CHAPTER 2

REVIEW OF LITERATURE

2.1 INCIDENCE OF PERIODONTITIS AMONG CHILDREN AT PUBERTY

According to Chauhan et al, among 12 year old children in Himachal Pradesh 60% female were affected by oral health problem. He also noted that girl children showed a higher percentage of health problems when compared to boys. Gingival bleeding was seen among 12.9% and calculus deposit was seen in 21.8% children. From his study he noted that children from rural area had unhealthy gingiva 23.9% when compared to those of urban area 17.1% [125].

A study conducted by Mahesh Kumar among 5 years and 12 years school going children in Chennai city, showed girls had lesser oral problems when compared to boys with a statistical significance of p=0.008. He also noted that children of both genders coming in the age group of 5 years had good oral hygiene when compared with adolescent children of 12 years. He also noted boys had a high Oral Hygiene Index Score when compared with girls in among 5 year olds. There was a reverse in this pattern among 12 year old children, where girls had a high OHI and showed increase in the rate of dental caries [126].

A study conducted by Dhar V. et al in Udaipur Rajasthan in 2007, on pubertal children of 14 years age it was noted that female children were more affected when compared to boys of the same age. Population size for the study was 1,587 out of which 760 were girls. The prevalence of gingivitis in the study was 84.37%. In his study he found that mild gingivitis prevalence was high among 5-7 years, whereas severe gingivitis was found to be more among 8-10 and 11-14 years. He attributed this change to mixed dentition of primary and permanent teeth, food habits, falling of primary dentition and oral hygiene. He concluded his study stating that highest prevalence of severe gingivitis was among 11-14 year olds [127].
In Kerala an epidemiological study was conducted by Jose among children of rural area. The study population was 1068. In his study he chose 578 female children between the age group 12 years to 15 years. He reported a lesser prevalence rate for gingivitis of 15%, which is lesser compared to other reports from India. In another finding from this study, he noted that 50% of children suffer from some form of dental diseases. He also discussed that gingivitis prevalence increased as age advanced. He also pointed out that girl in the age group 12-13 years were most affected by gingivitis [128].

Vyshalee et al conducted a study among school children in rural area of South India. According to her study it was found 45% of students had good Oral hygiene and 38% had poor oral hygiene. In her study population only 17% brushed twice [129].

Nakagwa et al conducted a study from prepubertal to pubertal children to find out the influence of sex hormones on gingivitis. In his study he confirmed that there was an increase in *Prevotella intermedia* and *Prevotella nigrescens* along with increase in the inflammation of gingiva. He stated that there was a significant relationship between Gingival Index and the level of sex hormones among those who are having gingivitis and in girls who are in the healthy group. But there was no significant relationship between the clinical parameters and the hormonal level [130].

Kobyashi et al tried to establish relation on the periodontal bacterial colonization pattern among Japanese children and their mother. Sample size for his study was 78 children and 68 mothers. The prevalence of *Capnocytophagea* spp. among children was found to be higher when compared with other periodontal bacteria like *Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* [131].
2.2 INCIDENCE OF PERIODONTITIS AMONG PREGNANT WOMEN

The prevalence rate of periodontitis among pregnant women depends on various factors such as age, socioeconomic status, ethnicity and the personal habits of each individual [132]. According to a study conducted by Chaithanya et al it was noted that the there was a high rate of periodontitis and presence of periodontal pathogens among women within the age group of 25-29 yrs. It was also noted that there was a direct relationship between the education status of the pregnant women and their periodontal health status [133].

In a study conducted in Sri Lanka on the prevalence of periodontitis by Karunachandra et al showed that the depth of periodontal pocket in pregnant women was in between 4mm -5mm [134]. Al Habashneh reported that the prevalence of gingival prevalence of pregnant women ranges from 36% to 100% [135]. Cross sectional studies was done on the relation between gingival disease and pregnant women as early as 1960s. According to Silness and Loe as pregnancy advances gingival disease also increases and persist till delivery. Post partum decrease in the gingival inflammation was noted [136].

Fernanda Campos Machado detected and enumerated periodontal pathogens from subgingival biofilm in pregnant women. He used FISH (Fluorescence in situ hybridization) technique to determine the quantitative and qualitative difference among 8 periodontal pathogens.

Fernando et al suggested that due to hormonal changes in pregnancy, there was an increase in the growth of Prevotella intermedia. Apart from this Campylobacter rectus and Prevotella nigrescens was also found high among pregnant women. He was also of the opinion that other anaerobic bacteria should be evaluated as they will be having some influence on preterm birth. In his study he had taken twenty pregnant and twenty non pregnant women. His study he noted that there was no marked difference between pregnant and the non pregnant group. He also did not find any significant difference in the isolation of Prevotella intermedia.
between pregnant and non pregnant women, even though they were isolated in high number [137].

Jagjit et al in 2013 did an evaluation on the various socio-demographic variables which may affect the periodontal health of pregnant women. The total number of subjects included for this study was 190 pregnant women who attended Govt. Medical College, Chandhigarh. In his study he did not find any statistical significance in plaque index score among pregnant women of various socio economic statuses. But he got significant differences on Bleeding on probing among pregnant women of different socio economic statuses. In his study he found that very few pregnant women had periodontal related issues. Jagjit et al attributed this to their maintenance of good oral hygiene [138].

A study was conducted in rural area of Belgaum, India among pregnancy related periodontitis and low birth weight. The aim of their study was to find out if there was any relation between low birth weight and periodontal disease. The sample size for the study was 240. In his study he found that education of the women had significant association with periodontitis and low birth weight [139].

Periodontal status among pregnant and non pregnant women was done by Surekha R et al, in Karnataka, India. She did not find any significant difference in the oral hygiene status in both the groups. Plaque index, gingival Index and clinical attachment loss had a significant difference among pregnant and non pregnant women. She noted that there was severe gingivitis and periodontitis among pregnant women [140].

In a similar study done in Faridabad city, Haryana by Gunjan S. et al., they found that 47% of pregnant women attending various Government Hospitals wound to be suffering from pregnancy related periodontitis. In this study, the sample size was 800 pregnant women. Out of which 168 belong to the age group of below 20 years, 556 belonged to the age group of 20 to 30 years and the rest belonged to the group above 30 years of age. They observed that 10.25% had some medical ailments during pregnancy. 700 (87.5%) had oral health related problems and 124
had bleeding gums. Out of 800 subjects 424 had a Community Periodontal Index 2. Calculus was found more in pregnant women within the age group 20-30 years. In pregnant women above 30 years the percentage of subjects with periodontal pockets of 4-5mm was 76.31%. 17-B estradiol and progesterone are known to alter the oral microbiota [141].

2.3 UNEVENTFUL OUTCOMES OF PERIODONTITIS DURING PREGNANCY

Marianna Vogit did a study to assess the factors associated with the prevalence of periodontal disease in low risk pregnant women in Brazil. The study population of her study was 334 pregnant women within the age group of 18 to 42. Out of this 157 were grouped as with Periodontal disease and 177 without periodontal disease. In this study they found high prevalence of periodontitis during pregnancy. 47% who had periodontitis belonged to the low income group. Bleeding on probing was found among 97.3% of women. Progression of pregnancy showed an increased damage to the periodontium. According to her bleeding on probing may act as a predictor for a later clinical attachment loss. She also suggested that for the well being of the prospective mother and her baby oral health should be looked into from the early stages of pregnancy [142].

Louis Muwazi did a study on post partum women on their periodontal conditions, low birth weight and preterm birth in Uganda, Africa. In their study they found that 26% of post partum women had gingival bleeding and 29.4% women had a 4mm or more periodontal pocket. They also found a significant association between gingival recession and low birth weight on bivariate analysis. But other parameters of periodontal disease did not show any significance with undesired pregnancy outcomes [143].

Pulikottil Shaju Jacob did a study in rural India to find out relation between low birth weight babies and periodontitis. He did a case control study with 340 post partum women. Among these the case consisted of 170 mothers who had babies weighing below 2,500gm and the control included mothers who had babies
weighing more than 2,500 gm. In his study he found that mothers who had Low Birth Weight babies, the frequency for periodontitis was found to be 58.9%. Among controls he noted that there was a reduction in the frequency of periodontitis to 27.6%. This difference was found to be statistically significant [144].

In a review by Aous Dannan, on Periodontal disease as a risk factor for Pre term birth and low birth weight supports the hypothesis that pregnant women who had periodontitis can be at risk in having a preterm birth or low birth weight babies [145].

A similar study on maternal periodontal disease as a risk factor for pre term delivery was done by Vitool Lohsoonthorn in Thailand. In his study he found there was no association between periodontal disease and the incidence of pre term birth among Thai women. He also did not find any linear increase in risk of preterm delivery with an increase in the severity of periodontitis. Periodontal pathogens may have access to fetoplacental tissues through blood. They trigger inflammatory responses and prostaglandin cascades, which induce pre term birth. He also quoted that some investigators were able to isolate periodontal pathogens from placental and fetal tissues [146].

In a study including 59 preterm delivery cases with a control 44 subjects done by Goepfert et al, the isolation rate of periodontal pathogens from preterm placentas was 2 out of 59. [147]. In control group he was able to isolate periodontal pathogens from 3 out of 44 cases. Dortbudak did not isolate periodontal pathogens from amniotic fluid [148].

### 2.4 EFFECTS OF PERIODONTAL THERAPY DURING PREGNANCY

R.Ovadia did a study to find out a relation between periodontal treatment and pregnancy. He did a controlled pilot study on second trimester pregnant women, to find out the effects of scaling, root planning and effects of the use of sonic tooth
brush on the incidence of pre term delivery. In his study he found that periodontal care and treatment during pregnancy reduced the incidence of pre term delivery. He also pointed out that if there is lack of periodontal treatment during pregnancy there was an increase in Gingival cervical fluid (GCF) IL-1β and GCF IL-6 levels, probing depth and plaque index. According to him treatment of periodontitis during pregnancy was safe and it improved periodontal health. In his study there was 3.8 fold reduction in the incidence of pre term delivery, a decrease in the periodontal pathogen load and a decrease in the levels of GCF. Chronic intrauterine infections have been linked with preterm delivery and increase in the C-reactive protein levels [149].

### 2.5 INCIDENCE OF PERIODONTITIS AMONG MENOPAUSAL WOMEN

Several studies have shown the relationship of bone mineral density loss and alveolar bone loss as a key factor for periodontitis. As such there is no direct study for the incidence of periodontitis among post menopausal women.

Maria Clarinda et al conducted a study to find out the effect of menopause on oral health. According to her, women are more vulnerable to oral health related problems after menopause. She is also of the opinion that when there is a reduction in the estrogen level, the calcium level in the bones and teeth is also decreased simultaneously. There may be a possible relationship between the density of bone in the jawline and skeleton apart from jaw. Hormone Replacement therapy helps in the preservation of bone in alveolar region. She also did a review on the influence of cytokines and periodontitis on systemic bone loss. According to her both osteoporosis and periodontitis are mediated by the same cytokines. She pointed out that removal of dental plaque and regular dental checkups will help in the reduction of osteoporosis which in turn will help in the reduction of periodontitis [150].

In a study done by Taguchi et al, on women who use Hormone therapy, he did not find any significant difference between the number of teeth present
among users of hormone and in non users. He attributed this to the, to the duration of therapy may have an influence on the number of teeth. Apart from this the number of subjects he included in his study was younger when compared to the other similar studies that have been done [151].

Mine Tezal et al made a study on post menopausal women on periodontal disease and incidence of tooth loss. In his study he noted that the mean alveolar Bone Loss had significance to the number of tooth lost. The follow up period of this study was one year. He noted that during the follow up examination there was 5% of tooth loss, one tooth per person. This is more predictive among post menopausal women when compared with other populations [83].

Laura Tarkilla did a two year follow up study on microorganisms that are associated with periodontitis in peri menopausal and post menopausal women irrespective of whether hormone replacement therapy is taken or not. He observed that there was a significant reduction in the number of \textit{T.forsythia} positive cases after 2 year follow up examination in both the study groups. \textit{Prevotella intermedia} also showed a significant reduction among women who are on Hormone Replacement Therapy who had periodontal pocket with 4mm depth. No significant difference was noted among non HRT users. In vitro studies shows oral microorganisms are capable of metabolizing sex hormones. Gingival inflammation may be induced due to metabolic products of progesterone. In this study he found that \textit{Treponema denticola} utilize host derived steroids. This may be attributed to the virulence nature of \textit{T.denticola}. He stated in his study that HRT reduces the incidence of periodontal bacteria significantly [152].

In a similar study done by Ricardo CA et al in Portugal on menopausal women and periodontal disease, he noted that premenopausal women had more number of teeth than menopausal women. In his study the subjects into two groups, consisting of 68 post menopausal women and 34 premenopausal group which made up the control group. After statistically adjusting tobacco smoking and plaque index, the incidence of tooth loss had statistical significance on menopause. But in the case of periodontal measurements there was no statistical difference among both the
groups. Sites for pocket depth was more in the case of Post menopausal group when compared with premenopausal women [153].

2.6 INFLUENCE OF OSTEOPOROSIS ON PERIODONTITIS

Snophia et al did a comparative study on bone mineral density of premenopausal and post menopausal women and status of periodontitis. The study was done in Tamil Nadu among women coming under the age group 44-55 years. The results of their study showed high incidence of osteopenia and osteoporosis especially in lumbar spine and femur region in post menopausal women who had periodontitis. This suggests alveolar bone loss can be taken as an indicator for systemic osteoporosis. Different clinical parameters like probing depth, clinical attachment loss showed statistical significance when compared with premenopausal group [70].

A review was done by Vahid Esfahanian et al on the relation between osteoporosis and periodontal disease. 17 studies were reviewed in this study. According to the study a mixed result of association of osteoporosis and periodontitis and no association was also seen. It was concluded that in subjects who already has periodontitis [154].

2.7 PREVALENCE OF PERIODONTITIS IN INDIA

A prevalence study on periodontal diseases in India was done by Vipin Agarwal et al in 2010. In 1995 the Ministry of Health and Family Welfare included National Oral Health care in principle. In rural India, dentist ratio is too less. According to Vipin Agarwal et al Indian population is approximately 1000 million, out of which rural population mounts upto 72%. He also quoted that the dentist: rural population ratio is 1:2.00, 000 [155].

Another prevalence study on periodontal disease was done by Shaju Jacob in 2011. According to him, there is lack of proper data in Indian population regarding prevalence of periodontitis. There is a close relationship between lifestyle and oral disease, like tobacco chewing, alcohol consumption and dietary habits. He
also suggested that impact of tobacco in all forms especially in developing and under developing countries will lead to an increase in the prevalence of oral disease like periodontitis, tooth loss and oral cancer. He pointed out that there should be a general representative survey in district level inclusive of all the districts in India, to study the prevalence of periodontitis [156].

Keerthi Sharma et al conducted a study to find out the prevalence of aggressive periodontitis along with the systemic manifestations in Moradabad district of Uttar Pradesh, India. She took a sample size of 3000 patients which included 1872 male subjects and 1128 female subjects. The mean age of the subjects was 30.99. In her study the proportion of gingivitis was 61.1%. 53.91% of males had chronic periodontitis where as among females 57% had aggressive periodontitis. She also noted that systemic manifestations like fatigue, anxiety/depression was greater among subjects who were suffering from aggressive periodontitis [157].

Chaithanya et al did a prevalence study in south Indian pregnant women on clinical periodontitis and periodontal pathogens. The study design he adopted was a cross sectional hospital based study. The study population included 390 pregnant women of a gestation period between 8-24 weeks. In this study he note that *Tanerella forsythia*, *A. actinomycetomcomitans*, *Eikenella corrodens* and *Capnocytophagea* spp., had no significance with gingivitis and periodontitis. According to him this difference may be due to geographical difference and may depend on the depth of periodontal pocket. From this study the magnitude of periodontal infection and gingivitis could be determined among South Indian Pregnant women [133].

2.8 PERIODONTAL PATHOGENS AND THEIR ASSOCIATION

N.Suzuki et al made a review on the role of Red Complex bacterial infection in causing periodontitis. The red complex mainly includes *Porphyromonas gingivalis*, *Treponema denticola* and *Tanerella forsythia*. According to him most of the therapeutic modalities in the treatment of periodontitis target for the complete removal of bacteria from the periodontal pockets. He pointed out that for better
treatment of periodontitis, the different association among the bacteria should be understood. There should be disruption of relation between the pathogenic bacteria and the host. There is difference in the oral microbiota of healthy and periodontitis subjects. According to him various models show synergestic pathogenesis, association of Porphyromonas gingivalis and Fusobacterium nucleatum, P. gingivalis and Tannerella forsythia, P. gingivalis and Aggregatibacter actinomycetomcomitans and also between P. gingivalis and Treponema denticola [158].

Daniluk T., et al did a study on aerobic and anaerobic bacteria in the subgingival and supra gingival plaques of periodontal patients. He pointed out the the bacterias of the red complex like Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola are closely associated with bleeding on probing and also responsible for increase in the depth of periodontal pocket. In his study he noted that the microbial flora was different in both supra gingival plaque and subgingival plaque. He also noted that Peptostreptococcus spp were in high prevalence in both supra and sub gingival plaque [30].

Aggregatibacter actinomycetomcomitans is having the ability to adhere to any surface in the oral cavity like the tooth, epithelial cells and other oral bacteria. They can also adhere to extra cellular matrixes. It is not confirmed whether A. actinomycetomcomitans is having more affinity towards soft or hard surfaces. According to Sliepen et al bacterial count was more on soft surface when compared with hard surfaces [159].

Gursoy M. et al, in his study tried to find out whether the frequency of Prevotella intermedia. The study population consisted of 30 pregnant women from Finland. The control group was non pregnant women which included 24 subjects. According to him Prevotella intermedia and Prevotella nigrescens should be separately studied. They studied 20 different strain of Prevotella intermedia with 16S ribosomal PCR. From the pregnant group 1817 isolates were obtained, out of which 95.3% were identified as Prevotella nigrescens and 2.5% were Prevotella intermedia. In the case of Non pregnant group 811 isolates were obtained. Here, the percentage of Prevotella nigrescens was 94.2% and that of P. intermedia was 5.5%.
In this study observed that among Finnish pregnant women the prevalence of *Prevotella nigrescens* was high when compared with *Prevotella intermedia* [160].

Tateishi F., *et al* in their study on the detection of *Fusobacterium nucleatum* in high risk pregnant women in chorionic tissues. Both oral tissue sample and chorionic tissue sample were taken from 24 high risk pregnant women. The control group consisted 15 normal pregnant women. PCR was used to detect *Fusobacterium nucleatum*. He observed that *F.nucleatum* was present in high risk pregnant women, and induced IL-6 and corticotrophin hormone (CRH). The Lipopolysaccharide (LPS) present in *F.nucleatum* stimulate the production of IL-6 and CRH. CRH coordinate and regulate the physiological condition during parturition. The levels of CRH will be high in the case of preterm birth when in comparison with the levels during term birth [161].

### 2.9 PERIODONTAL BACTERIA

Magda Feres *et al* emphasized that periodontal pathogens do not cause infection alone. Subgingival area does not contain pure culture of any particular type of bacteria. The host-beneficial bacterial presence and their levels are important for the initiation of periodontitis as well as for the control of post therapeutic infection. *Veillonellaparvula Actinomyces sp.*, *Streptococcus sanguis* and *Strep.mitis* are considered as host- beneficial bacteria. These bacteria are mainly isolated from sites that had less active disease and they responded to periodontal therapy. Species within the periodontal complexes had close association with one another. Red and orange complex showed close association. A strong interaction was found among Green, Yellow and Purple complex. This could indicate a possible pattern or a sequence. He examined different types of therapies apart from the use of antibiotics, on both clinical and microbiological aspects of periodontal disease. He considers that control of supra gingival plaque is very crucial in controlling periodontal disease. Another form of mechanical therapy is SRP (Scaling and Root Planning). Clinical benefits of SRP are reduction in inflammation and decrease in periodontal pocket depth. According to him surgical therapy is more effective than non surgical procedures in reducing pocket depths effectively. Pocket depth of 6mm responded
favourably to both modes of treatment. Periodontal surgeries are mainly performed for regeneration, esthetics and also for the control of infection. He also noted that patients who had SRP therapy showed disease progression when compared with those who had surgical procedures [162].

Mohammed Hossein Salari et al conducted a study on the rate of cultivable subgingival periodontopathogenic bacteria from patients suffering from chronic periodontitis. Even though more 400 oral bacterial species have been identified only very few have been recognized as periodontal pathogens. The sample size for his study was 203 patients, out of which 111 were females and 92 males. He used conventional methods for identification of bacteria to species level. He also made use of commercially available rapid test. He was able to identify both anaerobic and capnophillic bacteria. There was no significant difference between the rate of isolation of the bacteria in males and females. In his study he found 74.9% of samples had monvalent bacterial growth. Polyvalent growth was shown by 18.2% of samples. In his study he found Aggregatibacter actinomycetomcomitans, Porphyromonas gingivalis, Eikenella corrodens and Capnocytophagea sputigena was isolated in highest proportions. In his study he found Peptostreptococcus micros and Fusobacterium nucleatum were isolated with least proportions. He noted that there is high prevalence of anaerobic bacteria in patients who are having the deepest periodontal pockets. He pointed out that there are likely uncharacterized bacteria which may have a role in the initiation of periodontal disease [163].

2.10 IMMUNOLOGICAL CHANGES

Mahmoud Roubhia did a review on the interaction between host and oral commensal in both the disease and in the health status. He stated that keratinocytes acts as immobile immunocytes. They are able to secrete different types of pro inflammatory cytokines. These cytokines can help in immunity against the periodontal pathogens. When microorganisms come in contact with the epithelial cells, they produce antimicrobial proteins and defesins. They are produced by neutrophils, epithelial cells and macrophages. In normal healthy conditions, the host maintains a non destructive inflammatory barrier. He pointed that it is unknown how
the host’s immune system detects normal commensal of the oral flora as pathogenic. He is of the opinion that as the microflora changes into pathogenic state from the commensal state, it breaks down the oral homeostasis and induces significant changes in the tissue structure [164].

2.11 VARIOUS TYPES OF CONVENTIONAL METHODS EMPLOYED FOR DETECTION OF ANAEROBIC BACTERIA

Nguyen Doan et al made a comparative study on three anaerobic culture systems. The three anaerobic systems he compared were (i) Coy Anaerobic chamber (ii) BBL Gas Pak System and (iii) AnaeroPack System. The coy anaerobic chamber is a glove box which is supplied with 85% N$_2$ and 10% H$_2$ and 5% CO$_2$. The chamber is also supplied with heated palladium catalyst. In BBL Gas Pak system a 2.5 liter jar is used. An anaerobic gas Pak envelope is used. The envelope was pre activated by adding 10 ml of water to the envelope. With the help of BBL anaerobic indicator strip, the anaerobic conditions were monitored. The CO$_2$ concentration in the jar is about 4-10%. In Anaero Pack system a rectangular container and Anaero Pack sachet is used. Here the concentration of CO2 is approximately 18%.

They found that all the three systems were efficient in isolating *Porphyromonas gingivalis* and *Prevotella* spp., *Campylobacter* spp., *Peptostreptococcus* spp., *Tannerella forsythia* and *Eubacterium* showed better results with Gas Pack system and Anaero Pack system. He concluded his study by suggesting Gas Pak anaerobic system is convenient in Oral Microbiology laboratories. According to him it is a more convenient and cost effective method for isolation of periodontal pathogens [165].

WEC Moore suggested that most of the anaerobes can be identified based on five characteristics. According to him characteristics that determine the genus are spore formation, based on gram staining, arrangement of flagella, general morphology and biochemical reaction. In order to differentiate between *Peptococcus* and *Peptostreptococcus* the fermentation products will be of use. He also said that fermentation products are reliable method that can be easily done in any laboratory.
Lactobacilli and Propionibacteria are mostly identified by the fermentation product lactic acid and propionic acid respectively [166].

Elle Jo proposed a rapid identification method for bacteria and Yeast especially Candida spp. According to him the key criteria for identification is colony morphology and Gram staining. Catalase test has been modified to 15% from 3% that is usually employed for the identification of aerobic bacteria. Woods Lamp is a ultra violet light with a long wave length. Most of the anaerobic bacteria will show the property of fluorescence. Based on the colour of the fluorescence bacteria can be identified. Many species of gram positive cocci are still classified under Peptostreptococcus spp. Since the proposed guideline’s publication 2 species of Peptostreptococcus have been named as Finegoldia magna and Micromonas micros [167].

Anaerobic Laboratory of Centre for Disease Control (CDC) made three quadrant plates. The Plates I II and III contained different types of differential media which was prepared from Lombard Dowell media. These plates contained 21 tests for the identification of anaerobic bacteria. Presumpto Plate I was developed to identify Bacteroides and Fusobacterium sp. Additional identification tests for gram positive species was given in Presumpto Plates II and III. Whaley et al evaluated the accuracy of these Plates. He pointed out that this method is not a rapid method of identification when compared with the newer tests based on enzymes. But they are potentially more accurate because the basis of Presumpto plates are conventional methods, which is more familiarized with microbiologists. Whaley et al gave the opinion that the presumpto plates are as accurate as any other commercially available enzyme tests. When compared with other tests Presumpto plates were less expensive [168].
2.12 QUALITATIVE DETECTION OF PERIODONTAL DISEASE

PCR based techniques and enzymatic tests are mainly employed for qualitative analysis. Enzymatic tests are an inexpensive method for the identification of bacteria. They are available commercially as kits. The main drawbacks for these tests are that they cannot be used for antibiotic selection and treatment. Another major drawback is they can be used only for the identification of a group of bacteria. Hydrogen sulfide producing periodontal bacteria can be identified and qualitatively examined with the help of bismuth chloride. This can be detected by absorbance measurement of the black precipitate.

PCR techniques are more sensitive than other methods used for identification and qualitative analysis. Species specific primers can be used for the identification of anaerobic bacteria to the species level. The major drawback for PCR based techniques is that it will detect bacterial genetic material irrespective of whether they are viable or not [169].

Claudia Nonnenmacher et al in their study employed real-time PCR for quantitative detection of periodontal pathogens. She noted that all the bacterial species that was used for the study were detected with Ct value from 14-35 which stands for $10^8$ to $10^2$ copies of plasmid respectively. In the case of quantitative analysis for the clinical samples they observed that the total number of Eubacteria was higher in the case of subjects with periodontitis (median: $5.4 \times 10^6$) when compared to non periodontitis group (median: $5.5x 10^5$) when detected with universal probe and primer set for the detection of all groups of bacteria. Similar results were observed for A.actinomycetomcomitans, Porphyromonas gingivalis, Dialister pnuemosonties and for Micromonas micros. These bacteria were found in higher numbers in periodontitis group than in non periodontal group. Prevotella intermedia did not have any statistically significant difference between the two study groups. She has stated that PCR protocol can be used for quantification and to differentiate periodontal pathogens from the subgingival plaque [170].