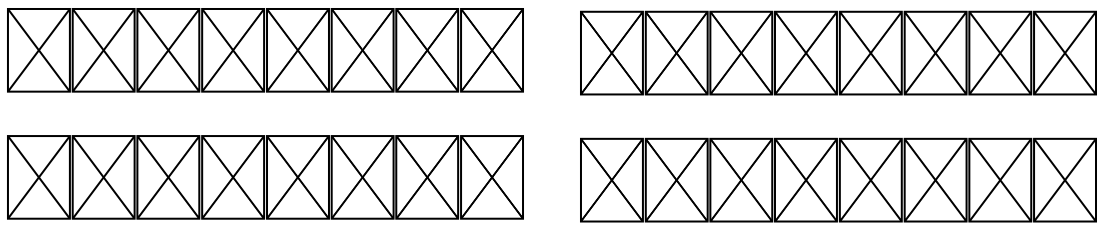
Appendix Ⅱ

Plaque Index



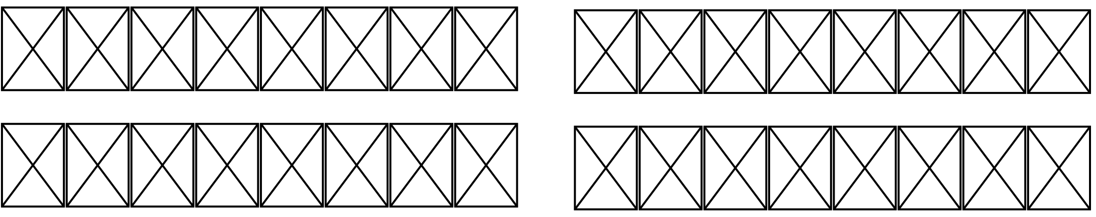
Mean of plaque index: --------

Gingival Index



Mean of gingival index: -----------

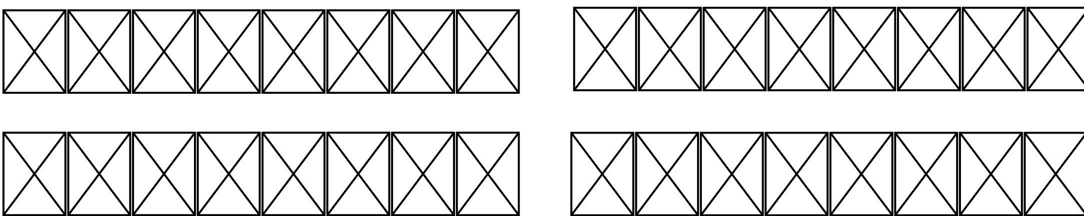
Bleeding on Probing



Percentage of bleeding on probing: ------------ %

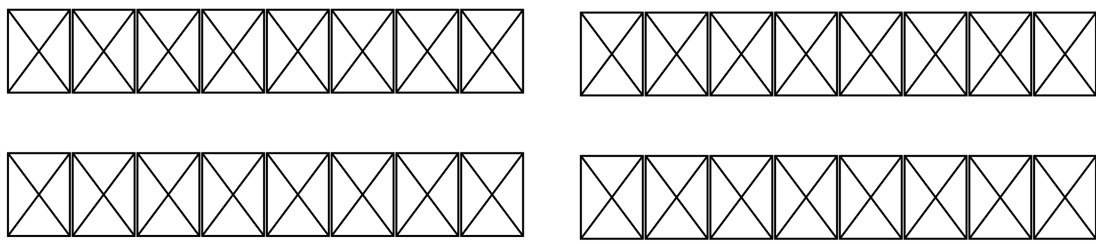
Appendix Ⅱ

Probing pocket depth



Mean of probing pocket depth: -------------

Clinical attachment loss



Mean of clinical attachment level: -----------

Appendix III

العدد: ١٢
التاريخ:٨ / ١ /٢٠١٩
رمز البحث: ٠١٢٦١٨

م/ قبول بحث

إلى الزميلة الدكتورة رهام عدنان راضي المحترمة
والزميلة الدكتورة الاء عمران علي المحترمة

نود اعلامكم بأن لجنة اخلاقيات البحوث في كلية طب الأسنان – جامعة بغداد اطلعت على مشروع البحث المقدم من قبلكم والموسوم:

The Estimation of Anticardiolipin antibody, Antiphosphorylcholine and High Sensitive C- reactive protein in serum of patients with chronic periodontitis in relation to Atherosclerotic cardiovascular disease.

ولا ترى اللجنة ما يمنع من القيام بالبحث من الجانب الاخلاقي.

مع التقدير.

د. أكرم فيصل الحويزي
رئيس لجنة اخلاقيات البحوث

الخلاصة

المقدمة: يعد مرض النساغ ومرض التصلب العصيدي من الأمراض الالتهابية المزمنة الواسعة الانتشار خلال العقدين الماضيين. زادت كمية الأدلة التي تثبت وجود علاقة بين بكتيريا الصفيحة الجرثومية للاسنان وأمراض الشريان التاجي التي تنشأ نتيجة تصلب الشرايين.

الهدف من الدراسة: لتقييم الحالة الصحية للانسجة ما حول الاسنان في مجموعتي الدراسة (مرض التصلب العضيدي لديهم النساغ المزمن) والمجموعة الضابطة, تقييم مستويات مضاد الكارديوليبين , مضاد الفوسفوريل كولين والبروتين الارتكاسي عالي الحساسية في مجموعات الدراسة والضابطة والمقارنة بينهم, وكذلك اختبار العلاقة بين مستويات مضاد الكارديولبين, مضاد الفوسفوريل كولين واالبروتين الارتكاسي عالي الحساسية مع مؤشرات ماحول الاسنان السريرية في كل مجموعة دراسية.

كذلك تحديد العلاقه بين المستويات المصليه للمؤشرات المناعية مضاد الكارديبولبين, مضاد الفوسفوريل كولين والبروتين الارتكاسي عالي الحساسية لدى مرضى التصلب العصيدي لديهم النساغ المزمن ومرضى النساغ المزمن.

المواد وطرق العمل

85 من الذكور والإناث أدرجوا في هذه الدراسة تتراوح أعمارهم بين (35-64) سنة ، تم تقسيمم إلى ثلاث مجموعات: مجموعتي الدراسة (30 مريض ، مرض التصلب العضيدي لديهم النساغ المزمن) و (30 مريض لديهم النساغ المزمن) ومجموعة السيطرة (25اصحاء سريريا, يملكون انسجة ما حول الاسنان صحية).

قدرت الحالة الصحية للانسجة ماحول الاسنان عن طريق قياس المؤشرات السريرية التالية (مؤشر الصفحية الجرثومية، مؤشرالتهاب اللثة, مؤشر النزف عند التسبير, مؤشر عمق الجيوب بالاضافة الى فقدان الانسجة الرابطة سريريا) احتسبت لكل الاسنان باستثناء الرحى الثامنة.وبعد اجراء الفحص السريري ، تم جمع5 مل من الدم من مجموعات الدراسة والضابطة. بعد اجراء الطرد المركزي للعينات، تم حفظ عينات الدم مجمدة بدرجة(-20). تم تحديد مستويات المصل لكل من مضاد الكارديوليبين,مضاد الفوسفوريل كولين باستخدام

تقنية مقايسة الانزيم المرتبط الممتز المناعي وتحديد مستوى البروتين الارتكاسي عالي الحساسية باستخدام تقنية (Immunoturbidimetric).

النتائج

أظهرت النتائج ان القيم التوسطه لمؤشرات ما حول الاسنان السريرية(مؤشر الصفيحة الجرثوميه,مؤشر التهاب اللثة بالاضافه الى فقدان الانسجه الرابطة سريريا) كانت اعلى في مجموعة (مرضى التصلب العصيدي لديهم النساغ المزمن) مقارنة بمجموعة مرضى النساغ المزمن مع فروقات معنوية ملحوظة.قيمة المتوسط الحسابي لمؤشر عمق الجيوب كان اعلى لدى مجموعة(النساغ المزمن) عن مجموعة(التصلب العصيدي لديهم النساغ المزمن) مع فروقات معنوية ملحوظة. هناك نسبه عالية لمؤشر النزف عند التسبير لدى مرضى(النساغ المزمن)عن مجموعة(التصلب العصيدي لديهم النساغ المزمن) مع وجود فروقات معنوية عالية.

مستويات المصل للبروتين الارتكاسي عالي الحساسية كانت اعلى لدى مجموعة (التصلب العصيدي والنساغ المزمن)عند مقارنتهم مع مجموعة النساغ المزمنة والمجموعة الضابطة مع وجود فروقات معنويه عاليه. في حين كان المتوسط الحسابي لمضاد الكارديولبين اعلى في مجموعة(التصلب العصيدي لديهم النساغ المزمن) مقارنة مع مجموعة النساغ المزمن والمجموعة الضابطة لكن لايوجد فرق معنوي بين مجموعات الدراسة،بأستثناء مجموعة (التصلب العصيدي والنساغ المزمن) عند مقارنتهم بالمجموعة الضابطة كان هناك فرق معنوي ملحوظ.

فيما يخص العلاقة بين مستويات المصل لمضاد الكارديولبين،مضاد الفوسفوريل كولين والبروتين الأرتكاسي عالي الحساسية ومؤشرات ما حول الأسنان السريرية في مجموعتي(التصلب العصيدي لديهم النساغ المزمن) و(النساغ المزمن) هناك ارتباط طردي ضعيف في كلا المجموعتين .

بالأضافة الى ذلك، فان العلاقة بين المؤشرات المناعية لمضاد الكارديولبين،مضاد الفوسفوريل كولين والبروتين الأرتكاسي عالي الحساسية ومؤشرات ما حول الأسنان السريرية في مجموعتي (التصلب العصيدي لديهم النساغ المزمن) و(النساغ المزمن) ، أظهرت وجود علاقة طردية قوية بين مضاد الكارديولبين ومضاد الفسفوريل كولين،علاوة على ذلك في مجموعة النساغ المزمن، لم يكن هناك علاقة بينهما.

الأستنتاج

أظهرت هذه الدراسة أن المستويات المصلية للبروتين الأرتكاسي عالي الحساسية ومضاد الكارديولبين كانت أعلى بكثيرفي مرض التصلب العصيدي لديهم النساغ المزمن مما كانت عليه في مرض النساغ المزمن،لذلك قد تترافق زيادة خطر الأصابة بالتصلب العصيدي بمستويات عالية من البروتين الارتكاسي عالي الحساسية ومضاد الكارديولبين التي ترجع الى النساغ المزمن.علاوة على ذلك،فان الزيادة التدريجية في مستوى مصل مضاد الفسفوريل كولين في مرضى النساغ المزمن ومرضى التصلب العصيدي لديهم النساغ المزمن بالمقارنة مع المجموعة الضابطة قد تشييرالى ثأثير النساغ المزمن على التصلب العصيدي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بغداد ـ كلية طب الأسنان

قسم أمراض وجراحة ما حول الأسنان

تقييم المستويات المصلية للبروتين الأرتكاسي عالي الحساسية،مضاد الكارديوليبين و مضاد الفوسفوريل كولين لدى مرضى النساغ المزمن مع وبدون مرض التصلب العصيدي

رسالة مقدمة الى كلية طب الاسنان /جامعة بغداد كجزء من متطلبات نيل درجة الماجستيرفي أمراض وجراحة ما حول الأسنان

من قبل

رهام عدنان راضي

بكلوريوس طب وجراحة الفم و الأسنان

بأشراف

أ.م.د. الأء عمران علي

ماجستير أمراض وجراحة ما حول الأسنان

1441هـ 2019 م