Republic of Iraq

Ministry of Higher Education and

Scientific Research

University of Babylon

College of Dentistry

Department of Microbiology

Immuno markers in periodontitis with rheumatoid arthritis patients and antibacterial effect of nanoparticles against *Porphyromonas gingivalis* .

Submitted as Partial Fulfillment for the Requirements of the Degree of Master of Sciences in Oral and Medical Microbiology to the Council of the College of Dentistry at the University of Babylon .

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ وَأَنْ لَيْسَ لِلْإِنْسَانِ إِلَّا مَا سَعَىٰ ٣٩ وَأَنَّ سَعْيَهُ سَوْفَ يُرَىٰ ٤٠ ثُمَّ يُجْزَاهُ الْجَزَاءَ الْأَوْفَى﴾

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سورة النجم الآية

(٣٩ ، ٤٠ ، ٤١)

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We certify that this thesis entitled (Detection of biomarkers in Periodontitis Patients with Rheumatic arthritis and efficacy of Nanoparticles on bacterial isolates .) was prepared by " Mustafa Ibrahim al - Amery" at the College of Dentistry , University of Babylon , as a partial requirement for the degree of master of science in oral and medical microbiology .

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We the Examining Committee, certify that we have read this thesis entitled**(Immuno markers in periodontitis with rheumatoid arthritis patients and antibacterial effect of nanoparticles against *Porphyromonas gingivalis*)** and have examined the student " **Mustafa Ibrahim Hashim Al_ Amery** " in its contents, and that in our opinion, it is accepted as a thesis for the Degree of Master of Science in Oral and Medical Microbiology with Excellent degree.

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Dedication

The Soul of my father

To my dear mother

To my beloved wife

To my beautiful daughters Layan and Naya

To all my family who supported me with all love , Brother (Bassam and wissam) and sister Hadeel

To all people who helped me during my research

Lastly for myself , for choosing to keep going on and never give up on hard times .

Mustafa

Acknowledgements

First and for most I thank ‘'Allah" for giving me the strength and patience to finish this project and presented it to you .

Many thanks to my teachers and supervisors Asst. Prof .Dr .**Huda Abbas Mohammed** and L. Dr. **Zeyad Nazar Majeed** for their advices and providing most of the requirements for this project and Thanks to L. Rafea T. Ahmed in Departmat Laser physics , collage of science for women , Babylon university.

Thanks to the Dean of the College of dentistry ,Babylon University Prof . Dr . Wissam wahhab sahib for his providing all the needed facilities which were essential for successful completion of the present work .

I would like to thank the Scientific assistant , Asst . prof Dr. Hassan Faliah AL- Watifi . Also ,I would like to thank the Head of Department of Microbiology Asst .Prof . Dr. Ahmed Mohammed Abbas for facilitating my research work as well as all members of the department ,without exception for their continuous support and encouragement .

Many thanks to Dr. Gamela Ali for helping me to complete this work . My gratitude to my friends and the people that helped and supported me. My thanks and best regards to all the seniors of the Periodontics Department for their efforts and kindness.and Grateful thanks and respect to all members at the college of dentistry University of Babylon ,especially Dr. Doaa Neamaa Ibrahim and Dr. Hind Haider Omran for their cooperation during sample collection. My thanks and respect to all the staff members of the biological center at Marjan teaching hospital for their help during sample collection. I thank Dr. Muhammad Abdullah Ajresh, Director of the Training and Human Development Center in the Babylon Health Directorate

I gratefully acknowledge the cooperation of 120 persons who took part in this study.

Mustafa

Summary

Inflammatory chronic disorders like rheumatoid arthritis and periodontitis are both common . Periodontal illness is popular this disease has a complex etiology , and a number of elements including the invasion of microorganisms the health of the host and external environmental factors contribute to its development it is yet unknown whether rheumatoid arthritis and periodontitis are related , This study includes three aims the first is to isolate the most common bacteria in periodontitis While the second aim includes detection Immunoglobulin G1, Interleukin 4 levels between healthy persons and patients with periodontitis and rheumatoid arthritis , and third aim includes studying effect of novel materials (Nanoparticles) on Biofilm formation which produced by the bacterial isolated from periodontitis-rheumatoid patients (in vitro) . This study is carried out in the college of Density/ University of Babylon and Merjan Teaching Hospital Rheumatology Unit Babylon from (December 2022 to May 2023) Patients with periodontitis and rheumatoid arthritis (80 patient male and female) with age range (20-60) years . In this study 40 people with rheumatoid arthritis , 40 participants had healthy and 40 participants had periodontitis with probing depths > 5 mm and Clinical Attachments Loss of 3 mm. and blood samples are obtained to Immunoglobulin G1 and Interleukin 4 concentrations were assessed using the Enzyme-Linked Immune Sorbent Assay (ELISA) , and Using polymerase chain reaction (PCR) the precise primer sequence for *Porphyromonas gingivalis* was identified .

Immunoglobulin G1 and Interleukin 4 levels in patient samples are frequently low or undetectable , and they stayed that way on average , in two groups (periodontitis and RA) on the same level to some extent , but the level Interleukin 4 and Immunoglobulin G1 samples obtained from controls were higher than the levels obtained from patients with

periodontitis and RA . In the current study The results show that the levels of Immunoglobulin G1 are high in healthy people compared to those with periodontitis and those with periodontitis and arthritis , where the levels in healthy people .

The prevalence of *Porphyromonas gingivalis* was also revealed by our investigation to be The target sequence for *Porphyromonas .gingivalis* - specific amplification was the transcribed region . Only 27 . 5 % (11 of 40) of the group of healthy participants had *Porphyromonas .gingivalis* whereas 25% (10 of 40) of the group of periodontitis had it , and 20% (8 of 40) of the group of rheumatoid and periodontitis had it .

Our analyses showed increasing rates for antibiotic non-susceptibility in all isolates of *Porphyromona gingivalis* to Amoxicillin ,Clindamycin, Imepenim , Oxacillin, Erythromycin and Azithromycin while The bacterial are isolates the antibiotic susceptibile to Amikacin and Penicillin.

where the prepared nanomaterials consisted of three types , namely copper oxide with silver(Ag+ CuO) , copper oxide (CuO) and silver (Ag) then the five bacterial isolates of *Porphyromonas .gingivalis* were treated with the three nanomaterials . The biofilms , which are one of the virulence factors used by pathogenic bacteria for disease events , were measured by measuring the optical density using the Elisa device , It is clear from the current results that the copper oxide with silver (Ag +CuO) nanomaterial was more efficient in inhibiting the growth of bacteria by inhibiting the ability of bacteria to form biofilms and thus reducing the ability of bacteria to settle in the teeth or gums , As for the silver (Ag) nanomaterial , it stimulated the growth of bacteria and increased their ability to form biofilms .

The novelty of this study is using Cuo + Ag nanoparticles which synthesis by pulsed laser ablation method to decrease ability of *P. gingivalis* to form biofilm, This finding is reported for the first time all over the world.

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List of abbreviation

Abbreviation	Details
µl	Micro liter
ACPA	Anti-Citrullated Protein Antibody
ACR	American College Of Rheumatology
Ag	Silver
AHR	Aryl Hydrocarbon Receptor
AK	Amikacin
AML	Amoxicillin
AZM	Azithromycin
B cells	B lymphocytes
BOP	Bleeding on probing
CAL	Clinical attachment loss
CD	Clindamycin
CRP	C-Reactive Protein
Cuo	Copper II oxide
DDDW	Double distilled deionized water
Dmards	Disease-Modifying Antirheumatic Drugs
DNA	Deoxyribonucleic acid
DW	Distil water
E	Erythromycin

EDTA	Ethylene Damien Tetra acetic acid
ELISA	Enzyme-linked immune sorbent assay
EULAR	European League Against Rheumatism
Fim A	Fimbrillin
FLS	Fibroblast-Like Synoviocytes
HIV	Human immunodeficiencyviruse
HLA	Human leukocyte antigen
ICD	International Classification Of Disease
IGG	Immuno globulin guanine
IL-4	Interleukin-four
IMP	Imepenim
LPS	Lipopolysaccharides
Luria broth	LB
ND-YAG	neodymium-doped yttrium aluminum garnet
NP	Nanoparticles
OD	Optical density
OMVs	Outer Membrane Vesicles
OX	Oxacillin
P	Penicillin
P. Gingivalis	*Porphyromonas gingivalis*
PAD2	Protein-Arginine Deaminase type-2
PCR	Polymerase Chain Reaction
PD	Periodontitis Disease

PPD	Probing pocket depth
PS	Plaque Score
RA	Rheumatoid Arthritis
RANKL	Receptor Activator of Nuclear Factor- κb Ligand
RF	Rheumatoid Factor
SLE	Systemic lupus erythematous
SPR	Surface plasmon resonance
T cell	T Lymphocyte
TBE	Tris Borate EDTA
Treg cells	T regulatory lymphocyte
UV	Ultra-Violate
WHO	World Health Organization
Pg / ml	Pico gram / milli liter

Chapter One

Introduction

1.Introduction

One of the most well-known chronic inflammatory non-transmittable infections is periodontitis (Niklander *et al*., 2021). It is a multifactorial illness in which many factors include not only the existence of pathogenic microscopic organisms and the immune system, but also the individuals' genetic backgrounds (Genco and Sanz ., 2020 **).**

Periodontitis is caused by the red complex, bacterial species which consists of three kinds of bacteria , Porphyromonas gingivalis , Treponema denticola and Tannerella forsythia. *Porphyromonas gingivalis* considered mostly causative bacteria for periodontitis, is a Gram-negative, commit anaerobic bacterium that lives in the mouth and is connected to periodontitis, and has destructiveness factors, for example, (the trypsin like protease, PrtH protease, glycosidases, Leucine BspA, and S layer) (Nagao and Tanigawa , 2019).

Periodontitis is an inflammatory disease of dental supporting tissues that including gingiva , periodontal ligament , and bone . It has been indicated as one causes for rheumatoid arthritis RA Ferreira *et al.*,(2019).Rheumatoid arthritis is the type of arthritis that is most well-known (Constantinou *et al*., 2021). A chronic B-cell inflammatory autoimmune disease with rheumatic symptoms, RA is impacted by both genetic and environmental factors. New immune system mechanisms (innate and adaptive) that can affect RA's various stages have also been the subject of research (Croia *et al*., 2019).

Numerous clinical research have demonstrated a connection between periodontal disease (PD) and rheumatoid arthritis (RA) (Zhao *et al.*,2018). According to (Joseph *et al.*,2013), the both conditions are classified as chronic destructive inflammatory illnesses that exhibit striking pathological and clinical parallels at the cellular and molecular levels. Those who have rheumatoid arthritis are more compared to healthy controls, are more likely to have severe periodontitis or missing teeth(Reichert *et al.*, 2013). Contrarily, those with periodontal disease were found to be more vulnerable to RA than those in good condition(Mercado *et al.*, 2000). Additionally, it has been demonstrated that the nonsurgical treatment of periodontal disease improves rheumatic problems (Cosgarea *et al.*, 2019).

Rheumatism is a set of illnesses that affect the joints, bones, muscles, blood vessels, and associated soft tissue. The majority of these illnesses are autoimmune diseases. It is generally recognized that cytokines contribute to the pathophysiology of a number of rheumatic disorders, including systemic lupus erythematosus, spondyloarthritides, and rheumatoid arthritis. Interleukin-4 (IL-4) has recently been found to have a function in the possible rheumatoid arthritis mechanism. According to reports, IL-4 plays a role in controlling the activation, differentiation, proliferation, and survival of many T cell types. Additionally, B cells, mast cells, macrophages, and many other cell types are immune modulated by IL-4.(Chen Dong *et al.*,2018).In addition, raised serum levels of immunoglobulin subclass 4 (IgG4) were demonstrated in rheumatoid arthritis (Lin and Li ,2010). IgG1 subclass allocation determined on some biochemical and immunological parameters.

Nanotechnology is science and technology involves the synthesis, characterization, and application of materials by controlling the shape and size at the nanoscale (Mazumder *et al*.,2018).Nanoparticles are being employed more frequently to target microorganisms, which stimulates a high degree of structural, morphological alteration and lead to cell degradation(Klasen,2000).Therefore ,this study aims to investigate the effect of nanoparticles on bacterial isolates obtained from dental plaque biofilm.

1.2 Aim of the study

1. Detecting the presence of bacterial *P. gingivalis* isolates.

2. Determining IgG1, IL-4 levels in healthy persons and patients with periodontitis and rheumatoid arthritis.

3. Studying the effect of broad spectrum of antibiotic against bacterial strains that causes periodontitis.

4. Determining the effect of novel materials (Nanoparticles) on Biofilm formation by bacteria isolate from periodontitis and rheumatoid patients (in vitro).

Chapter Two

Literature Review

Literature Review

2 . 1 Autoimmunity

Immune tolerance refers to the process by which the immune system detects and destroys non-self-antigens but not self-antigens (Jerne,2004). Autoimmune illnesses develop when the immune system detects and attacks host components as a result of the immune system's loss of tolerance and inability to distinguish between self- and non-self antigens . According to Ercolini and Miller ,(2009) , autoimmune illnesses can arise as a result of environmental stimuli , aging , and hereditary factors.

The two types of tolerance are central tolerance , which affects the thymus and bone marrow and results in high-affinity B and T cells going through apoptosis , and peripheral tolerance , which affects lymph nodes and peripheral organs . However , affinity-free B and T cells mature into immune cells . If tolerance is unsuccessful , autoimmune disease is caused by the interaction of a defective gene with an unfavorable environment , (Zhang and Lu , 2018) . The microbiota can influence immune cells during autoimmunity through interactions with the immune system . Host immunity is significantly influenced by microbiota , and microbiota is in turn regulated by immunity . Innate and adaptive immune responses carefully regulate and arrange this relationship (Brown , 2019).

As a result of autoimmunity , autoimmune disorders can either be severe and fatal if immune cells attack life-sustaining tissues or chronic with recurrent inflammatory symptoms. As a result of genetic and environ mental factors , autoimmune diseases are characterized by dysregulated immune responses (Wu *et al*., 2019).

There are numerous diverse autoimmune illnesses , some of which affect a single organ only and others a number of organs , each with a unique immunological disorder . The most prevalent are listed below: Multiple sclerosis ,which harms the myelin sheath; Addison's disease ,which affects the adrenal glands; Graves' disease ; Sjögren's syndrome which impacts the glands responsible for lubricating the lips and eyes, Type1 diabetes ; arthritis ;Myasthenia gravis ,which interferes with nerve impulses ; systemic lupus erythematosus ; Coeliac disease ulcerative colitis and primary biliary cirrhosis(Wang *et al* .,2015).

2.2. Arthritis

More than 100 different forms of arthritis exist, and they can affect various bodily parts . Rheumatoid arthritis , osteoarthritis , gouty arthritis , calcium pyro phosphate deposition disease , psoriatic arthritis, viral arthritis , spondylo arthritis , and other related autoimmune illnesses are among the most common forms (Roy *et al* .,2019) .

2.2.1 Rheumatoid Arthritis . (RA)

Rheumatoid arthritis , is the type , of arthritis that is most well-known (Constantinou *et al*, 2021) . A chronic B-cell inflammatory autoimmune disease with rheumatic symptoms , RA is impacted by both genetic and environmental factors , New immune system

mechanisms (innate and adaptive) that can affect RA's various stages have also been the subject of research (Croia *et al.,* 2019) .

According to the Calabro *et al.*,(1976), RA is more common among women and in industrialized countries , where its popularity fluctuates between 0 . 3% and 1%. Before this, rheumatoid arthritis affected 1% of Iraqi patients with joint problems and 1.02% of those in the province of Babylon . (AL Rawi *et al*.,1978) . Then grew in Babylon , Iraq , to 1 . 60% in 2001 and 3 . 02% in 2011 (Alkazzaz , 2013) .

Epidemiological studies have suggested that obesity may raise the risk of RA development (George and Baker, 2016) . Women were found to have greater incidence rates than men , and rates grew with advancing age (Alkazzaz ., 2013; Nair *et al.,* 2019). Initial symptoms of rheumatoid arthritis in patients frequently include pain , swelling , and hardness in several joints (Diederik *et al.,2019*) . There are two main sero groups of RA that may be identified immunologically based on the prevalence of autoantibodies: seropositive RA and sero negative RA (Terao *et al* .,2019, Nijjar *et al* .,2021).

2.2.2 Innate Immune Response

The innate immune system acts as the body's initial line of defense against foreign invaders and is essential for non-specific antigen recognition and activation of the phagocytosis process, and the release of particular chemical mediators at the invasion site . The innate immune system's specific receptors enable a variety of immune cells , including macrophages/monocytes , dendritic cells , natural killer cells , neutrophils , eosinophils , and mast cells , to function (Croia *et al.*,2019, Smiljanovic *et a l* .,2018) demonstrate that monocytes are activated in

the joint , suggesting the relevant role of monocyte for local disease-specific stimuli in RA (Smiljanovic *et al* .,2018) .

Following tissue damage , soluble mediators (proteins and chemokines) secreted by necrotic cells stimulate monocyte migration to the site of inflammation and aid in the inflammation's amplification (Cecchinato *et al* .,2018). By introducing a brand-new rheumatoid arthritis experimental paradigm, Hagert *et al* .,(2018) demonstrate how innate immunity can cause persistent arthritis without the assistance of adaptive immunity . It appears that macrophages and the complement cascade (innate immunity) cooperate critical roles in the development of RA independently of the participation of B and T cells (adaptive immunity) through the stimulation of macrophage TLR4.

2.2.3 Adaptive Immune Response

According to numerous studies , the pathogenesis of RA is significantly influenced by the adaptive immune response through a number of molecular and cellular mechanisms. The second line of defense against foreign invaders is the adaptive immune system , which plays a critical role in the precise recognition of antigens T and B cells, Wang *et al* .,(2018b)finds that down regulation of T regular cell differentiation caused dysregulation of T-regular cells in the RA Furthermore , it was found by Luo *et al*., (2018) that periodontitis expression on T cells in RA patients was significantly higher in people with a high RF titer and correlates with disease activity.

2.2.4 Pathophysiology of Rheumatoid Arthritis.

The origins and pathophysiology of rheumatoid arthritis are complex and multifaceted. Several cellular components, soluble mediators, adhesion molecules, and autoantibodies are reported to contribute to the spread of inflammatory and structural changes in joints and internal organs by innate and adaptive immunity through genetic and epigenetic factors in a(Croia et al. 2019) review of one year in RA.

2.2.5 Etiology of Rheumatoid Arthritis .

As previously stated and subsequently , RA is an autoimmune chronic disease caused by a variety of genetic and / or environmental effectors , including : epigenetics (de la rica *et al.*,2013) , microbiome change in mouth and gut (Zhang *et al* ., 2015) , psychological factors Ylmaz *et al* ., (2017) microbiome change in synovial fluid (hammad *et al* .,2019) .

2.2. 6 Epigenetic and Rheumatoid Arthritis.

The term "epigenetics " which literally translates as "over and above the genome" (epi) , was coined by Waddington in 1942 and is of Greek origin.Its definition is "the study of mitotically (and occasionally meiotically) heritable changes of a phenotype , such as the gene expression of particular cell types that do not result from changes in the genetic code.

Phenotypic variations can arise via interactions between the genome and the epi genome under environmental control such as nutrient , stress, toxicants , pathogen , and others , rather than always coming from chromosomal alteration (Norouzitallab *et al.* ,2019).

Epigenetics provides a useful method to connect genetics , the control of gene expression , and environmental variables (Klein and Gay ,2015) . DNA methylation and post-translational histone changes (de)acetylation , methylation , and sumoylation , which have been linked to the onset of rheumatoid arthritis , are examples of the epigenetic (Klein *et al* .,2012) . Additionally, epigenetics involves the dysregulation of non-coding RNAs , which is crucial to the pathogenesis of RA and offers therapeutic promise (Karami *et al* .,2020) According to the research by Ai *et al*., (2021) , there were higher methylation variations in T cells depending on the location of the RA (blood vs synovium). When cells divide , daughter cells receive the epigenetic information which can make cells more aggressive and cause cancer-like behaviors in RA Fibroblast- like synoicytes (FLSs) (Sánchez-Pernaute *et al* .,2008, Mousavi *et al* .,2021).

2.2.7 Age and Rheumatoid Arthritis.

At age 30 to 35 , bones reach their largest and strongest levels , but at age 35 to 40 , when bone "resorption" outpaces bone formation , the process of bone deterioration starts (Public Health Service , 2004) . These findings help to clarify the fundamentals of advancing age in humans associated with progressive bone loss by molecular mechanisms by elevating the production of bone-resorbing osteoclasts , which maintains the hypothesis that human marrow cells and their products can contribute to skeletal aging (Chung *et al* .,2014)

According to (Weyand *et al* ., 2003) , Immunological degradation caused by age-inappropriate modification of the T-cell population results in autoimmunity in RA. Therefore , RA patients who appear at a later age tend to have more severe joint damage (Mangnus *et al* .,2015) .

However, increased age-dependent genomic DNA damages may contribute to the development of RA and/or its increased severity (Ogawa *et al* .,2013).

2.2.8 Sex and Rheumatoid Arthritis.

Studies on autoimmunity should be planned according to gender because gender has a substantial impact on the progression of autoimmune disease (Ngo *et al* , 2014 , Ad'hiah *et al* ., 2018 , Almutairi *et al* ., 2021 ,Delay *et al* ., 2021).

Females are more prone to developing RA than males due to numerous factors including hormonal , behavioral , genetic , and environmental ones , have been linked to the higher prevalence of women with autoimmune illnesses like RA (Oliver and Silman ., 2009) . A recent article from 2018 suggests that different Sex susceptibilities to autoimmune illnesses (Cincinelli *et al* ., 2018).

2.2.9 Smoking and Rheumatoid Arthritis.

One of the environmental risk factors for RA is smoking . There are numerous hypotheses about how smoking may cause RA . Smoking increases the amount of citrullination in the lungs via increasing the peptidyl arginine deiminase 2 (PAD2) enzyme's expression (Makrygiannakis *et al* ,.2008) . Aryl hydrocarbon receptor (AHR) and its gene expression were found in peripheral blood mononuclear cells (PBMC) of rheumatoid non-smoking and smoking patients with considerably higher expression in the latter group . This was discovered by (Cheng *et al* ., 2019) . By activating AHR , smoking can worsen arthritis and promote T cell differentiation (Talbot *et al* .,2018).

2.2.10 C-Reactive Protein and Rheumatoid Arthritis .

The acute phase reactant C-Reactive Protein (CRP) , which is generated by the liver and released into the blood during inflammation (Sproston , 2018) . Tillet and Francis discovered CRP in 1930 , and it got its name from how it reacted with the capsular (C)-polysaccharide of pneumococcus . According to (Spasovski and Sotirova 2014) , CRP is an effective diagnostic for evaluating RA patients.

Al-Taee *et al* ., (2019) found that 30% of healthy controls and 70% of Iraqis with RA had positive CRP levels , CRP is effective in bony destructive process in RA by induction of expression of receptor activator for nuclear factor-κB ligand (RANKL) on peripheral blood monocytes and osteoclast precursors, after that, direct development of mature osteoclast from these cells (Kim et al., 2015)**.**

2.2.11 Medical Conditions and Complications.

Berman *et al*., (2018) were list condition and complications developed with rheumatoid arthritis under paper named emergent complications of rheumatoid arthritis" as following: complications of airway, cardiovascular, pulmonary, neurologic, renal, gastrointestinal, vascular, hematological/lymphatics, dermatological and musculoskeletal; and endocrine, head, ears, eyes, nose, and throat complication. Paget disease is a primary bone disease with adjacent joint involvement that commonly coexise with rheumatoid arthritis (Borz-Baba, 2019), on the other hand, the review of (Panoulas et al., 2008) discuss the association of increased blood pressure in RA patients. While, "An 15 Year Longitudinal Study" of (Agca et al., 2019), concluded that incidence rate of cardiovascular events in RA was more than double that of the general population. Whereas the

cardiovascular diseases is prevelance in RA and DM2, with frequencies of 12.9% and 12.4, respectively (van Halm et al., 2009)

2.2.12 Signs and Symptoms of RA.

Signs and symptoms of RA are fast onset and include: Multiple joints are affected by joint enlargement, stiffness , and pain Symptoms that are symmetrical and present on both sides of the body and Morning stiffness that lasts longer than 30 minutes(Bandyopadhyay,2018). Additional symptoms like malaise, fever , exhaustion , and appetite loss. The factors that predict the onset of RA are comparable to those that predict the persistence of undifferentiated inflammatory arthritis (De Rooy *et al* .,2010) . In patients with newly developed arthritis , various factors have been identified as predictors of persistent RA . These factors were divided into three categories: clinical characteristics , laboratory diagnostic , and imaging.

2.2.13 Diagnosis of Rheumatoid Arthritis.

In contrast to the1987American College of Rheumatology(1987 ACR) classification criteria for rheumatoid arthritis , a joint working group from the American College of Rheumatology(ACR) and the European League Against Rheumatism (EULAR) developed more sensitive classification criteria for diagnosis of RA . These criteria are based on features at earlier stages of the disorder rather than late stage features. The objective of the 2010ACR/ EULAR Criteria , also known as the 2010 ACR/ EULAR Rheumatoid Arthritis Classification Criteria is to prevent people from developing the chronic RA state (Aletaha *et al* .,2010). The classification as ‘definite RA’ in the new criteria set is dependenton the : "confirmed presence of synovitis in at least one joint, absence of an alternative diagnosis better explaining the synovitis, and

achievement of a total score of 6 or greater (of a possible 10) from the individual scores in four domains: number and site of involved joints (range 0–5), serological abnormality (range 0–3), elevated acute-phase response (range 0–1) and symptom duration (two levels; range 0–1)", table (2.1).

Table.(2 -1): Rheumatoid Arthritis Classification Criteria (Aletaha *et al .,2010)*

Classification	**Points**	**Parameter**	**Score**
Joint Distribution	**0- 5**	1)large joint(	0
		(2-10)large joints	1
		1-3) small joints , (large joints not(counted)	2
		(4-10) small joints , (large joints not counted)	3
		>10) joints , (≥ 1 small joint)(	5
Serology	**0-3**	Negative ACPA and negative RF	0
		Low positive RF or low positive ACPA	2
		Positively RF or positively ACPA	3
Symptom Duration	**0–1**	< (6)week	0
		≥(6)week	1
Acute Phase Reactant	**0-1**	CRP and ESR levels are normal	0
		abnormal ESR or CRP levels	1

2.2.14 Treatment and management strategies.

Early RA patients with early diagnosis and therapy are prevented from progressing to joint damage ; RA is treated in two ways (Sailaja ., 2014). First line: Ibuprofen and naproxen are two examples of non-steroidal anti-inflammatory drugs (NSAIDs) that are used to treat swelling , pain and inflammation in the body . However taking NSAIDs with food or taking additional drugs might minimize their gastrointestinal adverse effects , which include pain , bleeding , and ulcers . Although corticosteroids are more efficient than NSAIDs , they have substantial side effects.

Second line: represented by Disease-Modifying Anti Rheumatic Drugs (DMARDs), that act to end progressive injure to cartilage, bone, and neighboring soft tissues DMARDs categorisied into non-biologic drug (as Methotrexate) and biologic drug, which subdivided into anti-TNF(as Infliximab) and non anti-TNF(as Rituximab)(Singh*et al*., 2012).

Disease-Modifying AntiRheumatic Drugs (DMARDs), a medication interferes with signs and symptoms of RA, DMARD improves physical function and inhibits evolution of joint harm. Whereas nonsteroidal anti- inflammatory drugs or pain medications just improve symptoms and do not avoid damage progression of joints and permanent disability. These type of drugs are not DMARDs and used during the short time until establish diagnosis of RA (Aletaha and Smollen, 2018).

2.2.15 Association of cytokine patterns with rheumatoid arthritis (RA).

2.2.15. A: Interleukin-4 (IL -4) The role of interleukin-4 in rheumatic diseases.

Rheumatism is a group of diseases, most of which are autoimmune diseases, that violate joints, bones, muscles, blood vessels and related soft tissue. As is well known, cytokines play a role in the pathogenesis of several rheumatic diseases, such as rheumatoid arthritis, spondyloarthritides, and systemic lupus erythematosus. Recently, the role of interleukin-4 (IL-4), which may participate in the mechanism of rheumatism, have been discovered. It is reported that IL-4 takes part in the regulation of T cell activation, differentiation, proliferation, and survival of different T cell types. IL-4 also has an immune modulatory effect on B cells , mast cells , macrophages , and many cell types (Dong, *et al*., 2018).

2.2.15.B: Immunoglobulin G (IgG)

Immunoglobulin G (IgG) constitutes about 75% of antibodies in the human serum and is the most common type of antibody in circulation. IgG is the essential antibody for the secondary immune response. Immunoglobulins of this class exist in balance in the intravascular and extravascular space, determining adequate general protection. They also bind to monocytes and macrophages, providing a better immune response. Due to the specific structure (their chain exists in four isotypic variations), immunoglobulins G (IgG) are divided into four types (subclasses): IgG1, IgG2, IgG3, IgG4. (Napiórkowska *et al* ., 2019).

2. 3 Periodontal Disease.

Periodontitis , a chronic inflammatory illness that gradually destroys the tissues supporting the teeth , is linked to a range of chronic conditions including cardiovascular disease (Hajishengallis and Chavakis , 2021). A persistent inflammatory illness called periodontal disease causes the periodontium , or the tissues that support the teeth , to deteriorate (gingival tissue , periodontal ligament , and alveolar bone) According to(Fernández and Gutiérrez , 2018) , periodontal disease is highly prevalent , affecting about 50% of people under the age of 30 and 70% of people 65 and older . In medical words , losing teeth is the outcome of not treating periodontal disease . (Van Dyke *et al* ., 2020).

Periodontal infection is often defined by the progressive degeneration of the periodontal complex's soft and hard tissues . This process is impeded by a relationship between dysbiotic bacteria populations and a variety of immune responses in the gingival and periodontal tissues (Radaic and Kapila ., 2021). When the local oral microbiota becomes dysbiotic and the inflammatory response results in tissue corruption , putative periodontal microorganisms are increased , resulting in an ongoing positive input pattern of aggravation , proteolysis , and improved periodontal microorganisms .

In addition , recently identified genetic and immunological pathways as well as organic host factors including nutrition and way of life have been acknowledged as additional contributing factors in periodontitis (Sedghi *et al* ., 2021).

These characteristics taken as a whole have widened our understanding of how periodontal disease progresses in the traditional sense. As a result , innovative techniques for preserving periodontal health and treating disease , such as the use of oral probiotics , have been evaluated to stop and limit the spread of infection . According to (D'Ambrosio *et al* ., 2022) systemic host diseases as diabetes and autoimmune disorders have been connected to the etiology of periodontal disease.

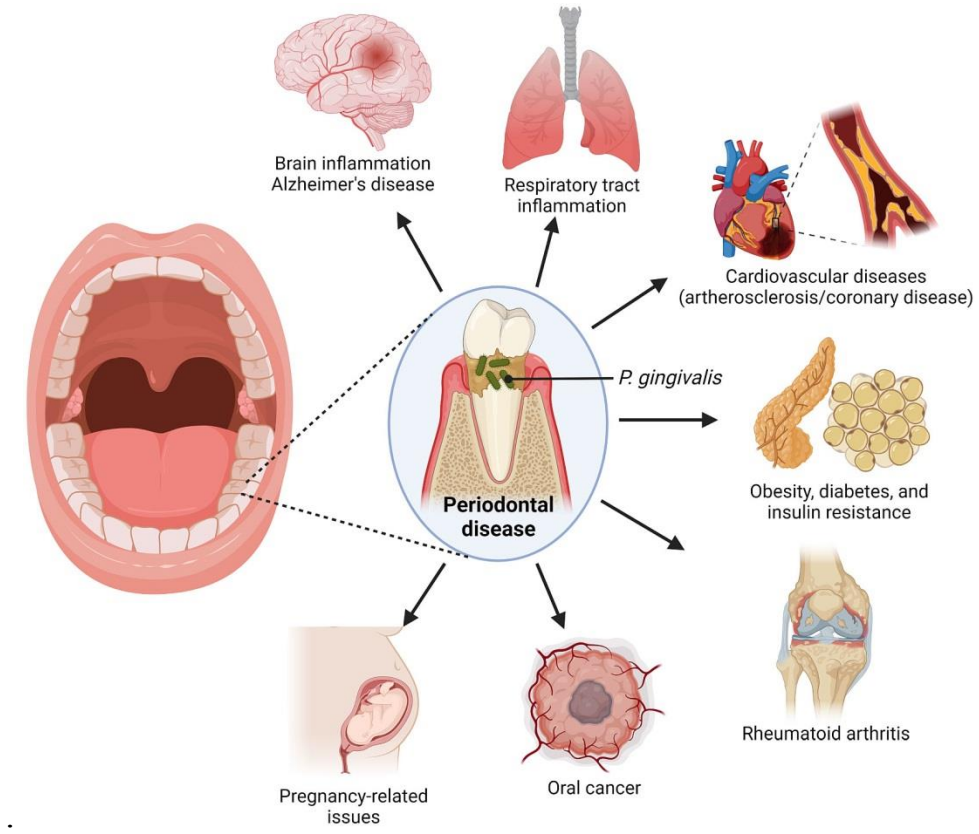


FIGURE (2 . 1) Link between *P* . *gingivalis* , an oral pathogen , with various systemic diseases . (Olsen *et al* ., 2018)

2.3.1 Periodontitis.

Periodontitis , which is a common oral disease caused by chronic inflammation of the periodontal tissues brought on by the buildup of excessive dental plaque , is seen in the figure (2 – 2)(Kushali , 2021).

Figure . (2 . 2) . Periodontitis. (Kushali , 2021).

Periodontitis should be early diagnosed to stay away from serious and unsalvageable harm to the protective and supportive structures. Nonetheless، based on periodontitis is a sign less sickness that advances gradually, hardly any people look for dental consideration in the early phases (Silva *et al* ., 2019) .

Periodontitis in early stages can be constrained by eliminating the biofilm and calculus subgingivally. Full dental cleanliness, as well as three month periodontal checkup, are basic for illness steadinessKönönen *et al*.(2019). Periodontitis common in adults and seniors around the world. In the United States, around 35% of individuals (30-90 years of age) are affected. With expanding age, the combined effects of alveolar bone loss, attachment loss, and pocket advancement become more noticeable. Periodontal harm is related with

age (Peres *et al*., 2019). The occurrence of early periodontal disease increments with age, with the most noteworthy rate happening between the ages of 50 and 60, and gingival recssion is the most well- known before the age of 40, while periodontal pocketing is the principle mode of destruction between the ages of 50 and 60. (Kornman et al., 2020).

2.3.2 Classification of periodontitis.

Three elements should be included in a system of classification for periodontitis: (Tonetti *et al* .,2018) A periodontitis case is recognized in a patient , the exact form of periodontitis is identified , the clinical course is represented , and numerous aspects that affect clinical care , prognosis and potentially more severe consequences on both systemic and oral health are reviewed.

The form of periodontitis are:

1.Necrotizing periodontal disease.

a. Necrotizing Gingivitis.

b. Necrotizing Periodontitis.

c. Necrotizing Stomatitis

2. Periodontitis as Manifestation of Systemic Diseases

Classification of these conditions should be based on the primary

systemic disease according to the International Statistical Classification of Diseases and Related Health Problems (ICD) codes.

3. Periodontitis Based on Severity and Complexity of Management, it was classified as:

a- Stages

Stage l: Initial Periodontitis

Stage II: Moderate Periodontitis

Stage Ill: Severe Periodontitis with potential for additional tooth loss

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

b- Distribution and extent: molar-incisor distribution , generalized , and localized

c- Grades: Signs of rapid advancement and predicted treatment response.

1 . Grade A: sluggish rate of development

2 . Grade B: a moderate rate of development

3 . Grade C: Fast rate of development (Herrera *et al* , 2018) .

2.3.3 Periodontitis risk factors.

Different periodontal risk factors can affect the frequency , occurrence , severity , and duration of infection progression . Smoking , poor dental hygiene , and ineffective plaque biofilm control are all serious risk factors (Wallis and Holcombe ., 2020) . The illness progresses gradually to reasonably , but the patient may have brief episodes of rapid progression (sometimes known as "explosions of annihilation") (Kwon *et al.*,2021). Local risk factors have been linked to periodontitis (for example tooth-related or iatrogenic variables) . The condition may alter and be linked to systemic diseases (such as diabetes or HIV infection) , but it may also be influenced by factors unrelated to

systemic disease , such as smoking , stress , anxiety , and depression (Renzo *et al.,2018)*.

2.3.3.1 Tobacco smoking.

Care should be taken however, when diagnosing a patient who smokes, as smoking can alter some of the results of an examination. In smokers, the gingiva are pale and fibrous and tend to bleed less while being probed due to the effect of nicotine on the vasculature by vasoconstriction them. Thus a lowered response is produced and this explains why incorrect data can be gained. There is also an increase in supragingival calculus alongside visible nicotine staining. The anterior dentition occasionally have recession and maxillary anterior and palatal surfaces are more adversely affected.

There is accumulating evidence for a higher level of periodontal disease among smokers (Idrissi Janati *et al*., 2022). Tobacco smoking exerts a substantial destructive effect on the periodontal tissues and increases the rate of periodontal disease progression. Risk factors including tobacco smoking modify the host response to the challenge of bacteria in microbial dental plaque (Ryder *et al*., 2018).

2.3.3.2 Diabetes mellitus.

Gum disease and periodontitis are significant mouth side effects of diabetes. Patients with untreated or inadequately oversaw type 1 or type 2 diabetes are higher risk periodontal sickness. Many investigations have tracked down a connection among diabetes and an expanded powerlessness to mouth contaminations, especially periodontal infection (Wu et al., 2020) Periodontitis is also associated with an increased risk of incident type 2 diabetes (Anton et al., 2021).

Mechanistic links between periodontitis and diabetes involve elevations in interleukin(IL)-1-β,tumour necrosis factor-α,IL-6(Sanz et al., 2018).

2.3.3.3 Cardiovascular disease.

Numerous studies have examined the link between periodontal disease and cardio vascular disease. Among the possible mechanisms are high cholesterol levels , the contribution of oral bacteria to atherosclerosis , and a potential increase in acute-phase proteins in chronic periodontitis (Falcao & Bullón ., 2019).

2.3.3.4 Drug-induced disorders.

Several drugs have a significant impact on salivary flow , including antimetabolites , analgesics , certain tranquilizers and sedatives , and anti hypertensives (Tripathi ., 2013) . The pH and composition of plaque are altered by various drugs , especially those that are dissolved in fluids with added sugar , which makes it easier for plaque to adhere to tooth surfaces (Herrera *et al*., 2018).

2.3.3.5 Obesity.

Obesity risk has been associated with periodontal disease . The relationship between obesity and periodontal disease can be explained in a number of ways (Li et al .,2018) . According to the information given , younger study participants may have different eating preferences from older research participants . An analysis of dietary patterns in adolescents aged 11 to 18 reveals a significant reduction in vitamin-rich foods including green leafy vegetables and potatoes .

Additionally teenagers are consuming less calcium while increasing their consumption of soda and avoiding citrus juices.This is essential for tooth health because low dietary intake of calcium and vitamin C has been associated to periodontal disease(de Almeida Bastos *et al*., 2018)

2.3.4 Periodontal pathogens.

More than 700 exceptional phylotypes are recognized in the oral bacterial microbiome, with about 400 species announced in subgingival plaque. The subgingival microflora in periodontitis can harbor hundreds of bacterial species but only a small number has been associated with the progression of disease and considered etiologically important (Buduneli,2021). Gram-negative anaerobic rods and spirochetes rule subgingival plaque from more profound periodontal pockets.

Porphyromonas gingivalis has implicated strong evidence in relation to periodontal disease progression (Niswade., 2022). Pathogenic bacteria must be able to colonize the host, avoid host
defensive mechanisms, and cause tissue damage. For many periodontal infections, mechanisms for each of these necessary phases in pathogenesis . have been found. The oral cavity is home to many different types of bacteria , which make up the oral microbiome Dahlen *et al*.,(2019).

The oral microbiome is composed of a unique and diverse ecosystem of microbial organisms that metabolically and physically interact. Such interactions result in the formation of complex biofilm communities in which physio - chemical gradients create distinct niches for microorganisms of differing metabolic needs (Berg *et al*., 2020).
Corynebacterium spp. anchor to early colonizers, such as *Actinomyces spp*. and *Streptococcus spp*. and radially extend outward to provide a

long, annulus structure. Attached at the tip of the annulus, *Haemophilus, Aggregatibacter,* and *Neisseriaceae* occupy the oxygen- and nutrient-rich periphery (Krsmanovic *et al*., 2021). Metabolic output from oxidative species at the periphery creates an anoxic environment at the biofilm center, in which anoxic capnophilic species, such as Capnocytophaga, Leptotrichia, and Fusobacterium thrive along the middle of the annulus (Sedghi *et al*., 2021). When the complex ecosystem of the oral biofilm is perturbed, microbial dysbiosis ensues. This disruption in microbial community dynamics plays a major role in the etiology of gingivitis and development of periodontal disease (Fragkioudakis *et al*., 2021).

Periodontitis is also characterized by immune dysregulation and inflammation and increased representation of periodontal pathogens that bidirectional promote one another and together drive destruction of the tooth supporting structures, including the periodontal ligament and alveolar bone (Curtis et al., 2020) . The impact of chronic inflammatory diseases at sites far from the oral cavity on periodontitis , and the emerging role of periodontitis in systemic inflammation , is also becoming recognized in the pathogenesis of periodontal disease (Meyle & Chapple ,2015)

2.3.5 Porphyromonas gingivalis (*P . gingivalis*).

P.gingivalis is one of the bacteria that form the classic„ red complex‟ described by(Socransky *et al*.,1998).It is a Gram- negative, obligate anaerobe rod, which produces black-brown colonies on anaerobic blood agar , and in the oral cavity is found mainly immersed in the sungingival microflora (Holt *et al*.,1999 , Mayorga Fayad *et al*.,2007 , Herrera *et al*.,2008). It is the bacteria most frequently found in patients with periodontal disease (Rafiei *et al*.,2017). *P. gingivalis* is pathogenic bacterium, commonly found in the human body and especially in the oral cavity, associated with periodontal lesions, infections, and adult periodontal diseases(Abitbol, 2001). It is produces several virulence factors, including outer membrane vesicles, adhesion's, lipopolysaccharides (LPS),hemolysis and proteinases (Grenier and Mayrand,2000, Grenier and Tanabe, 2010).

Arg- and Lys-gingipain cysteine proteinases are the main endopeptidases produced by *P. gingivalis* and are both extracellular and cell bound(Kadowaki *et al*.,2000). Enzymes production by this organism are responsible of the virulence of *P. gingivalis* are trypsin like proteinase, collagenase production, a series of proteases, super antigens, endotoxins, fatty acid, hydrogen sulfide, ammonium, cytolysis and hemolysis that collaborate to the destruction periodontal tissue (Haraldsson , 2005). *P. gingivalis* is able to invade oral epithelial and endothelial cells (Darveau *et al*.,1995, Socransky and Haffajee.,2005) and effectively induces pro-inflammatory cytokine production of monocytes , neutrophils , as well as macrophages. It is also able to modify the functions of immune cells in vitro and in vivo(Ulevitch and Tobias,1995 ,Baumgartner *et al*.,2002).

Porphyromonas species detected in endodontic infections include:

• *P. endodontalis.*

• *P. gingivalis.*

Table 2 . 2 Classification Porphyromonadaceae (Coykendall et al . 1980)

Domain	Bacteria
Phylum	Bacteroidota
Class	Bacteroidia
Order	Bacteroidales
Family	Porphyromonadaceae
Genus	*Porphyromonas*
Species	*P . gingivalis*

2.3.6.1 Virulence Factors.

Fimbriae , hemolysin , hemagglutinins , capsule , outer membrane vesicles (OMVs) , lipopolysaccharides (LPS) , and gingipains are among the virulence factors of *P. gingivalis* (Table 2-3) . Most strains of *P. gingivalis* contain fimbriae , which are tiny , thin filaments . They sever *P . gingivalis'* outer membrane , which encourages bacterial invasion of host cells , the production of biofilms , and bacterial adherence to host cells (Hasegawa and Nagano ., 2021) . *P . gingivalis* has both long and short fimbriae . Mfa1 protein subunits are used to build short fimbriae , whereas FimA protein subunits are used to build long ones (Hasegawa and Nagano , 2021) . Several compounds ,

including lactoferrin , staterin , fibrinogen , fibronectin , and proline-rich proteins and glycoproteins , are used by *P* . *gingivalis* fimbriae to connect to host tissues and cells (Xu w *et al*.,2019). *P* . *gingivalis* can attach to host tissues and cells through fimbriae , which it uses to communicate with other oral bacteria and create biofilm(Xu w *et al.*,2019) .

Table(2 - 3) *Porphyromonas gingivalis* virulence factors and its function (Xu w *et al.*,2019) .

Virulence Factors	**Function**	**REF**
Fimbriae	Fimbriae encourage the development of biofilms , bacterial movement , adhesion , and penetration of host cells .	(Hasegawa and Nagano , 2021)
Hemolysin	Serves to supply heme	Smalle and Olczak ., 2017
Hemagglutinin	Serves to supply heme	Smalle and Olczak , 2017 .
Capsule	Increased phagocytosis resistance is linked to encapsulation .	Xu , W *et a*l 2020
Outer membrane vesicles(OMVs)	bacteria's reactions to stress , food metabolism , other periodonto gens , and host cells	Zhang , *et a*l 2021
Lipopolysaccharides (LPS)	possess the capacity to trigger an inflammatory response	Xu , W *et al* 2020
Gingipains	provide for 85% of all proteolytic activity	Xu , W *et a*l 2020

2.3.6.2 Role of *Porphyromonas gingivalis* in periodontal disease and rheumatoid arthritis.

There is a considerable body of evidence suggesting that there is a strong link between PD and RA (Mikuls et al., 2014). Research indicated that periodontal pathogen Porphyromonas gingivalis is considered one of the etiological factors for periodontitis(Kaur *et al.*, 2013, Scher and Abramson, 2011).

P. gingivalis is one of the known pathogens that found to increase the expression of peptidylarginine deiminase (PPAD). PPAD is found to be involved in the citrullination of arginine-containing peptides. It has been thought that the production of citrullinated antigens is important for promoting the adaptive immune system which may be found to be limited in RA patients. Studies indicated that the levels of *P. gingivalis* were correlated with(ACPA)(Hitchon *et al*.,2010, Mikuls *et al.*, 2014). In addition, the same trend of findings was also found the levels of *P. gingivalis antibody* were associated with the RA-related autoantibody Mikuls *et al*., (2012), suggesting that potential role of this pathogen in RA development is supported by many kinds of attacking factors like LPS, fimbriae, and haemagglutinin. These factors are thought to enable the pathogen to deposit and targeting the periodontal pockets Hajishengallis *et al*., (2011). In addition, research indicated that the presence of extracellular cysteine proteases is important helping the pathogen relying on the immune system of the host towards its side (Guo *et al.*, 2010, Krauss *et al*., 2010). Furthermore, the destruction of antimicrobial peptides by gingipains could promote other microorganisms to form a pool in the gingiva . (Ford *et al* ., 2010, Liu *et al*., 2010).

2 . 4 Nanoparticles (NPs).

Nanoparticles (NPs) . play a crucial role in the creation of novel antibacterial substances utilized to obliterate a range of pathogenic microorganisms (Patra & Baek , 2017) . Rising pureness NPs can be produced by the laser ablation mechanism; this upper-lower position physical process depends on the size of the ancestors of the separator metal ions for the mineral atoms . In this study , NPs were created using this technique(Aadim & Jasim ., 2022) . Some of the energy of the laser pulse is reflected by the sample's surface as it reaches the surface . It should be emphasized that the reflectivity is material- and laser-dependent (Liu 2005) . The sample absorbs energy which is subsequently transferred from optical photons to electrons then to the lattice which disperses the energy throughout the material (Zhigilei *et al* ., 2009).

The energy of the laser and the quantity of laser pulses are two crucial variables that affect the color of the nano solutions . The NP concentration will rise when the laser energy exceeds 100 mJ , which will increase the absorbance (Sanchez-Ramirez , *et al 2008)* . This is consistent with Veeradate *et al* . (Piriyawong , *et al 2012*)and is caused by the surface plasmon resonance (SPR) beam's diameter decreasing as energy increases .

The resulting NP is of high concentration and huge nanosize Some of the laser's energy is reflected by the sample's surface when it comes in contact with it . It should be noted that the wavelength of the laser and the material have an impact on reflectivity(Liu , 2005) . The energy that the sample absorbs is sent from the optical photons to the electrons and subsequently to the lattice , which disperses the energy throughout

the material (Zhigilei *et al* ., 2009) . Atoms and molecules may be drawn away from the surface as a result of photochemical reactions brought on by the pulses of extremely high energy . In these conditions , the evaporation process moves quickly because the surface temperature might become close to the critical temperature (the level of evaporation). Plasma , which is made up of ionized atoms and electrons is created during evaporation . A small portion of the incident laser energy may be absorbed by the plasma cloud at subsequent pulses , allowing that energy to briefly reach the metal's surface.

By absorbing successive photons , plasma expands into a bubble shape , heats up , bursts , and then the vapor of the substance and the scattered particles (Aerosol) cool in the liquid medium and start to form The remaining energy diffuses into matter via heat conduction . The steps of the laser ablation process can be broken down into the following categories . (Liu , 2005; Zhigilei *et al* ,2009).

Chapter Three

Materials and Methods

3 . Materials and Methods

3 . 1 . Study subjects.

This study is carried out in the Collage of Density at University of Babylon and Merjan Teaching Hospital, Rheumatology Unit in (Babylon city from (November 2022 to May 2023), patients with periodontitis and rheumatoid arthritis (80 patients male and female) with age range (20-60) years. 80 patients were grouped into two subdivision (each 40) included ,40 patients with periodontitis only, 40 patients with periodontitis in addition to rheumatoid arthritis .The control group included 40 people apparently healthy (males and females). All subjects in this study had been taken an agreement to participate in this study , depending on the clinical and serological parameters according to 2010 ACR/ EULAR criteria .

3 .2 Inclusion Criteria

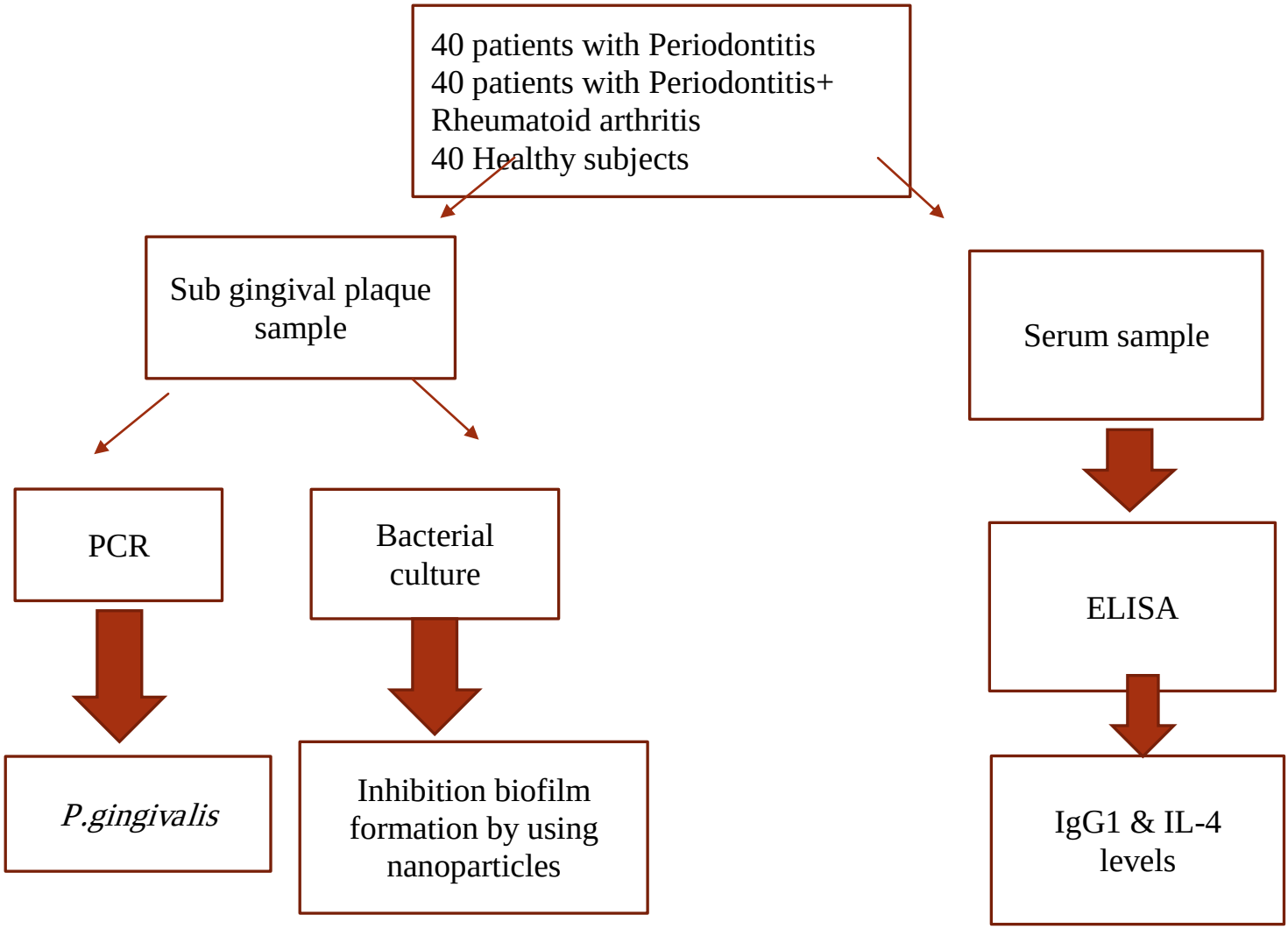
- Patients with periodontitis and at least 16 teeth , with pocket depth ≥ 4mm and/or with clinical attachment loss
- Patients with rheumatoid arthritis in addition to periodontitis.

3 .3 Exclusion Criteria

- Patients that are take antibiotic therapy in the last 3 months.
- Pregnant women.
- Smoker

3 . 4 Study Design

The steps of study are mentioned in figure (3 . 1) .



Figure(3-1): Scheme of main steps of the current study**.**

3 . 5 Materials

3 . 5.1 General laboratory Equipments

The primary technical instruments used in this work Tables (3-1) .

Table (3-1): Materials and equipment used in the study.

Equipment or Instrument	**Company**	**Country**
Autoclave	Stermite	Japan
Balance	Radwage	Poland (EU)
Burner	Turkey	UK
Centrifuge	Memmert	Germany
Candle jar	OxideLTD	Japan
Deep Freeze	GFL	Germany
Different size tips	Meheco	China
Disposable syringe of 5ml	Medical ject	Syria
Distilled water		Iraq
ELISA Reader	Biotek	USA
ELISA Washer	Biotek	USA
Filter paper		China
Gel electrophoresis	Bioneer	Korea
Incubator	Memmert	Germany
Inoculating Loop	Memmert	Germany
Light Microscope	Stermite Olympus A	Japan
Microwave oven	LG	Korea
Micropipettes 0 . 5-10µl , 10-100 µl , 10-100 µl	Oxford	USA
Para film	Bemis	USA
PCR	Clever	USA
Refrigerator	Concord	Italy
Vortex	Heidolph	Germany
Water bath	GFL	Germany
Wooden stick	Supreme	China
Williams' periodontal probe advanced		

3. 5 .2 Chemicals

Table (3-2) lists the biological and chemical substances that were used in the investigation

Table (3 - 2): The chemical and biological materials

Item	Company	Country
Agarose	Himedia	India
Ethanol (95%) , (70%)	Scharlau	Spain
Ethidium bromid	Bio basic	Canda
Glycerol	B . D . H	England
Gram stain kit	Crescent	KSA
Normal saline	Pharmaline	Egypt
Oxidase reagent (Gordon-Mcleod reagent)	Himedia	India
TBE Buffer 10x	Condo	USA
Blood agars	Liofilchem	Italy
Mueller Hinton agars	Liofilchem	Italy
Phosphate buffer	Liofilchem	Italy
Brian heart infusion	Liofilchem	Italy

3.5 .3 . Antibiotic Disc.

Table (3-3) lists the antibiotic discs that were utilized in this study.

Table (3 – 3) Antibiotics disc utilized in the study.

Antibiotics	**Abbreviation**	**Conc . mcg**	**Company**
Clindamycin	CD	2	Liofilchem/ Italy
Amikacin	AK	30	
Imepenim	IMP	10	
Penicillin	P	10	
Amoxicillin	AML	25	
Erythromycin	E	15	
Azithromycin	AZM	15	
Oxacillin	OX	1	

3 . 5 . 4 Biomarkers kits:

Tables (3-4): Immunological kits.

NO	Type of kits	Company	country
1	IL-4	BT LAB (Catalog No:E-0092Hu)	China
2	IgG1	BT LAB (Catalog No :2038Hu	China

3 . 6 . Methods

3 . 6 . 1 Collection of Samples

3 . 6 . A. Sub Gingival Plaque Sample

A total of 360 subgingival plaque samples were collected , three samples were gathered from every subject The sample collection takes place at two locations from each periodontitis patient .Sites with the deepest pocket depth and clinical attachment loss were selected for sample collection . The test sites were gently cleaned of supragingival plaque and then air-dried and maintained dry using cotton rolls, sterile paper points size(30) mm were placed in the chosen location (periodontal pockets or gingival sulcus) about 30 seconds to extract the subgingival plaque. Any paper point contaminated with blood was thrown away . The paper's stance from every sampling location was put right away into a microfuge tube with 1ml of phosphate buffered saline in it. The samples for the PCR analysis were then stored at -20 C° .

3.6.B Periodontal Examination Parameters

3.6.B.1 Bleeding on Probing (BOP)

Bleeding on probing (BOP) is a clinical indicator of periodontal disease development and stability . BOP has been utilized as a clinical indicator to establish the presence of periodontal disease in the past and its development (Joss *et al* ., 1994) .

3.6.B.2 Plaque Score (PS)

To disclose the tooth surface , a revealing solution was painted on when the patient thoroughly cleans his mouth . In the absence of plaque , the individuals received a score of zero , and in the presence of plaque , a score of one was given (O'leary *et al* .,1972) .

3.6.B.3 Clinical Attachments Loss (CAL) .

The cement- enamel junction(CEJ) and the bottom of the periodontal pocket are measured linearly by CAL , according to(Eke *et al* .,2015) .

3.6.B.4 Probing Pocket Depth (PPD)

A measurement of the distance from the gingival margin to the point where the probe encounters physical resistance from the connective tissue attachment is provided by probing pocket depth (PPD) (Reddy , 1997).

3.6.C . Blood Samples

Serum separation takes just a few minutes , thus 3 ml of blood were centrifuged for 5 minutes at 4000 rpm in a gel tube . For the purpose of determining the concentrations of IgG1 and IL-4 , three Eppendorf vials of serum were separated then frozen at -20 C° .

3.7 Bacteriological Study

All samples were obtained using paper points size (30) and transferred for PCR and bacteriological investigation. In terms of bacteria , This procedure comprised several consecutive phases , beginning with culturing on blood agar . The colonies on blood agar plates turned black due to heme buildup after 6 to 10 days of incubation at 37 C° in candle jars with CO2 (Shah and Collins , 1988). The samples were then stained with Gram stain and inspected under a light microscope Following that, biochemical tests were used to identified bacterial isolates .

3.7.1 Blood Agar Medium

It was initially made by mixing 40gm of Blood Agar with 1L of D . W, autoclaving it for 15 minutes at 121 C° , letting it cool to 50 C° , and then adding 5% of sheep blood To culture bacterial isolates and test them for blood hemolysis using this medium (McFaddin , 2000) .

3.7.2. Müller-Hinton Agar Medium

Müller-Hinton agar medium was prepared according to the manufacturing company. It was used in anti-bacterial susceptibility testing (MacFaddin, 2000).

3.7 3 Antibiotic activity test of nanomaterial

Suspected isolates were cultured on Mueller Hinton Agar to determine how susceptible they were to different antibiotics. A (0 . 1ml) of bacterial suspension was added to the culture mixture in three replicates , spread with a spreader , and put in Petri dishes . The diameter of the inhibitory zone was then measured after the plates were cultured for (24hours at 37C°) with four holes drilled into the culture media using a cork borer. (Brown & Kothari ,1975).

3.7.4 In vitro antimicrobial susceptibility testing (AST)

Patient isolates of *Porphyromonas gingivalis*, are subjected to antimicrobial susceptibility testing (AST). After subculturing, bacterial suspensions were prepared from pure cultures were inoculated onto Blood Agar with 5% sheep blood supplemented with haemin and vitamin K1 (Oxoid™/Fisher Scientific) . Antibiotic discs (Oxoid™/Fisher Scientific) for *in vitro* susceptibility testing contained 25 μg amoxicillin (AML), 2 μg clindamycin (DA), 15 μg azithromycin (AZM), 15 μg erythromycin, penicillin (P)10 μg Amikacin 30 μg(AK) Imepinim (IMP) 10 μg oxacillin (OX)μg After 3–5 days under anaerobic conditions zone diameters were measured with a calliper and interpreted according to (Dubreuil, 1995; Podbielski et al., 2007). Zone diameter breakpoints for resistance were defined accordingly, for example, susceptibility was defined if zone diameters were equal to or lay above the following: amoxicillin ≥24 mm, , azithromycin ≥20 mm, clindamycin ≥15 mm (Dubreuil, 1995).

3.8 Immunological Study

Immunological study is carried out on the same patient by collecting blood sample to be used for ELISA to determine the (IL-4 and IgG1) concentration according to study design .

3.8.1 ELISA technique

ELISA was achieved according to the method described by the manufacturing company (BT LAB /China).

A .Principle of ELISA

Sandwich-ELISA is the technique used in this ELISA kit. An antibody specific to human IgG and IL-4 has been pre-coated on the micro-ELISA plate included in this kit. The micro-ELISA plate wells are filled with stands or samples, which are then mixed with the appropriate antibody. Then, each microplate well receives an addition of an Avidin-Horseradish peroxidase (HRP) combination and a biotinylated detection antibody specific for human IgG1 and IL-4 before being incubated. Free elements are removed by washing. To each well, the substrate solution is applied. The only wells that will be blue in color are those that have human IgG1, IL-4, biotinylated detection antibody, and Avidin-HRP conjugate. By adding stop solution, the enzyme substrate reaction is stopped, and the color changes to yellow. When evaluated spectrophotometrically at a wavelength of 450 nm +/-2 nm, the optical density (OD) value is proportional to the levels of IL-4 and human IgG1. Human IgG1 and IL-4 concentrations can be calculated. by measuring the difference between the sample's OD and the standard curv**e.**

B . Protocol of ELISA

1. The reference material or sample was injected into each well in an amount of 100 microliters , and it was incubated at 37C° for 90 minutes .
2. One hundred microliter of biotinylated detection Ab was added to remove the liquid and incubate for 1 hours at 37C°.
3. The plate was aspirated then washed for three times .
4. one hundred microliter was added of HRP conjugate . Incubate for 30 minutes at 37C°.
5. The plate was aspirated then washed for 5 time .
6. Substrate reagent in an amount of 90 microliters was next added . at a temp of 37C°for 15 minut .
7. Stop solution containing 50 microliters was added at 450nm , the OD value was calculated .

3.9 Evaluation of the Results

The average OD for each standard was plotted on the vertical axis against the concentration on the horizontal axis , and the best fit curve was created using the points on the graph . Regression analysis can provide the best fit line , and computerized curve-fitting tools can simplify these computations .

3.10 Molecular Study

3.10.1 DNA Extraction

The genomic DNA purification Kit provided by the manufacturer Bioneer served as the basis for this method's creation (Korea). The acquired chromosomal DNAs served as the templates for all PCR experiments . A Thermal Cycler was used to carry out the PCR reaction The following procedure was followed to perform a DNA profile prior to the PCR experiment utilizing bacterial DNA and loading buffer without thermal cycling conditions:

1 . A 1 . 5 l micro centrifuge tube was filled with 10 mg of minced tissue .

2 . The sample was thoroughly lysed after being treated at 55 C° for 3 hours with 100 ml of LB14 and 20 ml of proteinase K (the majority of tissues needed longer) .

3 . It was carefully inserted into a clean micro centrifuge tube after being centrifuged at (12 , 000g)for5 minutes .

4. A 500 l addition of BB14 .

5 . After centrifuging the entire mixture at 12 , 000 g for 30 seconds , it was put to a spin column and the flow-through was discarded .

6 . After adding 500 cc of WB14 , step 5 was once again performed .

7 . It was centrifuged for two minutes at 12 , 000 g to eliminate any remaining WB14.

8. The 1 . 5-l micro centrifuge tube was sterile and used to contain the spin column.

9 . After that 30-100 ml of sterile distilled water (PH>7 . 0) or Elution Buffer (preheated to 65 c) were poured to the column's center . The column was then centrifuged at 12 , 000g for 1 minute to elute the isolated DNA after being incubated at room temperature for 1 minute .

10 . Step 9 was repeated once to recover additional DNA , After that DNA isolate was kept at -20 C° .

3 . 10 .2 . Determination the Purity and Concentration of DNA:

The extension coefficient of dsDNA at 260 nm which is 50 g/ml , is evaluated using a spectrophotometric technique to determine the optical density of the DNA . The purity of a DNA solution can be determined by the ratio of OD260/OD280 , which ranges from 1 . 8 to 0 . 2 for pure DNA (AL- Huchaimi *et al* ., 2018) .

3 . 10 .3 . Specific Primers Used in the Current Study:

Table (3-5): Specific Primers sequence of *P . gingivalis* (Slots *et al,1995)*

Primer name		(Sequence 5′_3**)**	Product size. bp	Reference
GIN	**F**	**5′AGG CAG CTT GCC ATA CTG CG-3′**	404	(Slots *et al)* .
	R	**5′ACT GTT AGC AAC TAC CGA TGT-3′**		

Table (3 – 6) PCR Cycle for *P. gingivalis*:

Stages	Temperature(° C)	Time(min)	No. of cycle's
1- First Denaturation	94	5	1
2-Denaturation	94	30 sec	45
3-Annealing	58	45 sec	
4-Extension	72	30 sec	
5-Last extension	72	5	1

3 . 10 .4 . Reaction Mixture for the PCR

DNA fragments are amplified during the polymerase chain reaction (PCR) using the Maxime PCR Premix Kit (i-Taq) from Intron bio/Korea . The PCR tube already includes the i-Taq DNA polymerase , dNTP mixture , and reaction buffer combined as given in the table (3–7) . The template DNA , forward and reverse primers , and Nuclease free water are then added .

Table (3-7) : The Reaction Mixture's Contents .

Reaction Mixture's Contents	**Volume** µl
Mix Master	12 . 5
Template DNA	8 . 5
Forward primer	2
Reverse primer	2
Total volume	25

3 . 10 .5 . Polymerase Chain Reaction PCR Condition

Using particular primer pairs , the DNA fragment is amplified using the traditional PCR process . Denaturation , annealing , and elongation are the three phases that normally take place in order in PCR to produce the desired output , or "amplicon" The table displays the PCR thermal cycling parameters . By electrophoresis in 1 . 5% (w/v) agarose gel to 1x TBE buffer and staining with ethidium bromide , the total size of the PCR products (5 l) was determined . By comparing the length of the product to a 100 bp DNA ladder , the product's size was calculated (Slots *et al,1995)*.

3 . 10 .6 . Gel Electrophoresis agarose

The best method for dividing DNA fragments is agarose gel electrophoresis . Gels can include (0 . 5% to 2%) agarose , depending on the sizes of the DNA fragments that need to be separated , Following extraction the genomic DNA (5–10 kb) was successfully separated using a 0 . 7% gel and the tiny PCR product fragments(0 . 2-1 kb) were successfully resolved using a (1 . 5%–2%) gel . However 100 ml of 1 TBE buffer was mixed with the same amount of agarose and cooked in the microwave until the mixture was clear . A final concentration of (0 . 5 g/ml) was achieved by adding 5 l of simply safe dye (10 mg/ml) to 100 ml of melting agarose gel after the agarose had cooled to (50–55)C° (Sambrook & Rusell , 2001) .

3 . 11 Nanoparticles study (NPs) .

3. 11.1 Nanoparticles synthesis.

3.11.2 Laser ablation and particles formation of nanopartices

The work was carried out on a silver chip with copper oxide in water (DDDW) , where the wavelength of the (Nd-YAG) pulsed laser (1064 nm) was used after laser focalization by a specular lens and in accordance with the threshold limit for the silver material and the fatigue limit for the taken copper oxide material , which are 80 mJ for silver and (100 millijoules) for copper oxide , and the frequency for the two materials is 5)) Hertz , as the frequency is When the (5) ml of water (DDDW)-immersed metal surface is struck by laser pulses , In the region of influence , a cloud will be produced with a powerful vibration wave that travels in all directions . A visible cloud of metal particles that adsorb beyond the metal's surface and scatter throughout the liquid is created by this cloud , which also generates light and sound . It was observed that as laser power or pulse count are increased , the color of the solution changes because this increases the concentration of nanoparticles , which in turn increases the intensity of the color of the solution . where copper oxide and silver nanoparticles were produced in the water , There are two significant elements that affect the color of nano solutions:

The number of laser pulses and laser energy come first and second . The concentration of NPS will grow as the laser energy is increased from (100ml) , and this results in an increase in absorbance , which is consistent with the reference Sanchez-Ramirez *et al*.,(2008). Increasing the number of laser pulses also results in this , The solution's color also changes , and when this happens , it indicates that the concentration of nanoparticles in both materials has increased .

Furthermore , when materials are converted into nanoparticles using high laser energy , the resulting NPS has a high concentration and a large nano size . This is because in line with the finding of the studyby(Piriyawong *et al.*, 2012) . the surface plasmon resonance (SPR) beam width decreases as energy increases (figure 3 - 2) .

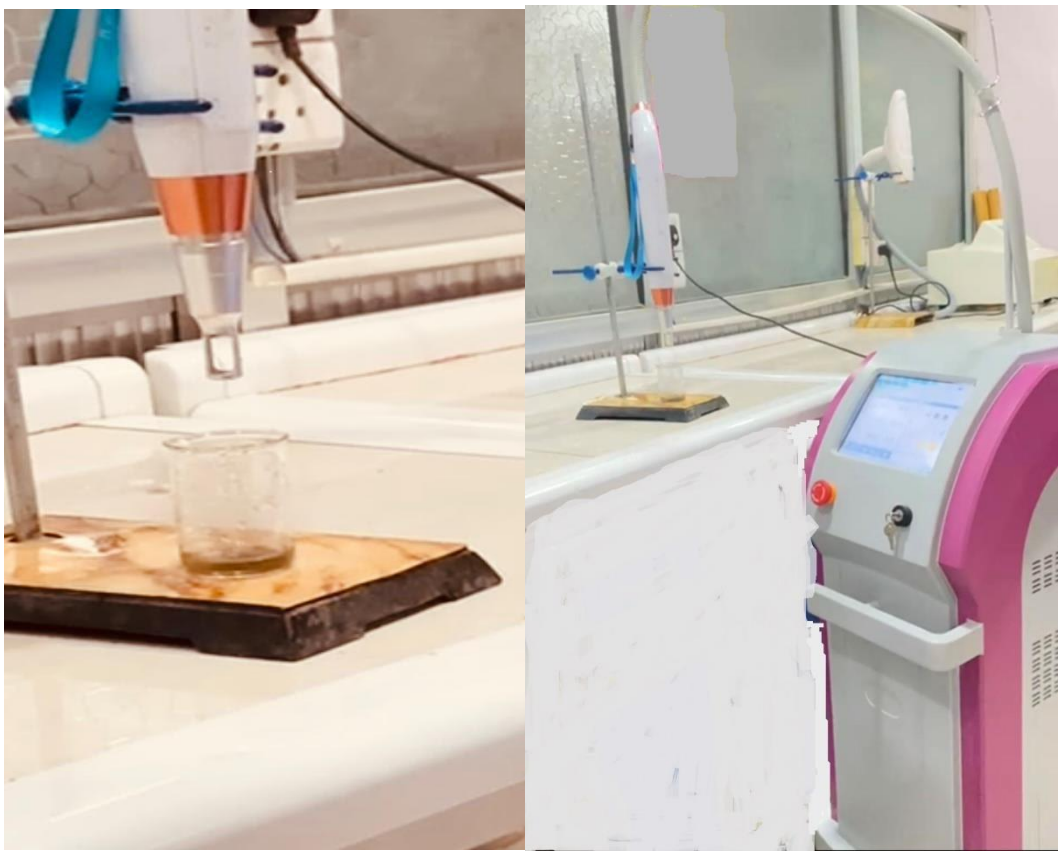


Figure (3. 2):. Preparation of nanoparticles by pulsed laser method in the current study .

3.11.3 Scanning the nanoparticles.

A 100 µl of the nanoparticles solution was laid on the aluminum slide and dried in a dark place at room temperature and then tested with SEM (Echlin ,2011).

3.11.4 Biofilm formation before adding nanoparticles .

A nutrient - rich medium called(Luria broth) (LB) is widely used to culture previously isolated bacteria in experimental situations . Isolated bacteria are cultivated for 48 hours . Isolates were dyed with (0 . 5%crystalviolet) after being fixed , at 60°C . Five isolates , each with around three copies , were put into the wells of a micro titer plate . The inverted micro titer plate was then used to remove any cells that had not attached . Micro titer plates are placed on absorbent paper , flipped over , and gently beaten to remove non-adherent cells from the paper's surface . Excess stains are then washed away with water , and the micro titer plates are then dried for (30 minut) (at37°C) With distilled water , the extra dye was washed away . The amount of biofilm was determined by measuring the absorbance of stained adherent film following treatment with acetone ethanol (20:80) using an ELISA instrument at a wavelength of (490 nm) (Schwedler C *et al., 2018)* At 490 nm , the dye's absorbance was determined . According to the optical density , mild , moderate , and strong biofilm producers for the bacterial species *P . gingivalis* were categorized as such (Stepanovic *et al* .,2004).

3.11.5 Biofilm formation after addition nanoparticles .

Each bacterial strain was given 100 µl of each of the NPs (Ag) , (CuO) and (Ag+CuO) , which were repeatedly injected into an ELISA apparatus with a weave length of 490 nm to form a biofilm (Muhammad *et al* ., 2022) , The four categories into which strains can be categorized are:nobiofilm producer , weak biofilm producer , moderate biofilm producer , and high biofilm producer (Stepanovic *et al* ,2004) . for a more simple analysis of the information .

3. 12 . Statistical analysis

All data were processed and analyzed with the statistical package SPSS version 23,2021. Results are expressed as (mean SD) person coefficient test was used to estimate the correlation between biomarker IL -4 and IgG1 with control . P-value below 0.05 are considered to be statistically significant.

Chapter Four

Results

4 . 1 Descriptive statistics of the demographic data .

4 . 1 . 1 Gender Distribution:

For this study, 120 participants were split into 3 main groups. In the first group, 40 participants had rheumatoid arthritis and chronic periodontitis, in the second, simply chronic periodontitis, and in the third, healthy controls (neither rheumatoid nor periodontitis). Ages of participants ranged from 20 to 60, 8 of the 40 patients in the RA+P group were male , while the other patients were female , making up (80%) of the total patient population . Likewise , there were twelve females and twenty-eight males in the P group , which led to the conclusion that men dominated this group and made up (70%) of the total population . Twenty (20) men and twenty (20) women made up the control group Table (4 . 1) and Figure (4 – 1).

Table (4 – 1) Male to female ratio: the groups under study**.**

Gender	**Statistical Analysis**	**Periodontitis +Rheumatoid arthritis Groups N(40)**	**Periodontitis Groups N(40)**	**Control Groups N(40)**
Male	**No .**	**8**	**28**	**20**
	Percentage%	**20**	**70**	**50**
Female	**No .**	**32**	**12**	**20**
	Percentage %	**80**	**30**	**50**

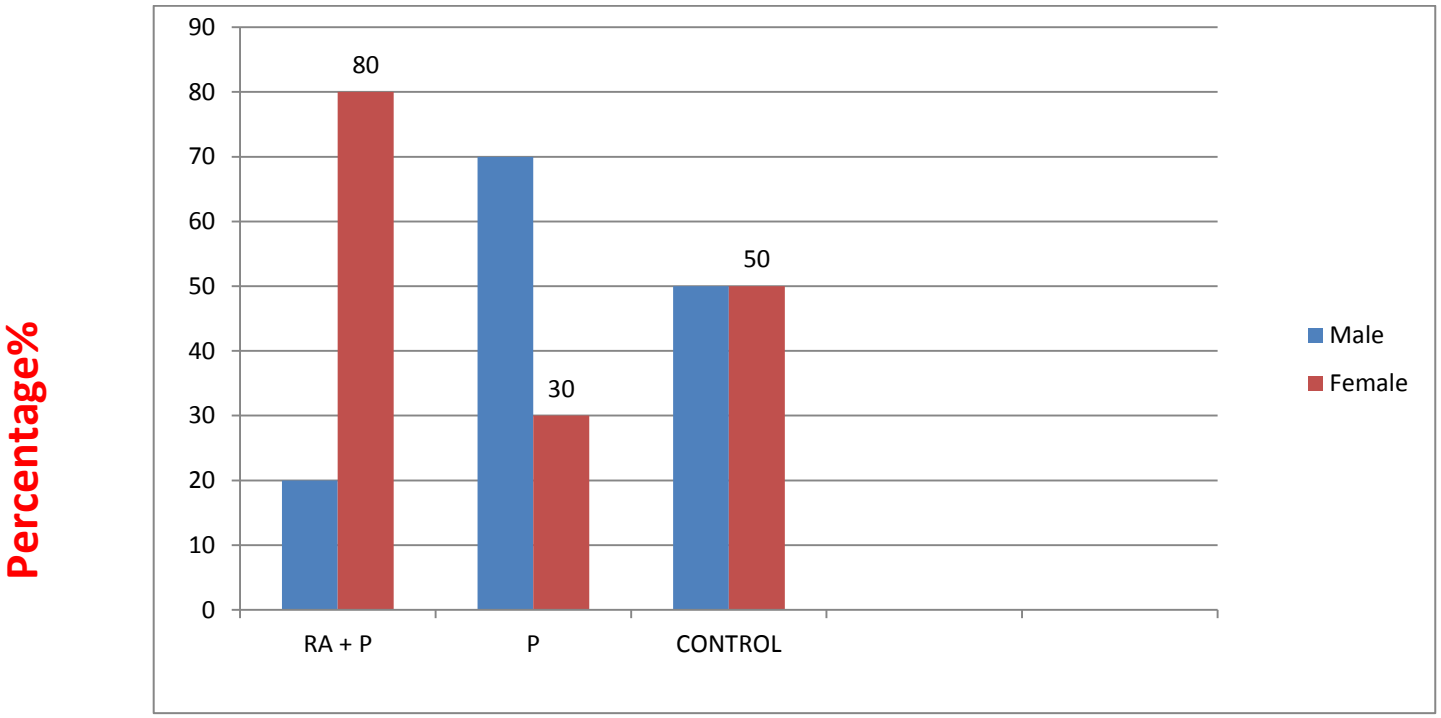


Figure 4-1: Bar chart for the percentage of males and females among the study groups .

4 . 2 Immunological Findings .

4 . 2 .1 Findings IL – 4 and IgG1

Table 4-2 demonstrate the levels of IL-4 and IgG1 among the study groups that involved in the current study . As it shown, there was no significant difference P>0.05 in the biomarkers levels among each group separately in all ages. In the current study , The findings demonstrate that healthy individuals have high levels of IgG1 . compared to those with periodontitis and those with periodontitis and arthritis , regarding the IgG1 levels in healthy people , it is 2 . 46±0 . 22 , 2 . 32±0 . 3 in the age groups 31:40 , 51:60 , respectively . While the levels of IgG1 are reached 2 . 27±0 . 09 , 2 . 25±0 in age group 41:50 and 20:30 respectively . In the patients with periodontitis , the age group 41:50 for people with periodontitis showed the highest level of IgG1 , reaching 2 . 18±0 . 55 , While the lowest level of IgG1 was for the age group 51:60 , reaching 1 . 77±0 . 77 , While the levels of IgG1 reaching 1 . 98±0 . 64 , 1 . 85±0 . 85 in the age group 31:40 , 20:30 respectively . In patients with rheumatoid and periodontitis , IgG1 levels reached 2 . 28 ± 0 . 13 in the 31:40 age group , while the lowest level of IgG1 in the age group 20:30 , 41:50 , 51:60 reaching 2 . 19±0 . 23 , 2 . 61±0 . 19 , 2 . 268±0 . 33 Table(4 – 2).

According to the findings of the current investigation , IL4 levels were higher in healthy individuals than in those with periodontitis and those with periodontitis and arthritis . 2 . 3±0 . 28 , 2 . 06 ± 0 . 55 , 2 . 05±2 . 341 . 82 ± 0 . 77 in the age groups 41:50 , 20\30 , 51:60 , 31:40 respectively . In contrast between periodontitis patients and patients with periodontitis and arthritis together ,

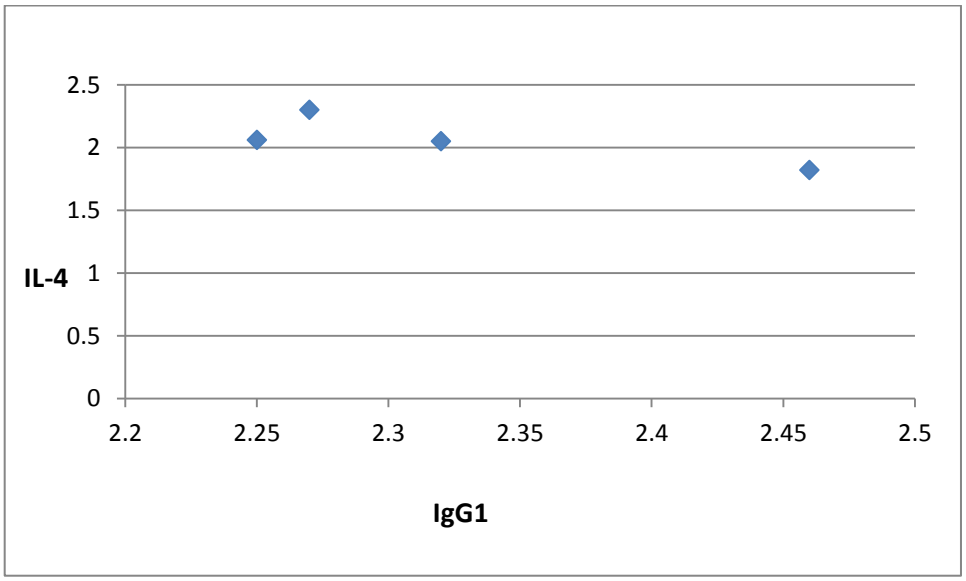
where the highest levels of IL4 were 0 . 95±0 . 56 in periodontitis patients within the age group 31:40 , followed by the age group 41:50 , 51:60 , 20:30 are reached 0 . 89±0 . 29 , 0 . 87±0 . 28 , 0 . 86±0 . 22 respectively . the results showed that the levels of IL4 were the lowest in people with periodontitis and arthritis together compared to healthy people and those with periodontitis , it is reached 0 . 70±0 . 09 , 0 . 73±0 . 17 , 0 . 77±0 . 20 , 0 . 82±0 . 32 in the age groups 20:30 , 31:40 , 51:60 , 41:50 The levels of IL-4 obtained from patients in age groups between 20-40 was significantly higher $P<0.05$ in control group rather than other study groups . On the other hand, no significant difference detected in IL4 levels among 41-60 years age groups. (Table 4 – 2).

Table(4 – 2) The levels of IL-4and IgG1 in Healthy, periodontitis, Rheumatoid and Periodontitis group.

Type of ABs	Groups	Age interval (Mean ± SD) Pg / ml				P value
		20-30	31-40	41-50	51-60	
IgG1	Periodontitis	1.85±0.85	1.98±0.64	2.18±0.55	1.17±0.77	0.555
	Rheumatoid arthritis and periodontitis	2.19±0.23	2.28±0.13	2.26±0.19	2.26±0.33	0.931
	Control	2.25± 0.31	2.46± 0.22	2.27 ±0.09	2.32 ±0.3	0.027*
IL-4	Periodontitis	0.86±0.22	0.95±0.56	0.89±0.29	0.87±0.28	0.943
	Rheumatoid arthritis and periodontitis	0.7±0.09	0.73±0.17	0.82±0.32	0.77±0.2	0.721
	Control	2.06± 0.55	1.82 ±0.77	2.3 ±0.28	2.05± 0.34	0.339

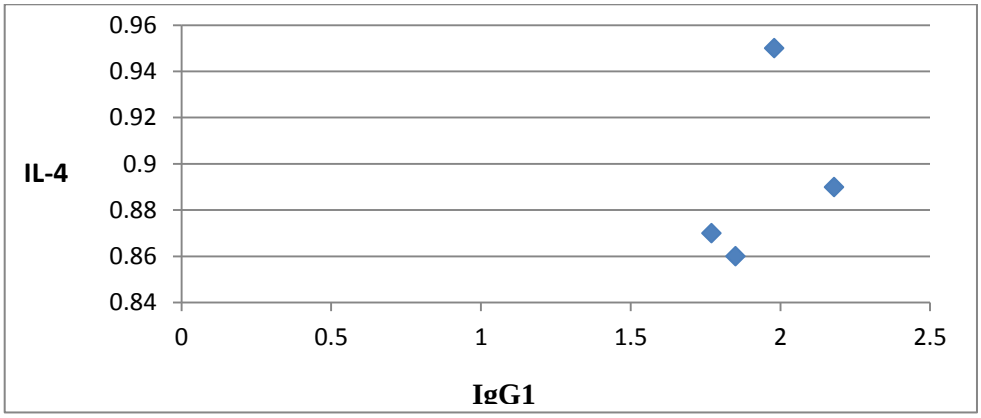
* L. S. D = 0.789 at $p \leq 0.05$

In the figures (4 - 2) (4 - 3) and (4 - 4) were shown negative Correlation a relationship between IL-4 and IgG1 in Control which V= -0.8 1798, while positive correlation between IL-4 and IgG1 in Periodontitis as well as in periodontitis with rheumatoid, which r = 0:3904 and 0.5586 respectively.

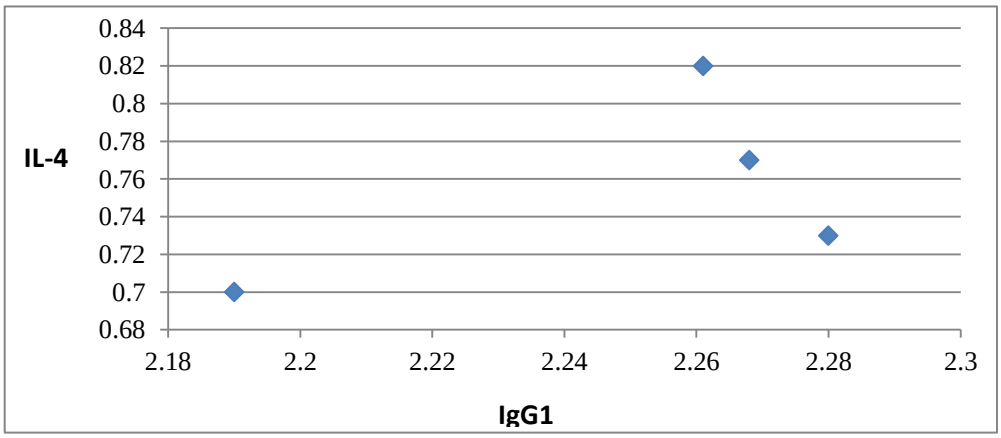


Figure(4-2): Correlation coefficient between IL-4 and IgG1 in healthy group (control).

*r= -0.81798375



Figure(4-3): Correlation coefficient between IL-4 and IgG1 in Periodontitis . *r= 0.3904



Figure(4-4): Correlation coefficient between IL-4 and IgG1 in Rheumatoid arthritis and Periodontitis . *r= 0.5586

4. 3 Molecular finding .

PCR , It is method should allow prompt and accurate identification of bacteria . The principle of the method is simple , when a pure PCR product of the 16S gene is obtained , sequenced , and aligned against bacterial DNA data base , then the bacterium can be identified. In the current study , *P . gingivalis* was diagnosed by PCR as it was isolated from swap of periodontitis group , periodontitis & rheumatoid arthritis group and healthy group .

In the figure (4 - 5) shows that the isolates (9 , 14 , 15 , 16) showed positive results for *P* . *gingivalis* , While (12 , 13 , 17 , 18 , 19 , 20) isolates are showed negative results for *P* . *gngivalis for periodontitis* group .

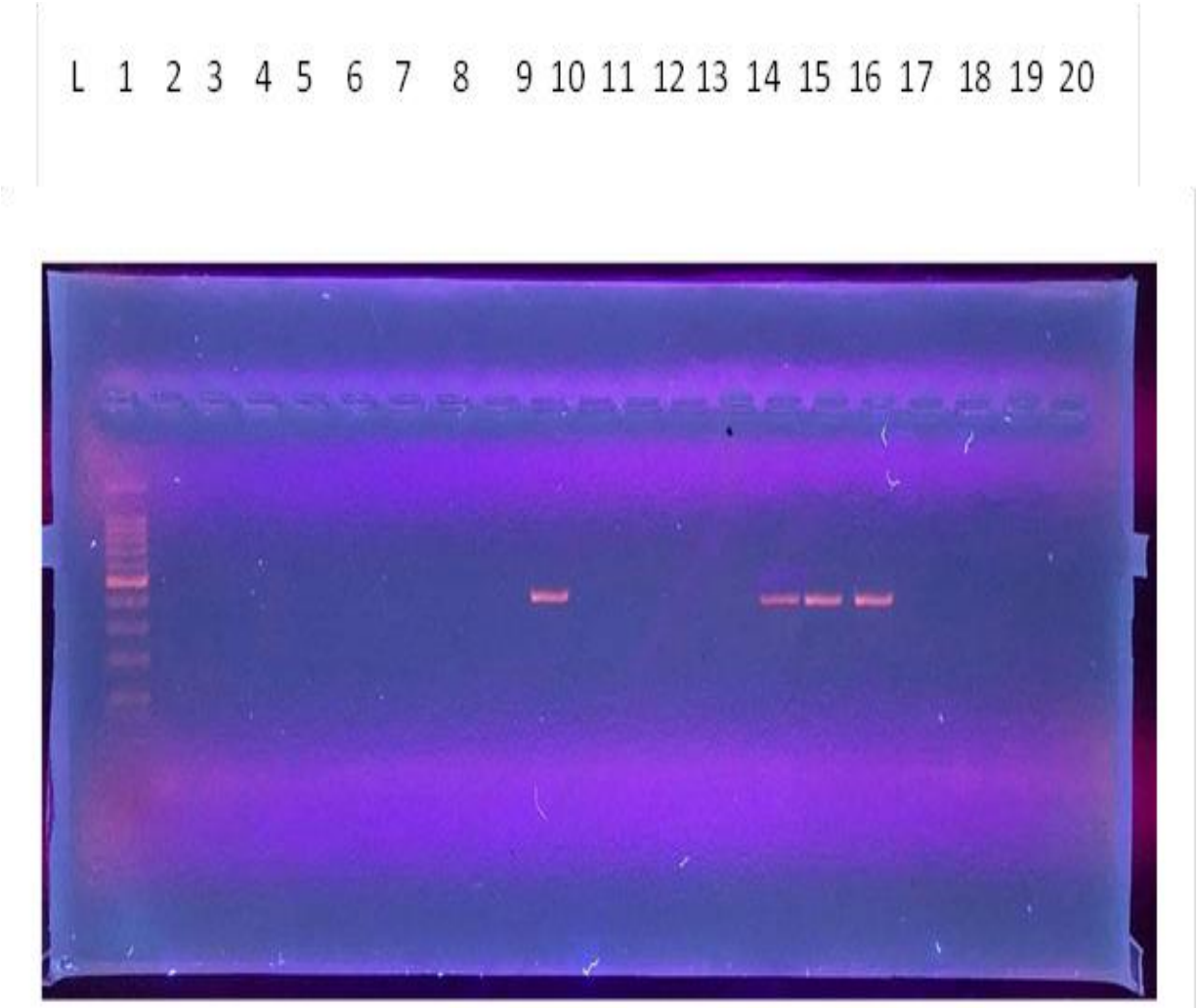


Figure (4 – 5) . Agarose gel electrophoresis (Ladder = 100 – 1000) , (pb404) . Agaros ; 1% , volt ; 80 , mA ;20 , time; 80 min of extracted DNA of *P* . *gingivalis*

In the figure (4 - 6) the isolates (3 , 6 , 13 , 14 , 18) showed positiveresults for P . gingivalis , While (1 , 2 , 4 , 5 , 7 , 8 , 9 , 10 , 11 , 14 , 15 , 16 , 17 , 19 ,20) isolates are showed negative results for *P . gngivalis* for Healthy group.

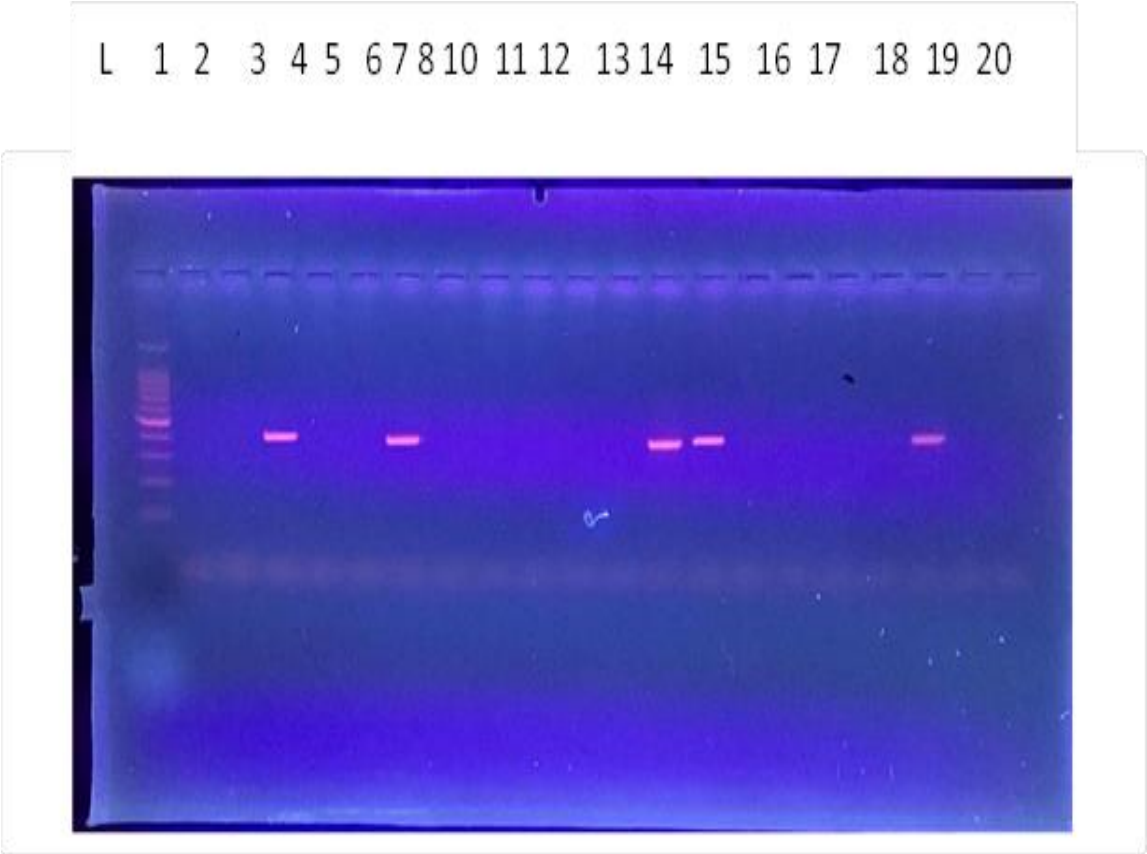


Figure (4 – 6) . Agarose gel electrophoresis (Ladder = 100 – 1000) , (pb404) . Agaros ; 1% , volt ; 80 , mA ;20 , time; 80 min of extracted DNA of *P . gingivalis* .

In the figure (4 - 7) the isolates (5 , 6) showed positive results for *P* . *gingivalis* , While (1 , 2 , 3 , 4 , , 7 , 8 , 9 , 10 , 11) isolates are showed negative results for *P* . *gngivalis* . (periodontitis and rheumatoid) group.

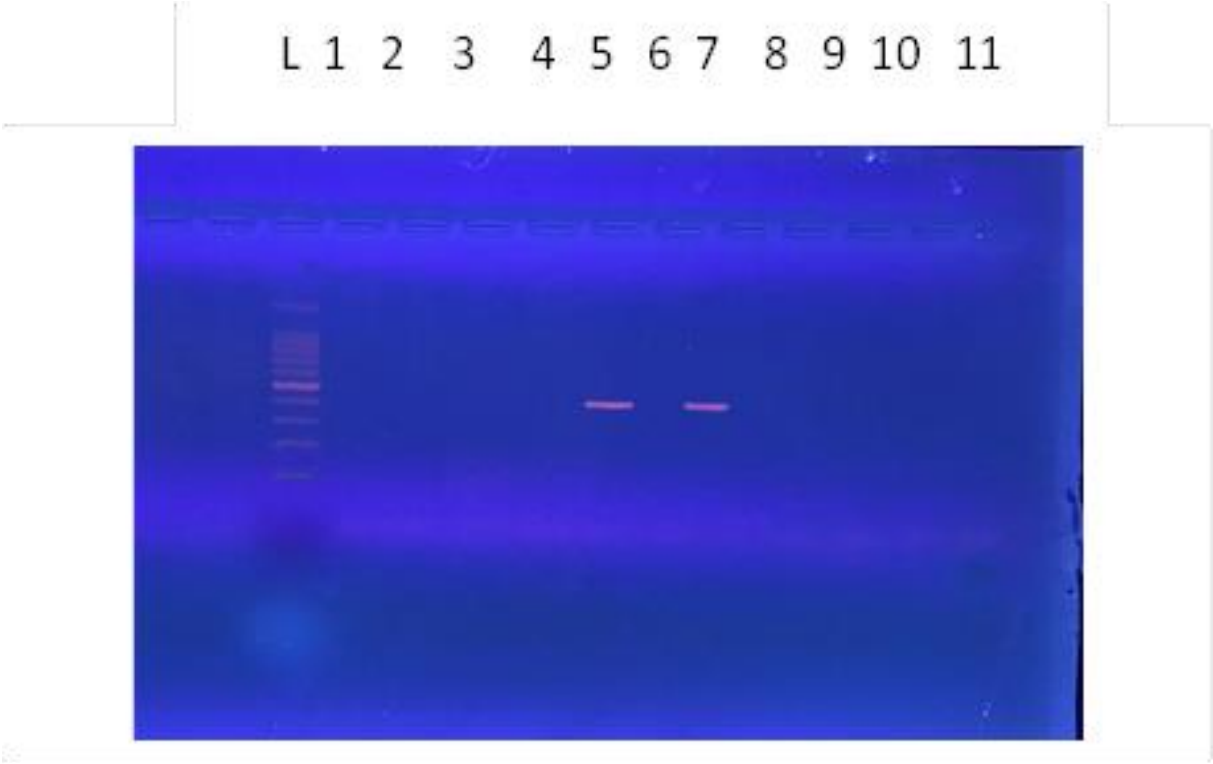


Figure (4 – 7) . Agarose gel electrophoresis (Ladder = 100 – 1000) , (pb 404) ,) . Agaros ; 1% , volt ; 80 , mA ;20 , time; 80 min of extracted DNA of *P* . *gingivalis*

4 . 4 Clinical Periodontal Parameters

Clinical periodontal parameterss such as the plaque score (PS) , bleeding on probing (BOP) , pocket depth (PD) , and clinical attachment loss (CAL) were measured as part of the intraoral examination . Six locations were used to evaluate PS , BOP , PD , and CAL for each tooth . All clinical indicators (PS , BOP , PD , and CAL) between the Healthy , periodontitis , and periodontitis with rheumatoid groups were significantly different (p0.05) according to the data analysis performed using the Kruskal-Wallis test in table (4 - 3) . Mainly between the healthy group and the groups (P and PR) , there was a substantial difference .

Table 4 - 3 : Clinical periodontal parameters .

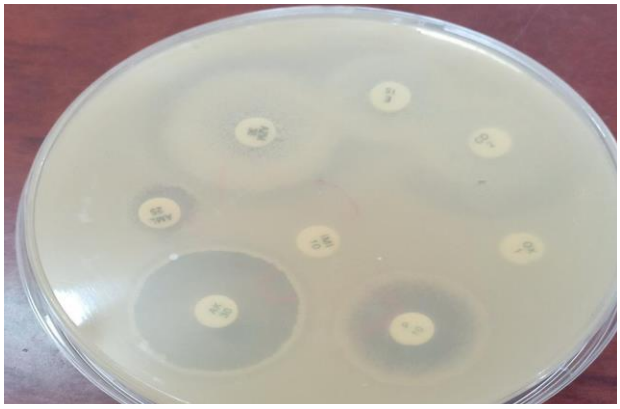
Clinical parameters	Healthy (n=40)	Periodontitis (n=40)	Periodontitis and rheumatoid (n=40)
PS (%)	7 . 945±2 . 194	35 . 965 ± 8 . 482	42 . 052 ± 12 . 278
BOP (%)	5 . 655 ± 1 . 280	33 . 222 ± 8 . 083	33 . 337 ± 8 . 752
PD (mm)	1 . 332 ± . 489	3 . 425 ± . 863	3 . 503 ± . 788
CAL (mm)	.000± . 000	2 . 917 ± 1 . 074	2 . 904 ± 1 . 107

* CAL=clinical attachment loss P . D . =pocket depth BOP=bleeding on probe P . S . plaque score

* P 0 .05 indicates a significant difference . the Kruskal-Wallis test .

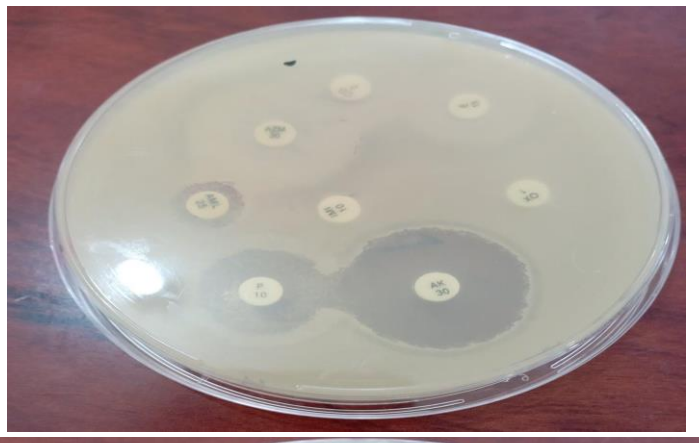
Table (4 – 4) The antibiotic discs that are shown the size zone .

Isolates	Antibiotics	Inhibition zone (mm)	Isolates	Antibiotics	Inhibiti on zone (mm)	Isolates	Antibiotics	Inhibition zone (mm)
1	Amikacin (AK)	15	3	Amikacin (AK)	0.1	5	Amikacin (AK)	15
	Imipenem (IMI)	0 . 1		Imipenem (IMI)	0 . 1		Imipenem (IMI)	0 . 1
	Amoxicillin (AML)	2 . 0		Amoxicillin (AML)	0 . 2		Amoxicillin (AML)	0 . 2
	Azithromycin(EZM)	2.0		Azithromycin(EZM)	0.2		Azithromycin(EZM)	1.2
	Clindamycin (CD)	2.0		Clindamycin (CD)	0.1		Clindamycin (CD)	0.1
	Erythromycin (E)	1.0		Erythromycin (E)	0.1		Erythromycin (E)	1.0
	Pencilin (P)	12 . 0		Pencilin (P)	0 . 1		Pencilin (P)	2.0
	Oxacilin (OX)	0.2		Oxacilin (OX)	0.2		Oxacilin (OX)	0.2
2	Amikacin (AK)	1.0	4	Amikacin (AK)	15			
	Imipenem (IMI)	15		Imipenem (IMI)	0 . 1			
	Amoxicillin (AML)	0.5		Amoxicillin (AML)	2 . 1			
	Azithromycin(EZM)	1.5		Azithromycin(EZM)	2.2			
	Clindamycin (CD)	12		Clindamycin (CD)	0.1			
	Erythromycin (E)	0.1		Erythromycin (E)	2.0			
	Pencilin (P)	0.2		Pencilin (P)	10			
	Oxacilin (OX)	0.1		Oxacilin(ox)	0.2			



Figure(4 -8) isolate number 1 the antibiotic disc that are used in the study are shown size zone.

where it appears increasing rates for antibiotic non-susceptibility in isolates of *P. gingivalis* , to AML , CD, IMP, OX , E, and AZM , while the antibiotic susceptibility to AK and P.



Figure(4 -9) isolate number 2 the antibiotic disc that are used in the study are shown size zone.

where it appears increasing rates for antibiotic non-susceptibility in isolates of *P. gingivalis,* to AML , CD, IMP, OX , E, and AZM , while the antibiotic susceptibility to AK and P.

Figure (4 -10) isolate number 3 the antibiotic disc that are used in the study are shown size zone.

where it appears increasing rates for antibiotic non-susceptibility in isolates of *P. gingivalis* , to AML , CD, IMP, OX , E, , AZM , AK and P.

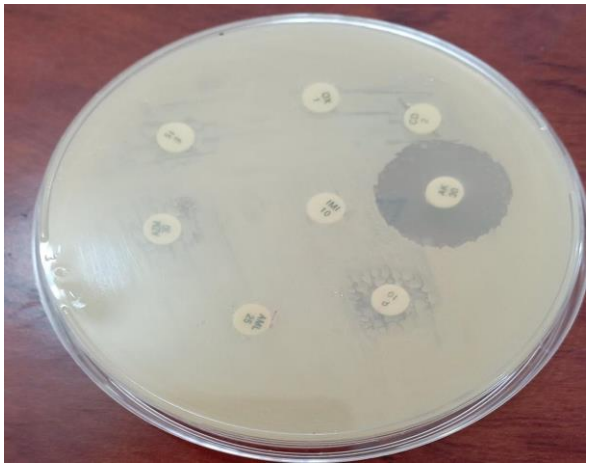


Figure (4 -11) isolate number 4 the antibiotic disc that are used in the study are shown size zone.

where it appears increasing rates for antibiotic non-susceptibility in isolates of *P. gingivalis* , to AML , CD, IMP, OX ,P, E, and AZM , while the antibiotic susceptibility to AK only.

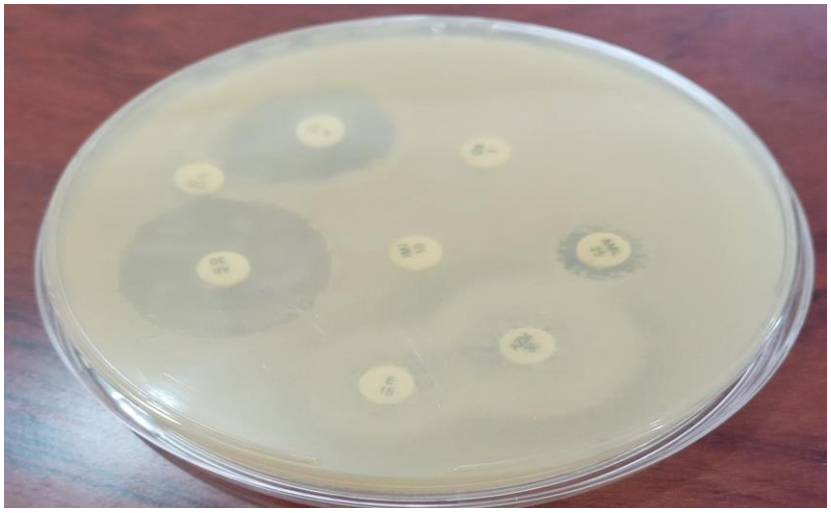


Figure (4 -12) isolate number 5 the antibiotic disc that are used in the study are shown size zone.

where it appears increasing rates for antibiotic non-susceptibility in isolates of *P. gingivalis,* to AML , CD, IMP, OX , E, and AZM , while the antibiotic susceptibility to AK and P.

4 . 5 Nanoparticles (NPs) activity findigs .

The nanomaterials were prepared by the physical method (pulsed laser ablasion), where the prepared nanomaterials consisted of three types, namely Ag+ CuO , CuO and Ag then the five bacterial isolates of *P. gingivalis* were treated with the three nanomaterials. The biofilms, which are one of the virulence factors used by pathogenic bacteria for disease events, were measured by measuring the optical density using the ELISA device, that treatment with nanomaterials Ag only that showed the unable to affect the bacterial isolates by increase the formation of biofilms , after it was treated with nanomaterials cuo only that showed the littel effect on bacteria isolates by simple reduction the formation of biofilms except for secend isoleat it incerased .

Finally it was treated with nanomaterials Ag +Cuo showed the ability to affect the all bacterial isolates by reducing the formation of biofilms were shown in table (4 _ 5) and figure (4-13).

Table (4 – 5) : Biofilm formation (optical density) of *P . gingivalis* in comparison to control (without nanoparticles) .

Isolates	Optical density and standard deviation			
	Without nanoparticles(Control)	Ag µ l	CuO µ l	Ag+CuO µ l
1	0 . 37±0 . 071	0 . 64± 0 . 06	0 . 34± 0 . 09	0 . 15± 0 . 008
2	0 . 32±0 . 03	0 . 74± 0 . 27	0 . 36± 0 . 02	0 . 14± 0 . 03
3	0 . 32± 0 . 02	0 . 50± 0 . 009	0 . 22± 0 . 01	0 . 13± 0 . 01
4	0 . 37± 0 . 05	0 . 51± 0 . 05	0 . 24± 0 . 07	0 . 3± 0 . 22
5	0 . 35± 0 . 005	0 . 62± 0 . 10	0 . 32± 0 . 03	0 . 21± 0 . 10

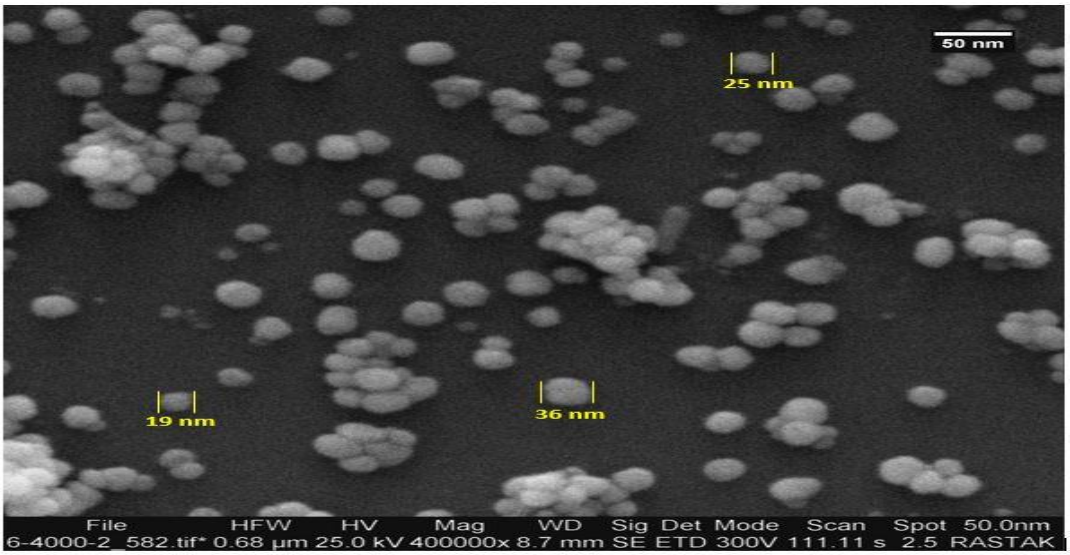


Figure (4 - 13) : scanning electron microscopy (SEM)graph showed the size of the prepared Nanoparticles.

Chapter Five

Discussion

5.1 Discussion

Rheumatoid arthritis and periodontitis are two chronic inflammatory illnesses that typically affect the elderly . It has been suggested that the two disorders are connected and affect one another's severity *P* . *gingivalis* , a periodontal pathogen has recently been linked to the pathogenesis and progression of rheumatoid arthritis (Agnihotri & Gaur.,2014).

There is a higher prevalence of severe perodontitis among RA subjects According to Karapetsa *et al.*,(2022) the clinical periodontal characteristics and the severity of RA are highly associated ,Many investigations on the periodontal health of RA and periodontitis patients have been motivated by the parallels between the two conditions and these investigations have revealed The prevalence of *P .gingivalis* is also revealed by our investigation to be the target for *P. gingivalis* -specific amplification. where it was found Only 27 . 5 % (11 of 40) of the group of healthy participants had *P* . *gingivalis* , whereas 25% (10 of 40) of the group of periodontists had it , and 20% (8 of 40) of the group of rheumatoid and periodontists had it Based on results from earlier examinations into the prevalence of *P* . *gingivalis* in both groups , it was determined that *P* . *gingivalis* did not differ in prevalence between the two groups in some studies . In some cases , the patient's health state and different forms of periodontal disease may be linked to this disparity (Riep *et al* .,2009; Kumar *et al*., 2006). Then again , it should be highlighted that periodontal pathogens can exist in both healthy individuals and patients , suggesting that periodontal illness is not always caused by periodontal pathogens (Yong *et al* ., 2015) . This difference could be due to a number of factors , including the patients' overall health and periodontal disease types .

Furthermore , it should be highlighted that the presence of periodontal infections in healthy individuals and patients may suggest that periodontal disease is not always caused by these pathogens (Yong *e al*., 2015) . In comparison to the periodontitis group , the rheumatoid and periodontitis group , and the healthy group , the ratio of *P* . *gingivalis* infections was higher in the healthy group . These findings demonstrate that *P* . *gingivalis* can live normally in a periodontally healthy dentition and link it to the formation of periodontitis . The studies comprised a range of periodontal disease types and population demographics (age , severity , comorbidities) , which may have influenced the findings . These include age , gender , smoking , drinking , and being a smoker . The prevalence of *P. gingivalis* differed between the studies that were included , as we observed . The two strain type may be one of the causes of the difference between. According to Singh *et al* .(2011) first strain *P* . *gingivalis* has a capsule that is recognized as a major antigen linked to the strain's pathogenicity .

Second stain *P* . *gingivalis* on the other hand , is devoid of this antigen and only displays a mild inflammatory response .These two strains are significantly dissimilar to one another as a result Michaud *et al* ., (2013). The findings of this study show that *P* . *gingivalis* is highly present in subjects with healthy periodontal tissue and that it is also present albeit less frequently , in those with periodontal disease . As a result , this bacterium may be regarded as a significant potential risk factor that raises the probability of periodontal disease . if the potential for illness exists .

Leukocyte permeation and cytokines induce alveolar bone loss , synovitis , and joint destruction in rheumatoid arthritis and periodontitis , two immunological inflammatory diseases .The development and expansion of B lymphocytes depend heavily on the cytokine interleukin-4 that is created by T_helper2 , cells . Because it effectively reduces the cytokines that cause inflammation , such as tumor necrosis factor-alpha (TNF-alpha) , are produced . IL-1alpha , IL-1beta , IL-6 , and IL-8 bymonocytes and macrophage , IL-4 has an anti-inflammatory impact (Fernandez-Solari *et al* ., 2015) .

The levels of IL-4 and IgG1 samples obtained from patients are mostly low or even undetectable , and remained , on average , in two groups (periodontitis and RA) on the same level to some extent but the level IL4 and IgG1 samples obtained from control higher than the patients with periodontitis and RA .

In the current study , The findings demonstrate that healthy individuals have high levels of IgG1 . compared to those with periodontitis and those with periodontitis and arthritis , regarding the IgG1 levels in healthy people . In the patients with periodontitis , the age group 41:50 showed the highest level of IgG1 , While the lowest level of IgG1 was for the age group 51:60 , In patients with rheumatoid and periodontitis , IgG1 levels high in the 31:40 age group , while the lowest level of IgG1 in the age group 20:30 , 41:50 , 51:60 This is consistent with the results of previous study showing that IgG1 concentrations were decreased in patients with arthritis (Schwedler *et al* ., 2018).

According to the findings of the current investigation IL4 levels were higher in healthy individuals than in those with periodontitis and those with periodontitis and arthritis . In the age groups 41:50 , 20:30 , 51:60 , 31:40 respectively . In contrast between periodontitis and with periodontitis and arthritis patients together , where the highest levels of IL4 were in periodontitis patients within the age group 31:40 , followed by the age group 41:50 , 51:60 , 20:30 are lowest , the results showed that the levels of IL4 were the lowest in people with periodontitis and arthritis together compared to healthy people and those with periodontitis , in the age groups 20:30 , 31:40 , 51:60 , 41:50. This is consistent with the results of previous studies , which showed that IL-4 concentrations decreased in patients with periodontitis as the disease progressed .

It is proposed that IL-4 may play a significant role in the remission of or improvement of periodontal disease (Rahim *et al* ., 2023). Statistical analysis revealed that there were significant differences below the level of significance $P \leq 0.05$ between IgG1 and IL-4 values in all patients with periodontitis and the value of L . S . D . about 0 . 789 compared to those with periodontitis and arthritis and healthy people , as no significant differences appeared between different age groups . There is a weak correlation between IgG1 and IL-4 in periodontitis group about 0 . 3904 , while in Rheumatoid and Periodontitis group , there is an intermediate correlation about 0 . 5586 . While in the case of healthy people , There is an inverse correlation between IgG1 and IL-4 about - 0. 81798 , This means that the higher the IgG1 , the lower the level of IL-4 in the blood of healthy people .

A negative correlation indicates two Variables that tend to move in opposite directions . A Correlation coefficient of -0.81798 indicate a strong negative relationship, it is mean perfect reverse Correlation while in Periodontitis as well as Periodontitis with rheumatoid, the correlation between IL-4 and IgG1, weak Positive correlation. A weak Positive correlation indicates that although both variables tend to go up in response to one another, the relationships is weak direct correlation (Ratner 2009).

Our analyses showed increasing rates for antibiotic non-susceptibility in all isolates of *P. gingivalis*, to AML , CD, IMP, OX , E, and AZM , while the antibiotic susceptibility to AK and P . Consistent with other investigators we observed non-susceptibility of *P. gingivalis* to AZM (van Winkelhoff *et al*., 2000a, 2000b; Jaramillo *et al*., 2005 ; Ardila *et al*., 2010; Ardila & Bedoya-Garcia2020) However, others reported *P. gingivalis* sensitivity to AZM (Pajukanta *et al*., 1994; Jaramillo *et al*., 2005).

While Susceptibility testing revealed a sensitivity of 100% of P. gingivalis to azithromycin (Japoni *et al*., 2011). Susceptibility testing revealed a sensitivity of *P. gingivalis* to E , CD , IMP, AML (Andrés*et al* .,1998). agree with our study we observed susceptibility of *P. gingivalis* to penicillin

It is important to mention at this study is the first study that investigate the effect of nanomaterial'son certain dental plaque biofilm bacteria. The nanomaterial's are prepared by the physical method (pulsed laser abrasion), where the prepared nanomaterials consisted of three types, namely Ag+ CuO , CuO and Ag then the five bacterial isolates of *P. gingivalis* were treated with the three nanomaterials. The biofilms, which

are one of the virulence factors used by pathogenic bacteria for disease events, were measured by measuring the optical density using the ELISA device, where it was found that the optical density of the first isolate before treatment with nanomaterials no effects, while it decrease biofilm after treatment with Ag +CuO . While the effect of Ag and CuO nanomaterials was less on the first isolate, where the optical density after treatment with these materials .

The Ag+CuO nanomaterial showed the ability to affect the second bacterial isolate by reducing the formation of biofilms, as the optical density before treatment it was high, while after treatment with this nanomaterial it lowest .

As for the nanomaterials, it did not show any effect on the second isolate, but on the contrary, a high optical density was observed, which indicates that the bacteria were activated when treated with Ag , CuO, , while the value of the optical density before treatment is high.

The Ag+CuO and CuO nanomaterials showed an effect on the second isolate, as the optical density value decreased after treatment with these materials, while the optical density increased after treatment with Ag nanomaterial,

When the fourth isolate was treated with CuO nanomaterials, a decrease in the optical density was observed, , as this material showed more efficiency in inhibiting *P. gingivalis* than the CuO nanomaterial, after treatment with this material, while the optical density of the fourth isolate before treatment it was high . On the contrary, the Ag nanomaterial did not show an inhibitory role on bacteria, as the value of the optical density increased and reached 0.51±0.05 compared to the optical density value of the fourth isolate before treatment.

The results show the efficiency of the Ag +CuO nanomaterials in inhibiting *P. gingivalis* through a decrease in the optical density value, followed by the CuO nanomaterials, where the optical density value after treatment with these materials , while the optical density value of the fifth isolate before treatment it was high.

The results also showed that when the fifth isolate was treated with Ag nanomaterials, the optical density value increased, compared to the optical density value of the same isolate before treatment .

The results of the current study agree with what was shown by Yoon *et al.* (2007), which showed that copper has an effective antimicrobial role compared to silver by fusing copper with–SH groups of key enzymes Where copper oxide is a relatively cheap compound and can be easily combine with water and polymers and is somewhat stable in terms of physical and chemical properties and has antimicrobial properties (Stoimenov et al. 2002). Previous studies showed that copper has a role in inhibiting the growth of *P. gingivalis* by preventing its adherence to the teeth and gums by preventing the formation of biofilms (Xinru *et al*.,2022) .

The mechanism of action of nanoscale copper oxide with silver nanoparticles as an antibacterial agent is represented by the production of different types of active oxygen, including hydrogen peroxide H2O2, which is toxic to bacteria and inhibits their growth. These nanocomposites have no effect on human cells if used in very small concentrations (Tavassoli *et al.*,2013).Copper oxide nanoparticles or copper oxide nanocomposites with silver can be used as an antibiotic to prevent tooth decay or prevent dental inflammation, and this is consistent with RA (Amiri *et al*.,2017).

It is clear from the current results that the Ag +CuO nanomaterial was more efficient in inhibiting the growth of bacteria by inhibiting the ability of bacteria to form biofilms and thus reducing the ability of bacteria to settle in the teeth or gums, As for the Ag nanomaterial, it stimulated the growth of bacteria and increased their ability to form biofilms. Therefore, the use of silver nanoparticles in dental treatment would help bacteria increase their spread in the mouth and destroy teeth, causing tooth decay and gingivitis.

Conclusions and Recommendations

Conclusions

1. Female age group (50-59) are more susceptible to RA than other age groups.
2. Concentrations of IL4 and IgG1 are decreased in patients compared to their concentrations in healthy people.
3. Antibiotic medication may therefore be effective for RA patients. *P. gingivalis* affects individuals on both sides..
4. Use of silver nanoparticles would increase the susceptibility of *P. gingivalis* to the formation of biofilms.
5. Use of Cuo + Ag NPs reduces the ability of *P. gingivalis* to form biofilms.

Recommendations

1. More research is necessary for the detection of other serum components associated with both disorders, Such as RANKL, IL-23, and IL-17.
2. Additional research is required to identify the periodontal pathogen responsible for chronic periodontitis and rheumatoid arthritis, as well as to determine the methods by which the peptidyl arginine deiminase (PAD) enzymes produce immunogenic peptides and control the autoimmune response.
3. More research is required, including examination of biosimilar medications and evaluation of their impact on the periodontal health of people with rheumatoid arthritis.
4. look into how other risk variables that is including dietary issues, stress, and lifestyle factors, relate to Periodontitis and RA.
5. Searching for nanomaterials manufactured by chemical or biological methods that have the ability to inhibit the bacteria to form biofilms, which is a measure of the virulence and pathogenicity of bacteria.

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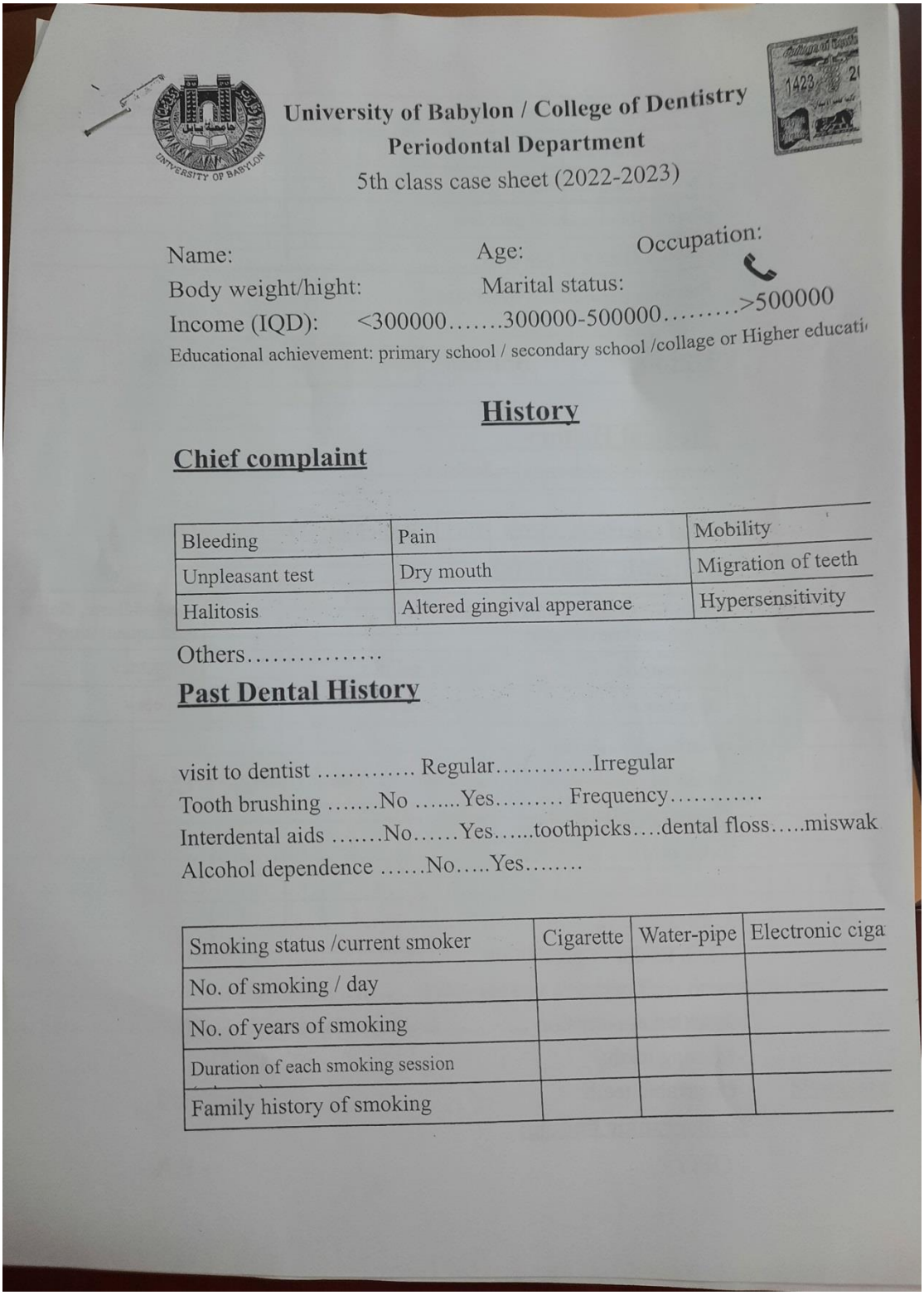
Appendices



University of Babylon / College of Dentistry
Periodontal Department
5th class case sheet (2022-2023)

Name: Age: Occupation:
Body weight/hight: Marital status:
Income (IQD): <300000…….300000-500000……>500000
Educational achievement: primary school / secondary school /collage or Higher educati

History

Chief complaint

Bleeding	Pain	Mobility
Unpleasant test	Dry mouth	Migration of teeth
Halitosis	Altered gingival apperance	Hypersensitivity

Others……

Past Dental History

visit to dentist ……. Regular…….Irregular
Tooth brushing …….No ……..Yes…… Frequency……
Interdental aids …….No……Yes…..toothpicks….dental floss…..miswak
Alcohol dependence ……No…..Yes……..

Smoking status /current smoker	Cigarette	Water-pipe	Electronic ciga
No. of smoking / day			
No. of years of smoking			
Duration of each smoking session			
Family history of smoking			

Smoking status /former smoker	Cigarette	Water-pipe	Electronic cigare
No. of smoking / day			
No. of years of smoking			
Duration of each smoking session			
Family history of smoking			

Previous periodontal treatment .....

Habits: Bruxism ☐ Clenching ☐

Medical History :

-sensitivity&abnormal reaction to

Local anesthesia ☐ Yes ☐ No Penicillin ☐ Yes ☐ No Others .....

Systemic disease(s)

Acquired and/or congenital heart disease	Angina M.I	Renal failure , dialysi &transplantation
Hypotension	Diabetes mellitus	Pregnancy
Hypertension	Infection disease	Epilepsy

others .....

Intake of medication(s).....No.....Yes.....Drugs type .....

Examination

Extra-oral.....

Intra-oral.....

-Ulceration.....

-Teeth tender to precaution.....

-teeth with negative vitality .....

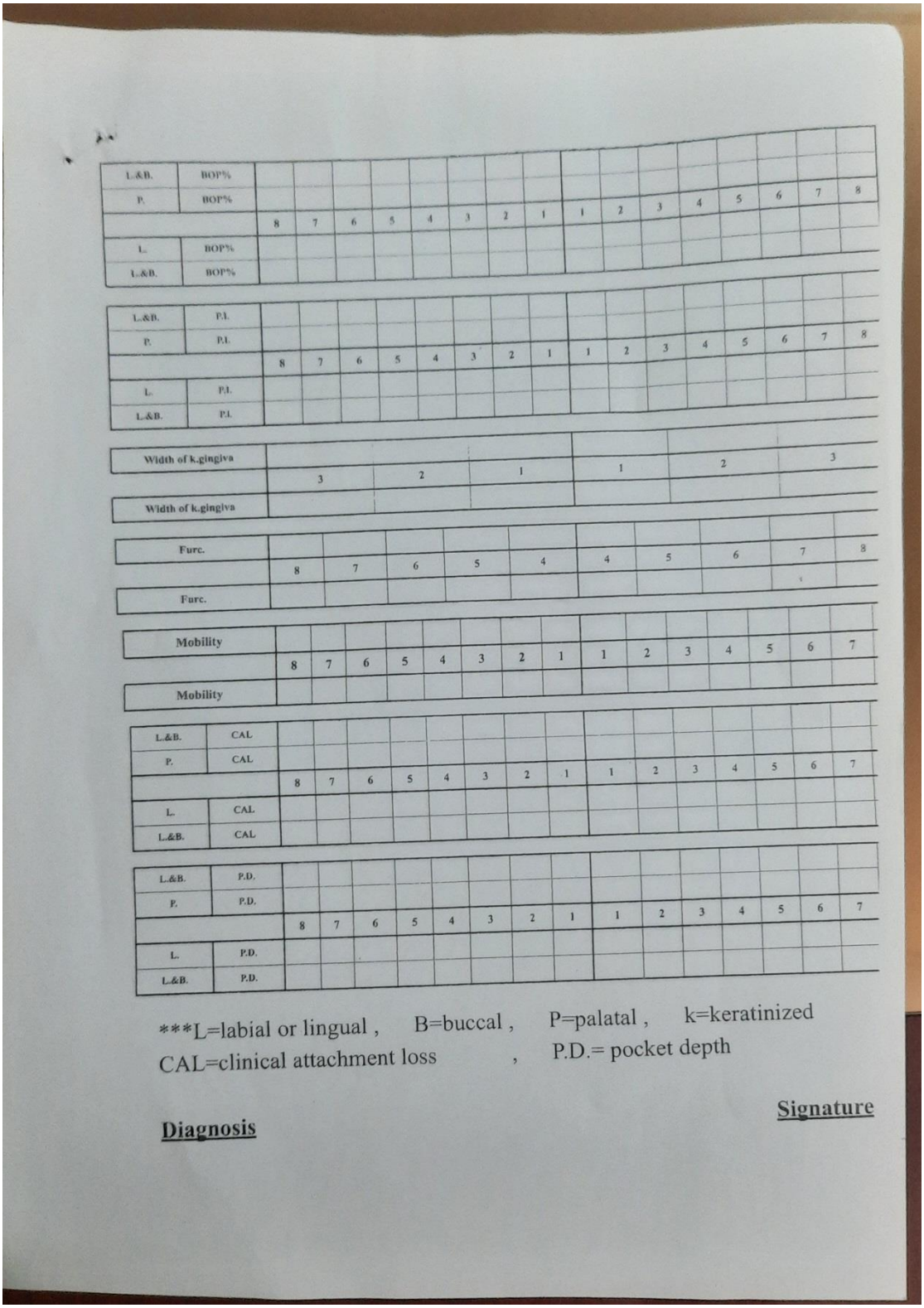
-Attrition & Abrasion .....

-Missing teeth:

-Unsavable teeth:

Radiographic finding:

- OPG:- - P.A:-



L.&B.	BOP%															
P.	BOP%															
		8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
L.	BOP%															
L.&B.	BOP%															

L.&B.	P.I.															
P.	P.I.															
		8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
L.	P.I.															
L.&B.	P.I.															

Width of k.gingiva																
		3		2		1				1		2			3	
Width of k.gingiva																

Furc.																
		8		7		6		5		4		4		5		6		7	8
Furc.																

Mobility																
		8	7	6	5	4	3	2	1	1	2	3	4	5	6	7
Mobility																

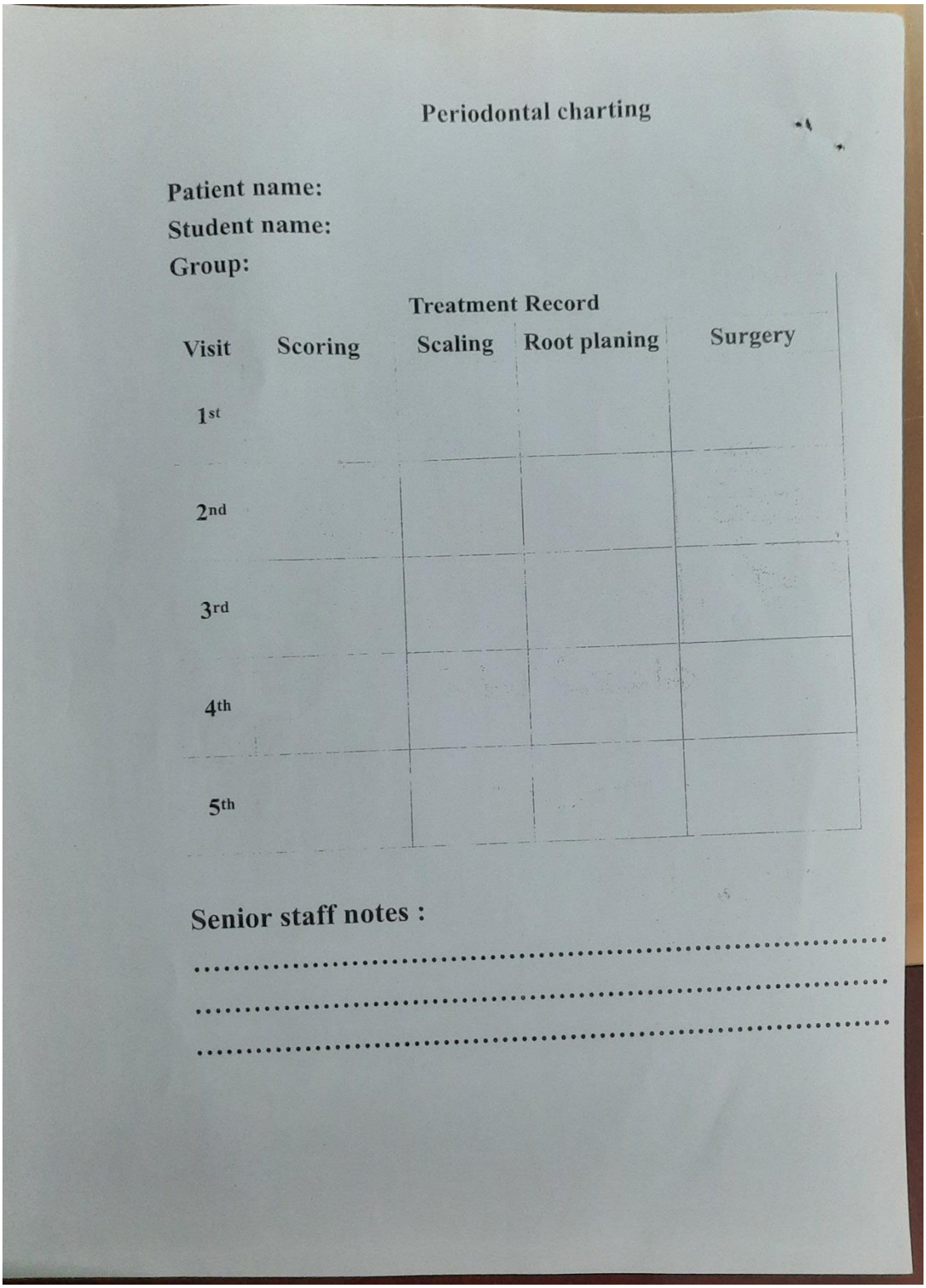
L.&B.	CAL															
P.	CAL															
		8	7	6	5	4	3	2	1	1	2	3	4	5	6	7
L.	CAL															
L.&B.	CAL															

L.&B.	P.D.															
P.	P.D.															
		8	7	6	5	4	3	2	1	1	2	3	4	5	6	7
L.	P.D.															
L.&B.	P.D.															

***L=labial or lingual , B=buccal , P=palatal , k=keratinized
CAL=clinical attachment loss , P.D.= pocket depth

Signature

Diagnosis



Periodontal charting

Patient name:

Student name:

Group:

Visit	Scoring	Treatment Record		
		Scaling	Root planing	Surgery
1st				
2nd				
3rd				
4th				
5th				

Senior staff notes :

.....

.....

.....

Appendix

موافقة للاشتراك في البحث العلمي

اسم الباحث :

عنوان البحث:

مكان اجراء البحث:

انت مدعو(ة) للمشاركة ببحث علمي سريري سيجرى في ---------- الرجاء ان تأخذ(ة) الوقت الكافي لقراءة المعلومات التالية بتان قبل ان تقرر(ي) اذا كنت(ي) تريد(ين) المشاركة ام لا . بإمكانك طلب ايضاحات او معلومات إضافية عن اي شيء مذكور في هذه الاستمارة او عن هذه الدراسة ككل من طبيبك .

في جال وافقت على المشاركة في هذه الدراسة ،سيبقى اسمك طي الكتمان لن يكون لأي شخص، مالم ينص القانون على ذلك ، حق للاطلاع على ملفك الطبي باستثناء الطبيب المسؤول عن الدراسة ومعاونيه .

موافقة المشترك:

لقد قرات استمارة القبول هذه وفهمت مضمونها . تمت الاجابة على اسئلتي جميعها. و عليه فإنني ، حرا مختارا ، اجيز اجراء هذا البحث و اوافق على الاشتراك فيه ، واني اعلن الباحث الدكتور _____ وزملاءه ومعاونيه او مساعديه سيكون مستعدين للإجابة على اسئلتي، وانه باستطاعتي الاتصال بهم على الهاتف _____ واذا شعرت لاحقا ان الاجوبة تحتاج الى المزيد من الايضاح فسوف اتصل بأحد اعضاء الاخلاقيات. كما اعرف تمام المعرفة بانني حر في الانسحاب من هذا البحث متى شئت بعد التوقيع على الموافقة دون ان يؤثر على العناية الطبية المقدمة لي .

اسم المشترك:

توقيع المشترك:

الاضطرابات الالتهابية المزمنة مثل التهاب المفاصل الروماتويدي والتهاب اللثة شائعة. تعد أمراض اللثة شائعة هذا المرض له مسببات معقدة، وعدد من العناصر بما في ذلك غزو الكائنات الحية الدقيقة لصحة المضيف والعوامل البيئية الخارجية تساهم في تطوره، ومن غير المعروف حتى الآن ما إذا كان التهاب المفاصل الروماتويدي والتهاب اللثة مرتبطان، تتضمن هذه الدراسة ثلاثة يهدف الأول إلى عزل البكتيريا الأكثر شيوعاً في التهاب اللثة بينما الهدف الثاني يتضمن الكشف عن مستويات الغلوبولين المناعي G1 والإنترلوكين ٤ بين الأشخاص الأصحاء والمرضى الذين يعانون من التهاب اللثة والتهاب المفاصل الروماتويدي، أما الهدف الثالث فيتضمن دراسة تأثير المواد الجديدة (الجسيمات النانوية) على تكوين الأغشية الحيوية التي تنتجها البكتريا المعزولة من مرضى التهاب اللثة والروماتويد (في المختبر).

أجريت هذه الدراسة في كلية طب الاسنان / جامعة بابل ومستشفى مرجان التعليمي وحدة الروماتيزم في بابل من (ديسمبر ٢٠٢٢ إلى مايو ٢٠٢٣) على مرضى التهاب اللثة والتهاب المفاصل الروماتويدي (٨٠ مريضاً من الذكور والإناث) للفئة العمرية (٢٠-٦٠) سنين . في هذه الدراسة، كان ٤٠ شخصًا مصابًا بالتهاب المفاصل الروماتويدي، و٤٠ مشاركًا يتمتعون بصحة جيدة و٤٠ مشاركًا يعانون من التهاب اللثة مع أعماق فحص أكبر من ٥ ملم وفقدان المرفقات السريرية بمقدار ٣ مم. وتم الحصول على عينات الدم لتراكيز الغلوبولين المناعي G1 والإنترلوكين ٤ وتم تقييمها باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA)، وباستخدام تفاعل البوليميراز المتسلسل (PCR) تم تحديد التسلسل التمهيدي الدقيق للبورفيروموناس اللثوية.

وفي الدراسة الحالية أظهرت النتائج أن مستويات الغلوبولين المناعي G1 والإنترلوكين ٤ مرتفعة لدى الأشخاص الأصحاء مقارنة بالمصابين بالتهاب اللثة والذين يعانون من التهاب اللثة والتهاب المفاصل. تم الكشف أيضًا عن مدى انتشار بورفيروموناس اللثوية من خلال تحقيقنا ليكون التسلسل المستهدف لبورفيروموناس. كان التضخيم النوعي للبورفيروموناس هو المنطقة الفاصلة المنسوخة . فقط ٢٧ . كان ٥% (١١ من ٤٠) من مجموعة المشاركين الأصحاء مصابين بالبورفيروموناس اللثوية، في حين أن ٢٥% (١٠ من ٤٠) من مجموعة التهاب اللثة أصيبوا بها، و٢٠% (٨ من ٤٠) من مجموعة التهاب اللثة والروماتويد. أظهرت تحاليلنا زيادة معدلات عدم حساسية المضادات الحيوية في جميع عزلات المتصورة اللثوية للأموكسيسيلين، الكليندامايسين، الإمبينيم، الأوكساسيلين، الإريثرومايسين والأزيثرومايسين بينما حساسية المضادات الحيوية للأميكاسين والبنسلين .

حيث تكونت المواد النانوية المحضرة من ثلاثة أنواع وهي أكسيد النحاس مع الفضة (وأكسيد النحاس والفضة) ثم تمت معالجة العزلات البكتيرية الخمس لبكتيريا البورفيرينية اللثوية بالمواد النانوية الثلاث. تم قياس الأغشية الحيوية التي تعتبر أحد عوامل الضراوة التي تستخدمها البكتيريا المسببة للمرض عن طريق قياس الكثافة الضوئية باستخدام جهاز ELISA، ويتضح من النتائج الحالية أن أكسيد النحاس مع مادة الفضة النانوية تم أكثر كفاءة في تثبيط نمو البكتيريا عن طريق تثبيط قدرة البكتيريا على تكوين الأغشية الحيوية وبالتالي تقليل قدرة البكتيريا على الاستقرار في الأسنان أو اللثة، أما مادة الفضة النانوية فقد حفزت نمو البكتيريا وزيادة حجمها. القدرة على تكوين الأغشية الحيوية.

الجديد في هذه الدراسة هو استخدام جزيئات الفضة مع اوكسيد النحاس النانوية التي يتم تصنيعها بطريقة الاستئصال بالليزر النبضي لتقليل قدرة المتصورة اللثوية على تكوين الأغشية الحيوية، ويتم الإبلاغ عن هذه النتيجة لأول مرة في جميع أنحاء العالم.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية طب الاسنان

العلامات المناعية في التهاب اللثة لدى مرضى التهاب المفاصل الروماتزمي والتأثير المضاد للجسيمات النانوية ضد بكتريا البورفيرينية اللثوية

رسالة
مقدمة الى مجلس كليه طب الاسنان في جامعة بابل كجزء من متطلبات نيل درجه الماجستير في العلوم / الاحياء المجهرية الفموية والطبية

من قبل

مصطفى ابراهيم هاشم العامري
بكالوريوس علوم حياة /جامعة الكوفة ٢٠١٤

اشراف

أ.م .د. هدى عباس محمد
.م .د زياد نزار مجيد

٢٠٢٣ م **١٤٤٥ ه**