CHAPTER 1

INTRODUCTION

1.1 PERIODONTITIS

Periodontitis is a highly prevalent oral disease which affects 90% of the world’s population. The term “Periodontitis” is derived from the word “Periodontium” meaning tooth supporting tissues. It is defined as an apical extension of gingival inflammation and involves the tissues supporting the tooth, including the periodontal ligament and the bone [1].

When the periodontium gets inflamed, the gums become red and swollen. A periodontal pocket is formed, when destruction of the fiber attachment occurs. The supragingival plaque invades deeper into the subgingival area. This leads to multiplication of the anaerobic bacteria [2]. This bacterial flora produce various types of toxins. The toxins lead eventually to further destruction of the bones and tissues of the supporting tooth. This will initiate the formation of the pocket between the tooth and the gum. Slowly, the pocket will deepen and spreads into the surrounding tissues and bones. The tooth will gradually become loose and will lead to the loss of tooth [3].

In addition to the microflora in the biofilm, environmental factors, habits such as tobacco chewing and smoking, genetic factor, influence periodontitis formation. Periodontal disease is found to have a role or an association with pregnancy related problems like Pre term birth and low birth weight, diabetes, cardiovascular disease and pulmonary disorders Gingivitis is a less severe type of periodontitis. At this stage there is complete cure by professional care and dental treatment. Gingivitis does not destroy the supporting tissues of the teeth and is reversible. This state if left untreated will lead into Periodontitis [4].
Figure 1.1 Structure of Periodontium showing normal gums and periodontitis

1.2 DIFFERENT TYPES OF LESION IN PERIODONTITIS / GINGIVITIS

According to Page and Schroeder the lesions that are formed during periodontitis or gingivitis can be categorized into four phases: namely Initial lesion, early lesion, Established lesion and The Advanced lesion. The initial and early lesions describe the histopathological condition of clinically healthy gingiva and the initial stages of gingivitis. Established lesion features the chronic gingivitis stage while the advanced lesion describes the stage where chronic gingivitis progresses into the periodontitic stage [5].

1.2.1 The Initial Lesion

As soon as plaque is formed on the gingiva, inflammation develops. This is evident within 24 hours. There will be marked changes in the dentogingival plexus as there is an increase in the blood flow to the area. The most prominent feature is the dilation of the arterioles and the capillaries which supplies blood to the gingiva. Within the microcirculation hydrostatic pressure develops and intercellular
gaps begin to form between the adjacent endothelial cells. This leads to microvascular permeability results in the exudation of proteins and subsequent fluids.

At this stage there is an increase in the Gingival Cervicular Fluid. Toxic wastes that are released from the biofilm are diluted in the gingival tissue and the crevice. The bacteria along with their products are washed into the saliva. GCF contains compliment, antibodies, protease inhibitors and other macromolecules. In a short period of 2-4 days the plaque facilitates a cellular response.

In the initial phase, the Polymorphonuclear cells (PMN) migrates to the crevice due to chemo attractant gradient and is further migrated with the help of other adhesion molecules which are present specifically in the junctional epithelial cells and by the microbial chemotactic factors that are present. Lymphocytes remain in the epithelial cells unlike PMNs and are not exuded into the oral cavity as these are able to bind to the adhesion molecules, antigens and cytokines [6].

1.2.2 Early Lesions

After several days of plaque buildup in the third of gingival tooth surface the lesions become more demarcated. There is an increased redness in the gingival area due to the increase in the number of capillaries and also due to the dilation of the capillaries. Polymorphonuclear cells and leukocytes are seen in greater proportion when compared to plasma cells. Degeneration of fibroblasts and collagen fibers is noted in the lesions. This will help in the infiltration of leukocytes. The alterations in the tissues lead to the decrease of the coronal portion of the junctional epithelium. A gap is formed in between the enamel surface of the tooth and the epithelium, which allows the formation of a biofilm in the niche [7].

1.2.3 Established Lesions

At this stage a pocket epithelium forms. The Gingival cervical fluid (GCF) increases. The plasma cells show an increase at this stage. Collagen depleted
spaces invades into the deeper tissues. These spaces will help in the infiltration of leukocytes. Theses infiltrated leukocytes begins to accumulate in these spaces. The pocket epithelium replaces the junctional epithelium. Migration of the biofilm occurs as there is loss of attachment of the pocket epithelium to the tooth surface. There is more permeability to the passage of substances in the connective tissues at this stage. Ulcer formation occurs. There are two types of established lesions, first type is the stable one, which does not progress further and the latter is the active type that can progress rapidly into the destructive one [8].

1.2.4 Advanced Lesions

At advanced lesion stage there is reduced resistance to periodontal probing. The main difference between established lesion and advanced lesion is the detachment of connective tissue and the alveolar bone. There is extensive damage to the collagen tissue. The lesion becomes generalized and no longer localized to the gingival tissue. According to Garant and Berglundh the most prominent cells in the advanced lesions are the plasma cells. The pocket epithelium penetrates deeper into the connective tissue from the cement-enamel junction [9-10].

![Figure 1.2 Progression of periodontitis from Healthy gums to advanced periodontitis](image_url)
From the year 1977 till 1989 the AAP (American Academy of Periodontology) increased the 2 main periodontal categories to 5 categories. In the year 1999 at the International Workshop for a Classification of Periodontal disease and Conditions further two more categories were added on [11].

Table 1.1 1999 Classification of periodontal diseases and conditions (Abbreviated version) [11].

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<td>C. Pericoronal abscess</td>
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<td>A. Combined Periodontic endodontic lesions</td>
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<td>VII</td>
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<td>A. Localised tooth related factors that modify or predispose to plaque induced gingival diseases</td>
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<td>B. Mucogingival deformities and conditions around the tooth</td>
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<td>C. Mucogingival deformities and conditions on endontous ridges</td>
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<td>D. Occlusal trauma</td>
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1.4 TYPES OF PERIODONTITIS

1.4.1 Gingival Diseases

Milder form of periodontitis is known as gingivitis. Gingivitis can be of two types. Plaque Induced Gingivitis and Non-plaque Induced Gingivitis.

1.4.2 Aggressive Periodontitis

Aggressive type of periodontitis is usually seen in clinically healthy patients. Rapid detachment of the gums along with bone destruction is characteristic feature of this type of periodontitis. Other features include presence of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis or either one. Higher concentrations of Prostaglandin E2 and Interleukin 1β are other feature of aggressive Periodontitis [12].

1.4.3 Localized Aggressive Periodontitis

Onset of Localised Aggressive Periodontitis is around Puberty. There is a high antibody response at this stage. Attachment loss is seen at the site of two permanent teeth mostly around the incisors and or first molars [13].

1.4.4 Generalised Aggressive Periodontitis

Generalised Aggressive Periodontitis is seen in patients above 30 years. Antibody response is very poor. Atleast three permanent teeth are affected other than incisors [13].

1.4.5 Chronic Periodontitis

There is a deep progression of lesion into the tissues. This condition is always associated with periodontal pockets. This type of periodontitis is a commonly seen in adults. Gingival hypertrophy or gingival recession, suppuration and bleeding on probing are the main symptoms of chronic periodontitis.
If less than 30% of the site is involved then it is classified as Localised chronic periodontitis. If more than 30% of the site is involved then it is classified as Generalized Chronic periodontitis. Depending on the severity of the infection they are classified into Slight Periodontitis, Moderate periodontitis and Severe periodontitis [14].

1.4.6 Necrotizing Periodontitis

Necrosis of gingival tissues and alveolar bone is seen at this stage. The main symptoms are ulcerated papillae with bleeding and pain. This is accompanied by fever, malaise, halitosis and lymphadenopathy.

Necrotizing periodontitis is seen among Immunosuppressed and malnourished patient. The main difference between Necrotizing Ulcerative Gingivitis (NUG) and Necrotizing Ulcerative Periodontitis (NUP) is that in the former only the gingivitis is involved where as in the latter case the whole tissue and bones surrounding the teeth is involved [15-16].

1.5 BIOFILM

The formation of biofilm occurs in several stages. With the help of host molecules and bacterial components an Acquired Film is formed on the tooth surface. Initially the bacteria interact with the other components by Van der Waals force and Electrostatic force which helps in the formation of a weak bond. This bond is strengthened by adhesins. Adhesions are specific components present on the bacteria which help in the attachment of bacteria to the surface of tooth.

In time co-aggregation takes place with newer bacteria. These bacteria multiply and adhere firmly onto the tooth surface. Periodontitis syndromes depend on various other factors like oral environment and the microbial agents involved. Severity of Periodontitis depends on oral hygiene and plaque deposit of the host.
The natural balance (eubiosis) of the biofilm can be disrupted by various pre-disposing factors like lack of oral hygiene, age of the patient, smoking and tobacco chewing. Dysbiosis or alteration of eubiosis leads to inflammation or infection [17].

### 1.6 PLAQUE FORMATION

Human body is exposed numerous microorganisms. Often these microorganisms that adhere to the hard surfaces of the body like the tooth shed periodically, thus preventing the accumulation of these bacterial deposits. The teeth provide non-shedding surfaces for the oral microflora to develop extensively. The microflora of the oral cavity leads to the formation of dental caries, periodontitis and gingivitis.

Bacteria are able to adhere to surfaces. This property of bacteria depends upon an intricate chain of interactions between the surfaces the ambience and the microorganisms. Initially a conditioning film is being formed by the hydrophobic macromolecules on an immediately cleaned surface of the oral cavity. This is termed as Acquired pellicle. The composition of this layer is salivary glycoproteins or the mucins and the antibodies. This has the ability to change the free energy of the surface which facilitates easier bacterial adhesion. Bacterial surface has specific structures like Fimbriae and adhesions for attachment on to the host surface. Once these bacteria attaches to surface they are able to multiply rapidly and are able to synthesize newer outer membrane. This bacterial mass increases due to the adhesion of newer microorganisms and rapid multiplication of the adhered bacteria.

As the thickness of the biofilm increases an oxygen gradient is developed due to the rapid utilization of the oxygen by the external layer of bacteria and there is a poor diffusion of oxygen into the lower layers of the biofilm. This facilitates the formation of a completely anaerobic condition. As the periodontal pocket deepens the bacteria starts using the metabolic products that comes from the periodontal tissues and the blood capillaries.
Anaerobic bacteria are able to produce hydrolytic enzymes, with the help of which they can breakdown complex molecules into peptides and amino acids. These enzymes are the main causative agent of the destruction of the periodontal tissues [17].

1.6.1 Specific and Non Specific Plaque Hypothesis

Specific plaque hypothesis explains the pathological changes that occur in the oral cavity. It also states that only certain bacteria are responsible for the development of dental cavities. Non Specific Plaque Hypothesis considers disease, as an outcome of the activity of the plaque and microflora. According to Non specific plaque hypothesis the bacteria present in the plaque produce specific factors that can trigger inflammation and can cause degeneration of the gingival tissues [18-19].

1.6.2 Ecological Plaque Hypothesis

Ecological Hypothesis is a combination of Specific and non Specific Plaque Hypothesis. According to the hypothesis, non mutants species of Streptococcus are responsible for maintaining the balance of the oral microflora. These bacteria are able to ferment carbohydrates and produce acids. The acid helps in the de-mineralization of the tooth enamel.

According to Takahashi, temporary drop in the acidic pH will be restored to normal homeostasis in the supragingival plaque. Once the pH becomes acidic bacteria other than mutans starts to colonize. Non-mutans species is the etiological agent of the disruption of the homeostasis of the biofilm [20].
1.6.3 Various Factor that Influence Dental Biofilm Composition

Dental biofilm is termed as dental plaque or bacterial plaque. Dental plaque can form supra gingivally or in the subgingival site like sulcus or in the periodontal pocket. The composition of the sub gingival plaque depends on the local availability of blood products, depths of the pocket, \( pO_2 \) and redox potential of the site. Dental plaque is a true biofilm composed of bacteria in a matrix which is made up of extra cellular biopolymers and gingival and salivary exudates [21].
1.7 SUPRA GINGIVAL PLAQUE

A thin pellicle of glycoproteins is formed on the tooth surfaces. This pellicle forms an important part in the adherence of bacteria on to the tooth surface. The adherence of bacteria occurs in two stages. The first stage is the reversible state, where the bacteria adhere loosely to the tooth surface. The second stage is the irreversible state where the adherence will become consolidated. A major factor which influences the plaque formation rate is the presence of gingivitis. During the initial stage bacteria tend to resist detachment and will start multiplication. Bacteria that are present in the adjacent region will also multiply simultaneously. At this stage, different types of bacteria will be benefitted by each other. Corncob configuration is seen due to the adherence of cocci on to the surface of filamentous bacteria or on to the surface of bacilli [22-23].

![Corncob formation, SEM image](image)

The presence of lysed bacteria along with dead bacteria will provide nutrients to the viable bacteria present. 25% of dental plaque is made up of inter microbial matrix. Saliva, gingival exudates and bacteria present in the dental plaque contribute to the formation of inter microbial matrix. Extracellular carbohydrate polymers and degenerating dead bacteria also contribute to inter microbial matrix.
A fibrillar component is formed by oral Streptococci due to the utilization of glucans and levans. In other parts of the matrix it appears either granular or homogenous. The microbial matrix in regions where gram negative bacteria are present the matrix is characterized by trilamminar membrane and vesicles. Endotoxins and proteolytic enzymes are present in the vesicles [24].

Fructans is another by product produced during the break down of dietary sucrose. They provide energy when there is a low supply of sugar in the biofilm. Other products produced during the break down of dietary sucrose are dextran and mutan, which is a skeletal structure in the matrix [25].

1.8 SUBGINGIVAL PLAQUE

There are only very few studies done on the biofilm of sub gingival plaque. This is due to the difficulty of getting sample undisturbed from soft tissues of the sub gingival site and the hard tissues of the tooth. A cuticle is formed between the sub gingival plaque and tooth. This is an electron dense region containing remnants of the epithelial attachment lamina along with deposits of gingival exudates. Sub gingival plaque resembles supragingival plaque structurally. Apart from gram positive and gram negative rods and cocci, spirochetes are also found in the subgingival plaque. In the deeper parts of the periodontal pocket there is a distinct difference from the surface layer of the biofilm and the layer adjacent to the tooth surface. The surface layer of the sub gingival plaque is less dense. Luekocytes are seen in between epithelial lining of gingival sulcus and plaque [26].

1.9 MICROBIOLOGICAL ASPECT OF PERIODONTITIS

The oral cavity harbors a wide range of bacteria. The microflora is encompassed of Aerobic and anaerobic bacteria including both gram positive and gram negative bacteria. Even though many of these bacteria make up the normal oral flora they tend to cause opportunistic infections. In both gingivitis and periodontitis anaerobic bacteria is predominately seen [27].
Reports from India and other countries show that there is a high predominance of anaerobic bacteria in periodontitis. From the various studies the prevalence of anaerobic bacteria ranges from 48% to 100% [28]. Whereas, in the case of healthy subjects the prevalence rate is 38% 80%. This difference is mainly due to the culture technique employed, demographic differences, biotechnological method that is employed and on the patient criteria [29].

As periodontitis develops, a shift in the balance of the normal microflora occurs in the subgingival area. In the case of a healthy individual the prevalence of Gram positive anaerobes such as Streptococcus sp along with some obligate anaerobes is higher in ratio. The Gram negative anaerobic bacteria that are being consistently isolated form periodontitic sites are Porphyromonas gingivalis, Prevotella intermedia, Capnocytophagea, some Spirochetes and Aggregatibacter actinomycetomcomitans [30].

1.9.1 Microbial Complex

According to Socransky et al, the bacteria which are present in the Subgingival plaque exists in complexes based on cluster analysis and classified the microflora into 5 complexes. The 5 complexes as told by Socransky are Purple complex, Yellow complex, Green complex Orange complex and the Red complex. Each complex is comprised of a set of bacteria. According to Socransky the bacterial species present in the Red complex showed more prevalence in the pocket depths and that Red complex is closely associated with Orange complex. The relationship of bacterial species within in a microbial complex was based on multiple cluster and community ordination analyses [31].

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<tr>
<th>Purple Complex</th>
<th>Yellow complex</th>
<th>Green complex</th>
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<tr>
<td>Veillonella parvulla</td>
<td>Streptococcus mitis</td>
<td>Eikenella corrodens</td>
<td>Prevotella Peptostreptococcus</td>
<td>Treponema denticola</td>
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<td>Actinomyces odontolyticus</td>
<td>Streptococcus oralis</td>
<td>Capnocytophagea sps.</td>
<td>Peptostreptococcus</td>
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<td>streptococcus sanguis</td>
<td>Campylobacter</td>
<td>Eubacterium</td>
<td>Porphyromonas</td>
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Figure1.5 Socransky’s periodontal complex
1.9.2  *Porphyromonas gingivalis*

These organisms are Gram negative anaerobic non motile bacilli. These bacteria are capable of producing black or brown pigment. These bacteria come under the group of Bacteroides. *P. gingivalis* is able to inhibit PMN migration across the epithelial cells. About 4-6% of the cultivable flora is constituted by pigmented species of anaerobic bacteria [32]. Various studies show that the presence of *P. gingivalis* is lesser in number or absent in healthy subjects, where as frequency of isolates increase in the case of destructive periodontitis [33]. Kawada points out that the presence of *P. gingivalis* is directly proportionate to the pocket depth [34]. In another report by Kamma *et al* increasing frequency of isolates was reported in the case of deteriorating periodontitis and also in progressive periodontal disease [35-36].

*P. gingivalis* is capable of elevating the immunological response in patients with different types of periodontitis [37]. In observations made by Ogawa *et al* showed that in the plasma cells from chronic periodontitis 5% antibodies belonged to the fimbriae of *P. gingivalis*. In many studies on the antibodies against the antigens of *P. gingivalis* showed higher levels in some subjects with detached periodontal tissues but no in all. This suggests that *P. gingivalis* would have entered the periodontal tissues and would have initiated Periodontitis [38].

1.9.3  *Aggregatibacter actinomycetomcomitans*

*Aggregatibacter actinomycetomcomitans* was earlier known as *Actinobacillus actinomycetomcomitans*. These bacteria are non motile Gram negative small rods. When grown on blood agar they are able to grow star shaped centered small convex colonies. This was considered as an important periodontal pathogen due its high isolation rate from periodontic lesions both in aggressive periodontitis as well as from localized periodontitis [39]. *A. actinomycetomcomitans* is considered to be a part of the commensal flora of the oral cavity especially in the supragingival and gingival crevices. These bacteria are divided into 6 serotypes a-f among these serotypes a, b, and c are the predominant ones [40].
The bacteria initially attach to the epithelial cells of the oral cavity and colonize. *A. actinomycetemcomitans* has an adhesion and with the help of which, they attach to the carbohydrate receptor present on the buccal cavity [41]. Fimbriae along with the carbohydrate polymer attach to the hard surface. From the supragingival area they move to the subgingival environment. From here they can attach and invade the epithelial cell of the periodontal tissue and penetrate the connective tissues [42].

### 1.9.4 *Prevotella intermedia/Prevotella nigrescens*

*P. intermedia* is the second most important black pigment producing Bacteroides which is a major etiological agent of periodontitis. *P. intermedia* is gram negative rods with rounded ends. These organisms are mainly isolated from (NUG) Necrotizing Ulcerative Gingivitis [43]. According to Williams *et al* *Prevotella* sp. constitute about 30% of the cultivable oral microflora [44]. About 10-20% of the isolated anaerobic bacteria is made up of the pigmented *Prevotella* species and the non pigmented strains constitute 30% from almost all cases of periodontitis [45].

### 1.9.5 *Treponema denticola*

*Treponema denticola* is spirochete. They are Gram negative helically shaped anaerobic bacilli. These bacteria are also commonly present in periodontal pockets. *T. denticola* has been seen in elevated proportions from tissue biopsy from the affected area [46].

Riviere *et al* used monoclonal antibody detect spirochetes that were present in supra and sub gingival plaque and those present in NUG. “Pathogen Related Oral Spirochetes” were mostly isolated from periodontitis patients [47]. Spirochetes prolong wound healing and delays tissue remodeling after periodontal treatment procedure. Hence it is an ever healing wound that is seen during chronic periodontitis [48].
1.9.6  *Tannerella forsythia*

*T.forsythia* are fusiform Bacteriodes. These are the third commonly present periodontal pathogen. These bacteria are very difficult to cultivate as it requires 7-14 days for the colonies to appear. They are gram negative spindle shaped bacilli and are highly pleomorphic bacteria [49]. The growth is enhanced by co-cultivation along with *Fusobacterium nucleatum*. N-acetyl muramic acid enhances the growth and bacteria appear in short rods instead of pleomorphic [50].

According to Hamlet *et al* adolescents whose gingiva harbored *T.forsythia* had had a greater risk for periodontal attachment loss when compared to whom this species was not detected [51]. It was also noted that those subjects who had colonization of *T.forsythia* had greater chances of loss of attachment, alveolar bone loss and tooth loss than those who do not have *T.forsythia* [52].

1.9.7  *Fusobacterium nucleatum*

*F.nucleatum* is another common bacteria that is being isolated from the periodontal pockets. They are gram negative spindle shaped bacilli [53]. They comprise approximately 7-10% of the total isolate from the sub gingival plaque. These bacteria facilitate apoptotic cell death in both mononuclear and polymorphonuclear cells [54].

There was a high titre of Immunoglobulin IgG and IgM against the Lipopolysaccharide of *F.nucleatum* in the serum of subjects with periodontitis [55]. These bacteria are able to induce release of cytokines, oxygen radical and elastase from the leukocytes [56].

1.9.8  *Eikenella corrodens*

*E.corrodens* is capnophilic Gram negative small bacilli with blunt ends. *E. corrodens* is mostly isolated from sites of periodontal destructions [57]. They are able to stimulate secretion of metalloproteinases and interleukin 6 and interleukin 8.
These bacteria are mainly isolated from subjects with Localized Aggressive Periodontitis [58].

1.9.9 *Peptostreptococcus micros*

These are Gram Positive anaerobic cocci. *Peptostreptococcus* is associated with oral infections. According to Tew *et al* level of serum antibodies is higher in Generalized Aggressive Periodontitis when compared to healthy subjects and Localized Aggressive periodontitis [59].

1.10 IMMUNOLOGICAL RESPONSE IN PERIODONTITIS [60]

Certain microbial constituents are able to activate the inflammatory and humoral immune response and cause damage to periodontal tissues.

1.10.1 Inflammatory Responses

Inflammation of tissue appears red swollen warm and painful. Vasodilation and an increase in the blood flow results in the warmth and redness of the injured site. Oozing of plasma proteins creates an osmotic gradient which retains fluid in the inflamed tissues. This leads to swelling of the affected area. There is accumulation of inflammatory cells. In the case of Chronic and Aggressive periodontitis, patients very rarely feel pain at the inflamed site. This may be due lack of function at the specified site.

1.10.2 Proteinase and Proteinase Inhibitors

During periodontal disease there is tissue degradation. Protease is released from both the host and the bacterial flora. Proteinase enzyme breaks down proteins by breaking the peptide bond. Two types of peptidases are present depending on the site of activity namely endopeptidase and exopeptidase. In several studies a reduction in endopeptidase was noted after treatment. These proteinase increases inflammatory reactions and destroys connective tissues.
Proteinase inhibitor slows down the inflammatory process. The two important proteinase inhibitors are alpha 2 macroglobulin (A2 M) and alpha 1 antitryosin.(A1-AT). A2M is found to inhibit gingival collagenase and A1-AT inhibits Polymorphonuclear Leukocyte collagenase.

1.10.3 Cytokines

Cytokines are produced by both innate and adaptive host response. They are soluble proteins. Cytokines are able to maintain and initiate inflammatory and immune response. Cytokines have negative feedback control on the cells that produced them. Among them the most important cytokines are Interleukins,(IL). IL-1a and IL-1b and Tumour Necrosis Factor (TNF) are able to stimulate and inhibit bone resorption.

1.10.4 Prostglandins

Prostglandins are an important mediator of inflammation. They are able to induce the production of Matrix Metalloproteins (MMPs), which are able to aggravate tissue destruction during gingivitis and periodontitis.

1.10.5 Matrix Metalloproteinases

Periodontium is composed of collagen, glycoprotein, tissue bound growth factor, elastin lipids and water. Neutrophil PMN collagenase is found in a higher concentration in inflamed gingivitis. They are involved in periodontal tissue destruction. The most metabolically active tissue in the body is the periodontal tissue. Periodontal tissues are able to constantly renew matrix components [60].

1.11 FACTORS THAT INFLUENCE PERIODONTITIS

1.11.1 Smoking

Apart from the accumulation of plaque and calculus there are other various factors that affect the oral health status of women. Various habits like smoking, tobacco chewing and usage of pan are found to be a main risk factor for
aggravating periodontitis. Tobacco is one of the various factors that affect the gingiva and periodontal disease in both male and female. This will increase the colonization of the microflora in shallow pockets [61].

The first person to state a relation between smoking and periodontal health was Poindborg in the year 1947 [62]. Smoking alters phagocytosis and neutrophil chemotaxis. Smoking increases Tumour Necrosis Factor Alpha secretion in the gingival cervical fluid. The secretion level of neutrophil collagenase and prostgalandins are simultaneously increased [63]. According to Karl and Hughes smoking will lead to loss of bone density in women [64]. There is difference in weight loss, the intake of caffeine, and the consumption of alcohol [65].

1.11.2 Osteoporosis

Osteoporosis is the reduction of the bone density and changes in the structure of the bone. This leads to fragility and fracture of the bone [66]. The common risk factors of osteoporosis are malnutrition, low calcium intake, consumption of excess alcohol and smoking and reduced physical exercises. During the post menopausal period due to the reduction in the level of estrogen there is reduced calcium absorption into the body. Hence here is a reduction in the bone density and ultimately leads to osteopenia and in severe case to osteoporosis [67].

![Figure 1.6 Bone mass among male and female](image-url)
Osteoporosis is classified into two categories. Primary osteoporosis is associated with increasing age and decrease of sex hormones while; secondary osteoporosis is associated with use of glucocorticoids, any systemic disease which leads to bone loss and low intake of calcium [68].

The mechanism of osteoporosis and periodontitis has been proposed by Jean WW.

1. Bone loss density leads to rapid resorption of alveolar bone when gingiva gets inflamed and colonized by periodontal pathogens.
2. Periodontal infection increases cytokines production which will lead to increase in the osteoclasts activity leading to an increased bone resorption.
3. Genetic factors which influence systemic bone loss which inturn affects the periodontal bones and tissues.
4. Smoking and amount of calcium intake is also a risk factor for the development of osteoporosis and periodontitis [69].

**1.11.3 Osteoporosis and Periodontitis among Post Menopausal Women**

In a study conducted by Snophia Suresh the results shows that the Post Menopausal women had significant higher Periodontal parameters such as probing depth, loss of tissue attachment and alveolar bone loss. It can also be noted that the bone mineral density was significantly lower when compared to Pre menopausal women. From the study a positive association between alveolar bone mineral density and periodontitis can be derived [70].

According to Genco and Grossi estrogen deficiency is a main risk for periodontitis. Deficiency of estrogen leads to an increase in the production of bone resorbing cytokines. When these come in contact with products from periodontal pathogens, it leads to resorbing of bones. The inflammatory response of the host to the biofilm, triggers inflammatory responses, which in turn leads to tissue
destruction, alveolar bone resorption and finally to tooth loss, which explains the increased prevalence of periodontitis in post menopausal women [71].

1.11.4 Hormonal Change and Oral Health Status of Women

Hormonal changes not only bring changes in the reproductive system of women, but also in the oral health and oral micro flora. Puberty, Menopause, Pregnancy and Oral Contraceptives have influence in the oral health status of women. Even though many women do not notice the change, reports show that during menstrual cycle some women experience swelling of gingiva and gingival bleeding [72].

![Relative oestrogen levels from puberty to menopause](image)

**Figure 1.7 Relative oestrogen levels from puberty to menopause**

Oestrogen and progesterone as well as Chronic gonadotrophic are having a high influence on the minute circulatory systems which supply blood to the gingival area. The fluctuation in hormones is due to the anterior pituitary gland secreting Follicle stimulating hormone (FSH) and Leutenizing Hormone (LH) which aids in the maturation of the ovary. The mature ovary secretes the estrogen and the progesterone hormone [73]. The overall effects these hormones have on periodontal tissues are the following
1. Estrogen changes the redox potential of salivary peroxidase, which is active against microorganisms [74].

2. Estrogen can stimulate collagen metabolism [75].

3. Both female sex hormones can modulate connective tissues and vascular responses in the gingival or in the periodontium [76].

4. The hormones can interact with the inflammatory mediators, which explain the increases inflammation during the hormonal changes [77].

Oestrogen and progestrone receptors are found on the gingiva. Receptors for estrogen are present on the fibroblasts of lamina propria, periosteal region and also of the periodontal ligaments.

1.11.5 Influence of Estrogen of Periodontium

Estrogen decreases keratinization, leukocyte production from the bone marrow, T cell mediated inflammation, PMNL chemotaxis and also inhibits proinflammatory cytokins. Meanwhile it stimulates PMNL phagocytosis, cellular proliferation, gingival fibroblasts proliferation, maturation and synthesis of gingival tissues and also helps in the inflammation of gingival tissues [78].

1.11.6 Influence of Progestrone on Periodontium

Progestrone increases vascular dilation, prostglandins production, breakdowns folate which helps in the repair of the issues. It inhibits vascular dilation, collagen and non collagen synthesis and proliferation of human gingival fibroblasts. It also alters collagen fibroblast production pattern and also influences the rate at which it is produced [79].
1.11.7 Puberty

Puberty marks the beginning of adolescent age. Several oral changes are characterized during this phase. The important changes are transition from primary milk teeth to permanent teeth, skeletal change and hormonal variation. When a child reaches 10-12 years the entire set of primary dentition will be replaced by the permanent set. The hormonal changes occurring during this phase of life makes them susceptible to gingival problems [80].

Studies show that during puberty there is change in the oral microbial environment. The female sex hormones Progestrone and oestrogen are seen in increased level during puberty. Some bacteria are able to colonize well at high concentrations of these hormones. In another study microbial change is attributed to a high blood circulation to the gums during Puberty, which leads to gums sensitivity. As a result the gums become tender and show greater irritation to food debris and plaque, leading to gingivitis [76].

When compared to a healthy gum at puberty there is an increased evidence of the presence of Bacteroides and other Gram negative anaerobes in the subgingival biofilm [81]. A noted increase in the proportion of Prevotella intermedia and Capnocytophagea spp is seen during puberty. Though there is no significant accumulation of plaque gingival inflammation bleeding on probing is seen during puberty [82].

1.11.8 Menopause

Menopause is usually accompanied by some physical and microbial changes in the oral cavity. There is a rapid decline in the level of estrogen when a woman reaches menopause, which in turn leads to bone loss. According to Mine Tezal et al the relation between decline in level of estrogen and bone loss is best explained by the severity of periodontitis and lower bone density level in Post menopausal women. In a cohort study conducted by Mine Tezal et al., he concluded that periodontal disease due to bone loss is a predictor for tooth loss in Post
Menopausal women [83]. Shapero states that there is low intake of calcium due to the reduction in the estrogen level which results in the bone change in the post menopausal women in the mandibular region [84]. Women at menopause and post menopause, experiences various discomfort in their oral cavity. Menopausal gingivostomatitis affects the gums. The gums becomes dry or shiny or either pale or deep red in appearance and they bleed easily [85].

Low oestrogen production increases the production of interleukins 1 (IL1), IL-6, 8 & IL-10. It is also associated with the increase of tumour necrosis factor and granulocyte colony stimulating factor. These factors are able to stimulate osteoclast and modulate proliferation of bone cells. They induce resorption of alveolar as well as skeletal bone [86-87].

1.11.9 Pregnancy

“A tooth for every pregnancy” is a very popular notion. The most common oral manifestation associated with pregnancy is gingivitis. Calcium is drawn in large amounts from the maternal bones and teeth to meet the fetal requirements. Though calcium is present in the teeth as stable crystalline form it is mobilized easily to meet the demands of the fetus. There is exaggerated inflammatory response to local irritants due to change in hormones and blood circulation to the gums during pregnancy [88].

The fiery red marginal gingiva and formation of interdental papillae is characteristic of inflamed gingiva. There is an increase in the depth of periodontal pocket. From the second month of pregnancy gingival changes can be noticed and increases till the eighth month of gestation. There is a reversal of gingivitis after childbirth, but need not return to a healthy condition. If pregnancy gingivitis is left untreated it may progress with lower severity [89].

Studies have revealed that Periodontitis during pregnancy is related to Pre term delivery (37 weeks of gestation) and Lower birth weight of the baby. In a study conducted by Carrillo-de Albornoz et al. they found that in pregnant women
there is a high microbial flora in the oral cavity when compared to post partum women. Gingival inflammation was found to be more severe in pregnant women with high periodontal pathogens. The study also shows that in the case of non-pregnant women there is no observable difference between the two check ups made at a 6 month interval. But in the case of pregnant women they showed significant difference during the first trimester [90].

Evidences from researches shows that about 36-100% pregnant women suffer from pregnancy periodontitis. Gingival inflammation is initially caused by plaque and advanced by endogenous sex steroid hormones. There is a 10 fold increase in progesterone and 30 fold increase of estrogen during pregnancy when compared with the normal menstrual cycle due to continuous production of these hormones [91].

Pregnancy gingivitis does not have much difference from other gingival inflammation, but only that there is no change in the plaque levels [92]. There is an increase in the prevalence of anaerobic bacteria *Prevotella intermedia*, as they are able to substitute vitamin K and Hemin with progesterone and estrogen. A high level of the hormone progesterone during pregnancy increases the permeability and dilation of the capillary system, which in-turn increases the gingival exudates [93].

1.11.10 Pregnancy Epulis

A pedunculated granulomatous lesion forms on the gingiva during pregnancy. This is referred to as epulis. This growth is mainly seen on the anterior papillae of the maxillary region of the teeth. There is a high risk of recurrence even though it is surgically removed during pregnancy.

Pregnancy granuloma or pregnancy tumour is also seen, in addition to generalized gingivitis. The tumor grows fast and reaches a maximum size of 2 cm. Post partum the tumor is removed surgically for complete recovery [94].
Low Birth Weight and Pre Term Birth Delivery

According to Namiro et al, Low birth weight and Pre term birth are most prevalent in developing countries than developed countries. In developed countries mostly pregnancies are planned and hence the complications are less. Two third of the world’s pre term birth are accounted from South Asian countries and African continent [95].

Varghese Chacko et al showed a relationship between the oral health of the mother and the infant. Pregnant women had little knowledge on infant oral health. According to the survey conducted by Varghese Chacko et al pregnant women were ignorant about antenatal dental check ups [96].

Clinical trials on bacteria associated with periodontitis, done at Center for Oral and Systemic Disease, University of North Carolina reported that there is a high prevalence of Porphyromonas gingivalis, Bacteroides sp., Aggregatibacter actinomycetomcomitans and Treponema denticola among mothers of LBW and PTB babies [97].

Laboratory as well as clinical studies has shown that mothers who have pre term delivery had intra uterine infections [98]. The most common route of intrauterine infection is ascending route through vagina. Hematogenous spread of infection from other parts of the body is another route of infection. Anaerobic bacteria like Fusobacterium nucleatum and Capnocytophagea sp. are of oral origin.
Reports of various studies points out that in majority of intrauterine infections *Fusobacterium nucleatum* is the most frequently isolated bacteria [99].

1.11.12 Oral Contraceptives

A major oral manifestation among Oral contraceptive users is found to be gingival inflammation. Long time users of oral contraceptives showed Aggressive periodontitis. The gingiva becomes fiery red and the patients suffer from hemorrhagic gingiva. Drastic change in the saliva has been observed in Oral contraceptive users. There is a decrease in sialic acid, hydrogen and other electrolytes. Studies also show that there is a decreased flow in saliva [100].

Brian H. Mullay *et al* reports that there is poor periodontal health among Oral contraceptive users. Aggressive periodontitis was prevalent among the group of young females who were on Oral contraceptives. They also had deeper periodontal pockets, gingival bleeding and there was a severe case of periodontal attachment loss when compared with those who were not on the pills [101].

According to Kornman and Loesche periodontal pockets shows the presence of progesterone and estrogen hormones and that these hormones influenced the bacterial colonization of the oral cavity [102]. Vittek *et al* was able to demonstrate the presence of receptors within the gingiva for the female sex hormones [103]. Kalkwarf reported that though there are low levels of plaque formation, there is an increased level of gingival inflammation [104].

Advanced formulations of the pills which contain lower levels of progestins and estrogen have lower risks, but long term usage may lead to periodontitis [105].

1.12 PERIODONTITIS IN INFERTILE WOMEN

Infertility is seen in 8-15% of couples at the reproductive age group. Among these 58% females and 25% male are having health problems and for 17%
of couples the reason for infertility is unknown. The treatment for infertility has increased drastically in the recent years. This may be due to successful and newer techniques and awareness among the population. The women who are undergoing treatment are mainly put under drugs like Human chorionic gonadotrophins, Follicle stimulating hormone, Pulsatile gonadotrophin releasing hormone and Clomiphine citrate. The latter is a non steroidal estrogen antagonist which triples the production of estrogen and progesterone.

In a study conducted by Godavarthi on Periodontal status in infertile women, the results show that women who had a history of infertility treatment and women who are undergoing infertility treatment had significant clinical attachment loss when compared to control group. Sulcus bleeding Index was also found to be in significantly higher proportion among women who are undergoing the infertility treatment [106].

According to Haytac et al periodontal diseases which are induced by the ovulation drugs may have an adverse effect on the success of the treatment. Hence the subjects should be given awareness on effective plaque control which could minimize gingival inflammation [107].

1.13 EPIDEMOLOGICAL STUDIES OF ORAL HEALTH STATUS IN INDIA

According to Vos T., et al chronic periodontitis is one of the most common oral infections which affects nearly 750 million people which accounts to about 10.8% of the population as per records of 2010. Like diseases which are intimately related to personal hygiene and basic medical check up and care, periodontitis is seen to be common in economically backward populations or areas. Its prevalence decreases with a higher standard of living conditions [108]. In Israel, Yemen, North-African, South Asian, and Mediterranean region have a
higher prevalence rate of periodontal disease when compared to individuals from Europe [109].

Sharma et al conducted a cross sectional study in Moradabad population, Uttar Pradesh, India on the prevalence of aggressive Periodontitis in relation to systemic manifestations like anxiety, depression, loss of weight and loss of appetite. In his study the sex ratio was 2.65:1 (male: female). He noted that 57% females had aggressive periodontitis which was in higher proportion when compared to males. He also noted that the aggressive periodontitis is associated with systemic manifestations [110].

According to report submitted by Shah to the National Commission on Macroeconomics it is stated that 40-45% of population of India may suffer from advanced periodontal pocket formation due to alveolar bone loss. Shah also points out that only 7 studies are documented with total incoherent data [111].

Ramfjord et al in a WHO survey discussed the prevalence rate of periodontal disease inclusive of gingivitis in India was 100%. According to him, this is not because of age, sex, the geographical conditions economic status and nutritional condition but is aggravated due to the accumulation of calculus and plaque along with debris [112].

Rao SP noted that there is a difference in the prevalence of periodontitis in rural area when compared to the urban area. According to him the prevalence rate is less in urban area. This may be due to the fact that there is a better health care system in the urban area [113]. At present the oral health services are present only in around 20% of the community health centers. This is seen till the tehsil level hospitals [114].
1.14 HORMONE REPLACEMENT THERAPY

The bone density in women can be increased by hormone replacement therapy. According to Kimmel et al there is an increase in the bone density within 2 years of treatment. Controversies have been seen in the use Hormone replacement Therapy due to the risk factors. The main controversy associated with HRT is endometrial cancer. And long term use of same has been associated with breast cancer. However modern combined formulations can reduce the risk to a great extent [115].

Bisphosphonates are known to increase bone mass among estrogen deficient patients. Bisphosphonates bind to bone hydroxylapatite crystals and are able to regulate mineralization thus preventing dissolution. They inhibit the activity of osteoclast. An early diagnosis of osteoporosis among menopausal women can be done by dentists. A dental check up for alveolar bone resorption will help menopausal women in checking their bone loss density. According to Ronit Haimov et al women who attained their menopause tend to lose their bone mass at 1-2% per year. Some women lose their bone mass rapidly at a rate 5-6% per year [116].

Post Menopausal Estrogen users were found to retain more teeth when compared to Non-estrogen users. Estrogen replacement Therapy studies were done on animal models [117]. In a study done by Reddy MS et al on periodontitis on beagle dogs there was no difference on gingival inflammation and clinical attachment loss on dogs who had estrogen replacement therapy and those who did not therapy. But it can be noted that there was a significant difference on the alveolar bone mass on beagles that had estrogen replacement therapy [118].

Hormone replacement therapy (HRT) has been reported to have side effects. In some reports, it has been said that there was mandibular avascular necrosis after undergoing Estrogen Replacement Therapy. There was an increase in
the risk of having bone necrosis following tooth extraction. This condition is termed as bisphosphonate associated bone necrosis [119].

The main controversy associated with HRT is endometrial cancer. And long term use of same has been associated with breast cancer. However modern combined formulations can reduce the risk to a great extent [120].

Even though Bisphonates increase bone mass through stimulation of osteoblast differentiation prolonged use is not recommended. Some reports have shown there are systemic side effects to the use of Bisphosphonates. Esophagus ulcer formation, low serum calcium levels and gastric irritation. This prevents long term usage of HRT [121].

Research on tetracycline’s modified non antimicrobial analogue has shown that they are able to inhibit matrix metalloproteinases. Matrix metalloproteinaes like collagenases and gelatinases which are able to assist in tissue destruction. These drugs also increase osteoblast activity, production of collagen and formation of bones [122-124].
1.15 AIM OF THE STUDY

(i) To determine the prevalence of periodontitis in women, covering comprehensively the entire life cycle of women, namely pre pubertal, pubertal, post pubertal, child bearing age, pregnancy and menopause.

(ii) To study the association of osteoporosis and periodontitis in the vulnerable population subset of elderly women.

(iii) To ascertain the aerobic and anaerobic bacterial spectrum in periodontal disease in women.

(iv) To confirm the identity of seven obligate anaerobes with Real Time PCR. In order to reach the above objectives, a null hypothesis was formed: the different stages in women like puberty, pregnancy and menopause do not have a direct influence of the periodontitis

1.15.1 Outcomes of the Study

1. The burden of the disease would be defined, so that suitable infrastructure for prevention and treatment could be planned.

2. The awareness created by this study is to help women to improve their oral health care.