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ROLE OF SUPRA-GINGIVAL PLAQUE IN THE PATHOPHYSIOLOGY OF PERIODONTITIS

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ABSTRACT

Antonie van Leeuwenhoek was the first person to describe oral bacteria. Since then, extensive research has been performed regarding the development and microbiology of dental plaques. In spite of the complexity of the developing flora of supragingival plaque, culture studies have shown a remarkably orderly succession of organisms. The concept of microbial specificity in the aetiology of periodontal diseases suggest that qualitatively distinct dental plaques are associated with different forms of periodontal disease. Cross-sectional and longitudinal studies of the cultivable microflora reveal that only a small number of species found in the human subgingival plaques are associated with periodontal disease. Periodontal disease can be treated by acombination of mechanical wound debridement and the application of an antimicrobial agent. The effectiveness of mechanical anti-infective therapy and successful oral hygiene has met the success in clinical practice for most cases of periodontitis in adults. This literature review highlights about supragingival plaque and its role in Periodontitis.

KEYWORDS: Supragingival plaque, microflora, periodontal disease, antimicrobial agent.

INTRODUCTION

Plaque is defined as soft deposits that form the biofilm adhering to the tooth surface or other hard surfaces in the oral cavity, including removable & fixed restorations - given by Bowen in 1976. It is the primary factor implicated in the aetiology of periodontal disease. Removal of supragingival plaque and resolution of gingivitis promises that there is a high positive

correlation between the amount of supragingival plaque and the development of gingivitis. Dental Calculus is an adherent calcified or calcifying mass that forms on the surface of natural teeth & prosthesis. Materia alba is a deposit composed of aggregate of microorganisms; leucocytes & dead exfoliated epithelial cells. Plaque is haphazardly arranged and loosely attached to the surfaces of the teeth and composed primarily of microorganisms, which exist within an intercellular matrix. The term biofilm describes the relatively indefinable microbial community associated with a tooth surface or any other hard, non- shedding material. (Wilderer & Charaklis 1989).

Supragingival plaque is found at or above the gingival margin or if it has a direct contact with the gingival margin, it is referred as marginal plaque. Sub gingival plaque is found below the gingival margin, between the tooth and gingival sulcular tissue. Gingivitis is a form of gum disease characterised by reversible gingival inflammation without destruction of tooth-supporting tissues, periodontal ligament or bone.[1] Periodontal disease comprises of a group of inflammatory conditions of the supportive tissues of the teeth that are caused by bacteria. Loe et al (1965), conducted a study on plaque and concluded that plaque is the main etiological factor in periodontal disease.[2] Schei (1959), Russel (1967) epidemiological studies shows a positive correlation between the amount of bacterial plaque and the severity of gingivitis.[3] Mechanical debridement is the most efficacious method of removal of plaque but Lindhe et al have shown that maintenance of proper standard of plaque control over time by mechanical means is extremely difficult. Hence, mouth wash may be used as an adjunct to traditional cleaning procedures.

COMPOSITION

Biofilm consists of one or more communities of micro- organisms embedded in glycocalyx, that are attached to solid surfaces. Intercellular matrix consists of a few host cells such as epithelial cells, macrophages & leukocytes, and forms 20%-30 % of total plaque mass. Organic materials are polysaccharides (30%) produced by bacteria. dextran, protein (30%) mainly albumin (originating from gingival crevicular fluid), glycoproteins from saliva., lipid (15%) which consists of debris from disrupted bacteria, host cells & food debris. Inorganic material predominantly consists of calcium, sodium, phosphorous, potassium & fluorides (in traces). Saliva is the prime source of inorganic material in supragingival plaque and gingival crevicular fluid exopolysaccharides is the basis for sub gingival plaque, which acts as the backbone and bulk of the biofilm. It consists of matrix which is predominantly composed of

water and aqueous solutes. This protects & maintains the integrity of the microbial cells by preventing desiccation and attack by harmful agents. They bind to the essential nutrients such as cations to create a nutritionally rich environment. It acts as a buffer, retain the extracellular enzymes (and their substrates) and enhance the substrate utilization by bacterial cells.

PLAQUE FORMATION

Dental plaque development on exposed tooth surfaces begins with the precipitation of a salivary derived pellicle which develop on the natural and artificial tooth surfaces and cultures, and the organic film derived mainly from the saliva gets deposited on the tooth surface. Saliva per se consists of 95% water and 5% soluble organic (potassium, sodium, calcium, chloride, carbonate and hypophosphate ions) and inorganic component (mucopolysaccharides - glycoproteins). Whole saliva also contains cellular elements, including bacteria, PMN cells, epithelial cells and erythrocytes. Socransky postulated that one gram of plaque contains approximately 2 x 10^{11} bacteria.[4] Pellicle contains few bacteria in its early stage and few hours after its deposition they adhere to the pellicle firmly, forming the basis for plaque accumulation. Microbiological studies of developing plaque formation have shown that bacterial adhesion to the pellicle takes place very rapidly, within a few minutes after pumicing the surface.

The first type of organism found on the tooth surface are cocci and rods, mainly, Streptococcus sanguis and gram-positive facultative pleomorphic rods like Actinomyces viscosus and Actinomyces israelii. Few or no gram-negative organisms are detected in these very early plaques.[5] Studies of plaque composition in healthy oral cavities over a period of 2 to 3 weeks led Loe et al. (1965) to describe microbial maturation of plaques associated with experimental gingivitis. Starting with a cleaned tooth surface, there are three relatively distinct phases of plaque formation with some deviation dependent on individual variations. The first phase (days 0-2) exhibits a proliferation of gram-positive cocci and rods and an addition of 30% gram-negative cocci and rods in which the proportions reflecting the usual distribution of these components in whole saliva. The second phase (1 to 4 days) is characterized by the appearance and increase in numbers of fusobacteria and filaments. The third phase (4 to 9 days) is characterized by the appearance of spirilla and spirochetes. Gingivitis is clinically detected at the time of formation of these complex and mature supragingival plaques are found in 4-9 days.

MICROBIAL COMPOSITION

Gingivitis associated with the tooth surface of with a variety of micro-organisms including coccoid as well as filamentous forms. The deeper layers of the bacterial mass are more common with the lysis and mineralization of cells. Filamentous bacteria are relatively more numerous with the dense masses of long filaments covering with cocci and gives rise to the "corncob" formation. Flagellated bacteria and spirochetes are observed in the apical portions of the plaque adjacent to the gingiva. In periodontitis, the plaques extend sub gingivally into an area bordered by the root surface and the pocket epithelium. Filamentous forms are prominent, but the morphological cell types encountered in the gingivitis sample are also observed in the periodontitis sample.

SUPRAGINGIVAL PLAQUE AS THE CAUSE OF PERIODONTAL DISEASE

Supragingival plaque is primary initiating factor in gingivitis. Composition does not differ significantly from gingivitis and periodontitis. It may influence the disease process in many ways. But subgingival plaque is entirely different from supragingival plaque. In a healthy gingival condition, supra gingival plaque forms as the result of organisms in saliva adhering in a selective manner to the tooth or to other bacteria.in the natural situation, this colonization is regulated by salivary factors, which alters the attachment or bacterial metabolism. Once established, these organisms receive their nutrition by means of the hosts diet and interbacterial feeding. According to Loeshe and Syed in 1978 if early plaque forms are allowed to remain on the tooth more than a few days, supragingival plaque undergoes a maturation which is characterized by an increased Actinomyces species and decrease in oxidation–reduction potential.[6] Gingival inflammation develops an association with plaque maturation and is characterized by the presence of supra gingival inflammation as well as increased flow of gingival crevicular fluid. As the result of inflammation, there is an increased cell turnover in the epithelial attachment and a loss of connective tissue which tends to open up the gingival sulcus.[7] The result is a new ecological niche which is somewhat protected from the supragingival plaque. These environments provide a favourable condition for the establishment of subgingival bacteria which attaches to gram positive rods such as Actinomyces species rather than to the tooth.

They are favoured in a low oxygen environment and feed altered nutrients from gingival crevicular fluid and products from the supragingival microenvironment and supragingival plaque, may influence the microbial balance in stable gingival area. The essential factor for

establishment of a periodontopathic subgingival microflora appear to be the presence of the organism and the presence of an environment appropriate for colonization. The appropriate environment appears to include gram positive bacteria which provide attachment of pathogenic sub gingival flora. The disease process may be influenced by either eliminating supragingival plaque or by altering the environment by altering the composition of plaque. Their view has been made for relating periodontal destruction with levels of supragingival plaque and calculus (Lovai et ai, 1958, Schei et al. 1959, Abdellatif & Burt 1987, Maizeis & Sheiham 1987), but it must be recognised that such an established correlation is not wholly supported in some other studies (Baeium et al, 1986, Ismail et ai. 1986, Loe et al. 1986, Baelum et al. 1988).[8]

CONTROL OF SUPRAGINGIVAL PLAQUE

The control of supragingival plaque by professional tooth cleaning and personal efforts, has been shown to bringabout the resolution and to prevent the recurrence of gingival inflammation both in children (Axeisson & Lindhe 1974)[9] and in adults (Lovdal et ai. 1961, Giavind 1977).[10] In adults' control of supragingival plaque has also been shown to prevent the onset of, and to arrest, established periodontitis either with (Axelsson & Lindhe 1978, 1981a) or without (Axelsson et al. 1991) close monitoring of personal plaque control. Other clinical studies have shown that high levels of oral hygiene combined with active periodontal therapy and regular 'maintenance' professional tooth cleaning can successfully manage periodontal disease, although the influence of any periodic subgingival instrumentation on the successful management remains unclear. A regime of three-monthly professional tooth cleaning, which included subgingival instrumentation as deemed necessary, has however been shown in another series of studies (Ramjford et al. 1982, Ramfjord 1987)[11] to apparently overcome any deficiencies in supragingival cleaning following active periodontal treatment. Whilst neglect of supragingival plaque control after active treatment of periodontitis has been shown to frustrate the cure of the disease (Nyman et al. 1975).[12]

ANTIBACTERIAL AGENTS IN SUPRAGINGIVAL PLAQUE CONTROL

A few antimicrobial agents have been studied in respect to the control of supragingival plaque and they can be divided into bisguanide antiseptics, quaternary ammonium antiseptics, phenolic antiseptics, other antiseptics, oxygenating agents, metal ions and natural products.[13]

BISGUANIDE ANTISEPTICS

Several bisguanide antiseptics possess anti-plaque activity, including chlorhexidine, alexidine and octenidine. Chlorhexidine gluconate, however, is the most studied bisguanide and is the one on which there is most information on toxicology.[14] Biguanide antiseptics can kill a wide range of microorganisms by damaging the cell wall. The anti-plaque properties of chlorhexidine are unsurpassed by other agents and it has much greater and more prolonged effects than other antiseptics of similar or greater antibacterial activity. This appears to be caused by the adsorption of the dicationic chlorhexidine molecule onto oral surfaces and its release at bactericidal levels over prolonged periods.

CHLORHEXIDINE

The digluconate of chlorhexidine (1:6-Di 4'-chlorophenyl-diguani-dohexane) is a synthetic antimicrobial drug which has been widely used as a broad-spectrum antiseptic in clinical and vetinary medicine since 1953. It has been available in Europe for more than 25 years and has been successfully used in the dental field during that period. As an antimicrobrial agent, chlorhexidine is effective *in vitro* against both Gram-positive and Gram-negative bacteriaincluding aerobes and anaerobesand yeasts and fungi. Its antibacterial action is due to an increase in cellular membrane permeability followed by coagulation of the cytoplasmic macromolecules. It has also beenshown that chlorhexidine can reduce the adherence of *Porphyromonasgingivalis* to epithelial cells. This effect is probably due to the binding of chlorhexidine to the bacterial outer membrane and therefore it could have similar results on the adherence of other plaque bacteria.[15]

It has been shown that a 0.2% chlorhexidine gluconate mouth rinse will prevent the development of experimental gingivitis after the withdrawal of oral hygiene procedures. It has thus been shown to be both a highly effective anti-plaque agent. However, when used as an adjunct to normal oral hygiene measures, variable results are achieved, suggesting that chlorhexidine is more effective in preventing plaque accumulation on a clean tooth surface than in reducing pre-existing plaque deposits.[16] Chlorhexidine is thus able to inhibit plaque formation in a clean mouth but will not significantly reduce plaque in an untreated mouth.[17] For these reasons' chlorhexidine mouthwash should never be given to patients before the necessary periodontal treatment has been carried out and then should only be used for the specific reasons set out below.

Substantivity of chlorhexidine

The ability of drugs to adsorb onto and bind to soft and hard tissues is known as substantivity and this property was first described for chlorhexidine in the 1970s. Substantivity is influenced by the concentration of the medication, its pH and temperature, and the length of time of contact of the solution with the oral structures. This property of chlorhexidine was associated with its ability to maintain effective concentrations for prolonged periods of time and this prolongation of its action made it especially suitable for the inhibition of plaque formation.

Safety of chlorhexidine

The safety of an antimicrobial agent should be tested in animal studies prior to its clinical use. Any side effects found are then carefully investigated in human studies. The effects of their metabolic products on the environment are also frequently studied. Animal experiments with radiolabelled chlorhexidine have shown that the primary route of excretion is through the faeces. There is minimal metabolic cleavage and there has been no reported evidence of carcinogenic substance formation. Chlorhexidine is poorly absorbed by the gastrointestinal tract and it therefore displays very low toxicity (oral LD50 is 1800 mg/kg and the intravenous LD_{50} is 22 mg/kg). No tetra genic alterations have been found following long-term use. The most common side effect of chlorhexidine is the formation of extrinsic stain on the teeth and tongue following its use as a mouthwash

QUATERNARY AMMONIUM COMPOUNDS

Quaternary ammonium compounds such as cetylpyridinium chloride (CPC) have moderate plaque inhibitory activity. Although they have greater initial oral retention and equivalent antibacterial activity to chlorhexidine, they are less effective in inhibiting plaque and preventing gingivitis. One reason for this may be that these compounds are rapidly desorbed from the oral mucosa. It has also been found that the antibacterial properties of these compounds are considerably reduced once adsorbed onto a surface and this may be related to the monocationic nature of these compounds. The cationic groups of each molecule bind to receptors on the mucosa producing the mucosal retention but because of the monocationic nature of these molecules this process leaves few unattached sites available for its antibacterial function.

A CPC pre-brushing mouth rinse used as an adjunct to mechanical oral hygiene has not been found to have an additional beneficial effect on plaque accumulation. With regard to

conventional use, one study compared the plaque-inhibitory potential of 0.05% and 0.1% CPC, 0.05% chlorhexidine and control mouth rinses used twice daily during a 4-day period of non-brushing. The 0.1% CPC-rinse had the lowest plaque scores, being around 26% lower than the control rinse, and 7% lower than the 0.05% chlorhexidine rinse. The 0.05% CPC and chlorhexidine mouthwashes were very similar in their effects. The relatively poor effect of the 0.05% chlorhexidine and CPC mouthwashes is undoubtedly due to the low concentration in these formulations yielding too low a total dose for the expected effect.

Also, the short duration of this study makes it impossible to detect any effect on gingivitis which would be expected from a normal chlorhexidine mouth rinse. It does, however, show that the CPC 0.1% mouthwash did produce a limited but statistically- significant reduction in plaque growth. A slow-release system containing CPC has been tried to increase the retention time for CPC in the mouth. The plaque inhibitory effect over 18 days of this device was compared with that of a CPC mouthrinse, CPC lozenges (Cepacol) and a chlorhexidine mouth rinse (Peridex). As expected, the chlorhexidine mouthrinse (Peridex) had the most profound effects and these were not approached by the other formulations. However, there were no differences between any of the CPC formulations which showed that the slow-release system had no effect on the efficacy of CPC. All the CPC formulations and Peridex produced tooth staining and this was worst with the CPC lozenges.

Phenolic antiseptics

Phenols, either alone or in combination, have been used in mouth rinses or lozenges for a considerable time. When used at high concentrations relative to other compounds they have been shown to reduce plaque accumulation. Listerine is an essential oil/phenolic mouthwash which has been shown to have moderate plaque inhibitory effects and some anti-gingivitis effects. There have been a number of short and long-term home-use studies which have shown that it has moderate plaque inhibiting effects and some anti-inflammatory effects in reducing gingival inflammation. On the basis of these studies, it has been accepted by the American Dental Association to be an aid to home oral hygiene measures.

Its effects on 4-day plaque regrowth during abstinence from mechanical oral hygiene has been compared with those from chlorhexidine and anti-adhesive mouthwashes. 0.2% chlorhexidine mouthrinse was significantly more effective than Listerine which was in turn more effective than the anti-adhesive mouthwashes alone or in combination with chlorhexidine, which it inactivates (see earlier). It was, however found to be slightly more

effective than triclosan mouthwash in plaque inhibition. Its anti-inflammatory effects shown in the home use studies may be due to its antioxidative activity. Thus, it has a moderate effect on plaque regrowth and some anti-inflammatory effect which may reduce the severity of gingivitis. Its lack of profound plaque inhibitory effect is probably because, unlike chlorhexidine, it has poor oral retention.

Hexetidine

Hexetidine has some plaque inhibitory activity but this is low in comparison with chlorhexidine. Its substantivity (oral retention) is between 1 and 3 hours, which accounts for the reported low plaque inhibitory effects of Oraldene, the UK product. Another study investigated its effect on the healing of aphthous ulcers and did not show any added benefit over mechanical oral hygiene alone. Moreover, this agent at concentrations greater than 0.1% can cause oral ulceration. It has also been shown that combining zinc with hexetidine improves its plaque inhibiting activities probably by acting synergistically with it.

Povidone Iodine

Povidone iodine appears to have no significant plaque inhibitory activity when used as 1% mouthwash and the absorption of significant levels of iodine through the oral mucosa may make this compound unsatisfactory for prolonged use in the oral cavity.[18] Also, it could cause a problem of iodine sensitivity in sensitised individuals.

Triclosan

Triclosan, a trichlora-2'-hydroxydiphenyl ether, is a non-ionic antiseptic which lacks the staining effects of cationic agents. It has been used recently in several commercial toothpastes and mouthwashes and produces moderate plaque inhibitory effects when used as a mouthwash in combination with zinc. In one study, the combination mouthwash produced inhibition of plaque regrowth during a 4-day period with abstinence from mechanical oral hygiene, but this study raised doubts as to the individual contribution of triclosan to this effect. The use of a combination of zinc and triclosan arose from the concept that agents with different modes of action might have synergistic or additive effects. The effects of combination zinc and triclosan mouthwashes were investigated in a 3-week clinical trial, where abstinence from brushing was produced by wearing an acrylic tooth shield over the test area of the mouth during brushing. Two experimental mouthwashes containing 0.4% zinc sulphate and 0.15% triclosan were compared with a 0.12% chlorhexidine mouthwash and a placebo (negative control) mouthwash.

As expected, the plaque and gingivitis scores were the lowest in the subjects using chlorhexidine mouthwash. Another crossover study compared the effect of 0.06% triclosan, 0.12% chlorhexidine and placebo mouthwashes on *de novo* plaque formation over 18 days at healthy and inflamed gingival sites of ten volunteers. No significant differences in the gingivitis scores were found between the three mouthwashes but both active mouthwashes produced significant reductions of plaque formation compared to the control mouthwash. These reductions were significantly greater for the chlorhexidine compared with the triclosan mouthwash. They also found that more plaque formed at inflamed sites than healthy sites regardless of which mouthwash was used.[19]

Moreover, there is evidence that triclosan may also act as an anti-inflammatory agent in mouth rinses and toothpastes. In this way it has been shown to reduce the inflammatory reaction produced on the gingiva and skin by sodium lauryl sulphate, and the skin reaction to nickel hypersensitivity. In addition, it has been shown to reduce histamine-induced dermal inflammation and reduce the severity and healing period of aphthous ulceration. The mechanism of this property has been investigated *in vitro* and triclosan has been shown to inhibit both cyclo-oxygenase and lipoxygenase, and thus reduce the synthesis of prostaglandins and leukotrienes which are key mediators in the inflammatory reaction. Thus, triclosan mouthwashes reduce plaque accumulation but to a much lesser extent than chlorhexidine.[20] However, the extent of their plaque inhibitory effect seems to be dependent upon the presence of co-polymers in the formulation to increase oral retention of triclosan. Any effects of triclosan on gingivitis levels are probably due to its anti-inflammatory effect. The anti-inflammatory effect of triclosan also depends upon its ability to penetrate the gingival tissues and this is in turn dependent upon the nature of the solvent(s) in the mouthwash formulation.

DISCUSSION

It is now recognised that several different forms of periodontitis can be distinguished in humans. Pageand Schroeder in 1982has termed these as Pre-pubertal- Juvenile periodontitis, rapid progressing periodontitis, Acute necrotizing ulcerative gingivitis. The association between plaque and other forms of periodontal disease is much less clear. This is mainly due to differenthost defence mechanism which results in a different microbial florawhich is seen in host individuals for detectable supragingival plaque according to Burmeister et al in 1984, the general clinical impression is one of very sparse plaque. The subgingival plaque bacteria

in these diseases appear to be less dependent on a supragingival plaque than the adult periodontitis. According to Slots in 1979 the subgingival length of the junctional epithelium appears to be compromised and organisms are not as associated as those associated with adult periodontitis. Since there may not be a clear relationship between supragingival and other forms of periodontitis one cannot ensure that supragingival plaque control will be as effective in controlling these diseases asin control of adult periodontitis. In the prevention of gingiva or the initiation of periodontitis, there are 3 basic approaches toprevent periodontaldisease according to Kanneth S. Kernman.

1) The elimination of all clinically detectable plaque.
2) The reduction of plaque below the individual's threshold for disease.
3) Alteration of the microbial composition of plaque such that adult periodontitis will not develop.

According to Ling et al in 1973. Adequate plaque removal has been repeatedly shown to prevent gingivitis and substantially limit the progression of periodontitis. In 1978 reported that frequent prophylaxis and good oral hygiene will inhibit plaque formation and gingivitis, but more importantly it will prevent loss of periodontal attachment. Inaddition, chlorhexidine prevents plaque accumulation and therapy prevent gingivitis. long term mechanical cleaning is extremely labour intensive but clearly effective. The secondapproach to prevent disease byplaque control is toreduce plaque below an individual threshold for disease. Thisis a reasonable goal and in fact, represents the status of most of the population at any given time point. In general, most individual perform regular plaque control measure, but most individuals cannot remove plaque effectively especially in interproximal areas.

According to Socransky et al in 1984, most sites, even those with plaque and gingivitis, appear to be either not progressing at the rate which is below clinical detection. Many factors such as stress, acute viral disease, fluctuation may substantially alterboth host defence mechanism and the microbial composition of the microflora. Third means of preventing the disease is to alter the composition of the plaquesuch that the disease does not initiate. According to Kornman and Holt in 1984, it is possible for chemical agent to prevent or retard the progression of periodontitis without reducing plaque or gingivitis. Prevention of gingivitis by regular efficient plaqueremoval, prevention of periodontitis seems most appropriately managed by relatively complete and regular plaque removal or by means of alteration in the composition of the plaque.[21] Tursky and Hill in 1983 suggested that once a periodontal

pocket has developed with periodontitis associated microflora, supragingival plaque removal alone is insufficient to control the microflora and most likely the disease. If supragingival plaque is not controlled there is tendency towards progressive loss of periodontal support. Listgarden in 1978 said that supragingivalplaque control in site with periodontitis not only eliminates clinical signs of inflammation and reduce probing depth but also produce shift in the subgingival microflora to one which has been associated with gingival health.[22] Plaque control with periodontal therapy is directed at the subgingival microflora will essentially halt progression of periodontal disease.

CONCLUSION

The effective control of supragingival plaque by individuals is paramount in both the prevention and resolution of marginal gingival inflammation and may also be central to the manage management of progressive periodontal disease. At the same time as the effects of supragingival plaque control on gingival inflammation in the absence of professional tooth cleaning are apparent, those on progressive periodontal disease are less clear.

REFERENCES

1. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J., 1975; 25: 229-35.
2. Loe, H., Theilade. E. & Jensen, S.B. Experimental gingivitis in man. Journal of Periodontology "it, 1965; 177-187.
3. Schei, O, Waerhaug, J., Lovdai, A. & Arno, A. Alveolar bone loss as related to oral hygiene and age. Journal of Periodontology, 1959; 30: 7-16.
4. Al-Yahfoufi Z, Mombelli A, Wicki A, Lang NP. The effect of plaque control in subjects with shallow pockets and high prevalence of periodontal pathogens. J Clin Periodontol, 1995; 22: 78-84.
5. Gibbons, R. j. & Van Houte, J., Bacterial adherence in oral microbial ecology. Annual Review of Microbiologv, 1975; 29: 19-44.
6. Loesche, W. J, & Syed, S. A. Bacteriology of human experimental gingivitis: Effect of plaque and gingivitis score. Infection and Immunity, 1978; 2: 830-839.
7. Attstrom, R. Does supragingiva! plaque removal prevent furtber breakdown? In: Periodonlology today, ed, Guggenbeim, B., 1988; 251-259.

8. Loe, H., Anerud, A., Boysen, H. & Morrison, E. The natural history of periodontal disease in man: Rapid, moderate and no loss of attachment in Sri Lankan laborers 14—46 years of age. Journal of Clinical Periodontology, 1986; 13: 431-440.
9. Lindhe, J., Hamp, S. E. & Loe, H. Plaque induced periodontal disease in beagle dogs. Journal of Periodon!al Research, 1975; 10: 243-255.
10. Lovdal, A., Arno, A. & Waerhaug, J. Incidence of clinical manifestations of periodontal disease in light of oraJ hygiene and calculus formation. Journal of American Dental Association, 1958; 56: 21-33.
11. Ramtjord, S. P. Maintenance care for treated periodontitis patients. Journal of Clinical Periodontology, 1987; 14: 433-437.
12. Nyman, S., Lindhe, J. & Rosling, B. Periodontal surgery in plaque-infected dentitions. Journal of Clinical Periodontology, 1977; 4: 240-249.
13. Siegrist, B. & Kornman, K.S. The effect of supragingival plaque control on the composition of the subgingivaJ microbial flora in ligature-induced periodontitis in the monkey. Journal of Dental Researches, 1982; 936-941.
14. Westfelt E, Rylander H, Dahlen G, Lindhe J. The effect of supragingival plaque control on the progression of advanced periodontal disease. J Clin Periodontol, 1998; 25: 536-41.
15. Karpinski T.M, Szaradkiewic A.K: Chlorhexidine – pharmaco-biological activity and application. European Review for Medical and Pharmacological Sciences, 2015; 19: 1321-1326.
16. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. J Clin Periodontol, Sep., 1988; 15(8): 488-498.
17. Eley BM: Antibacterial agents in the control of supragingival plaque – a review. British dental Journal, 1999; 186: 286-296.
18. Addy M, Griffiths C, Isaac R. The effect of povidone iodine on plaque and salivary bacteria - a double blind cross-over trial. J Periodontol, 1977; 48: 730–732.
19. Jenkins S, Addy M, Newcombe R. A comparison of cetylpyridinium chloride, triclosan and chlorhexidine mouth rinse formulations for the effect on plaque regrowth. J Clin Periodontol, 1994; 21: 441–444.
20. Moran J, Addy M, Roberts S. The comparison of a natural product, triclosan and chlorhexidine mouthwashes on 4-day plaque regrowth. J Clin Periodontol, 1992; 19: 578-582.

21. Kornman, K. S., Holt. S. G. & Robertson, R B. The microbiology of ligature induced periodontitis in the Cynomolgus Monkey. Journal of Periodontal Research, 1981; 16: 363-371.
22. Listgarten, M. A. & Ellegaard, B. Experimental gingivitis in macaca mulatta. Journal of Periodontal Research, 1973; 8: 199-214.