Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/11866
DOI URL: http://dx.doi.org/10.21474/IJAR01/11866

RESEARCH ARTICLE

PERIODONTAL VACCINE- A SHOT IN THE ARM!

Dr. Apeksha Gajghate[1], Dr. Salman Ansari[2], Dr. Rajvir Malik[3], Dr. Namrata Khetal[1] and Dr. Rashmi Bele[4]
P.G Student, Department of Periodontology, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Wanadongri Road, Hingna, District Nagpur- 441110

Manuscript Info

Manuscript History
Received: 15 August 2020
Final Accepted: 18 September 2020
Published: October 2020

Key words:-
Periodontitis, Vaccines, Immunity, Pathogen.

Abstract

Periodontal diseases are immune inflammatory responses induced by dental plaque in which microorganisms harboured within a susceptible periodontium contributes to tissue destruction, bone loss and eventually tooth loss. The etiopathogenesis of periodontal disease is multifactorial which includes host associated factors, genetic factors, immune system dysfunction and environmental factors. Existing treatment modalities have resulted only in arresting the disease progression but have not cured the disease completely, nor do they avert the recurrence. Hence there is a need for therapeutic modalities which may include vaccines targeting periodontal pathogens. Vaccination is induction of immunity by injecting a dead or attenuated form of pathogen. Till date, no pre-emptive modality exists for periodontal disease, the availability of periodontal vaccine would prevent the progression of periodontal diseases. The aim of this review article is to confer the various approaches associated with periodontal vaccine.

Introduction:-

Periodontium comprises of two soft tissues namely Gingiva and Periodontal ligament and two mineralised tissues, vascular tooth supporting Alveolar bone and avascular Cementum that covers the anatomical root of tooth. Periodontal Diseases includes 'Gingivitis' where inflammation is confined to the Gingiva and 'Periodontitis' where it spreads into the Periodontal ligament. Typically, two main forms of periodontitis have been recognized along with syndromic forms and those associated with systemic diseases. The term 'Chronic Periodontitis' is known to affect chiefly, not necessarily, those aged above 35 years. The other term 'Aggressive Periodontitis' is usually associated with age group less than 35 years of age. However, According to American Academy of Periodontology workshop (1999), the criteria of age has been omitted since both forms of the disease can affect young and older age groups. In addition, the Gingival diseases, necrotizing periodontal diseases, Abscesses, Developmental and Acquired forms of periodontal diseases, and Endodontic-periodontal lesions are other classes.[1]

Etiopathogenesis of periodontal diseases:

Since Periodontitis is a multifactorial disease, it involves interaction among host, micro-organisms and environmental factors which includes genetic factors.[2] There are over 300 species of microorganisms that have been found to colonize the periodontal tissues, of which the following are considered to be the primary pathogens causing periodontitis:

1. *Porphyromonas gingivalis (P.gingavalis)*
2. *Aggregatibacter actinomycetemcomitans*

Corresponding Author:- Dr. Apeksha Gajghate
P.G Student, Department of Periodontology
Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Wanadongri Road, Hingna, District Nagpur- 441110

3.Tannerela forsythia (T.forsynthia)

These bacteria produce an array of antigens that stimulate pro-inflammatory cells and leads to the production of a wide variety of cytokines.[3] These antigens may stimulate T-helper cells-1 (Th1) or T-helper cells-2 (Th2) cells. Antigens are taken up by dendritic cells and presented to CD-8 or CD-4 cells along with MHC antigens.[4] The host produces anti-bacterial substances such as defensins, cathelicidins and saposins, which protect the host tissues from bacterial products and forms the first line of defense. However, sometimes these are inactivated by the bacterial virulence factors. Once bacteria break this barrier, cytokines are produced, which can be both proinflammatory and anti-inflammatory. Production of inappropriate cytokines results in periodontitis.[5]

Host response in periodontal disease:

The presence of periodontal pathogens triggers the host immune response. The host defense against periodontal pathogens includes of Innate and Acquired immunity. Saliva, Gingival crevicular fluid (GCF) and epithelial cells play a key role in innate immune response.[6] Neutrophils, Macrophages and Dendritic cells are also important innate immune cells which express pattern recognition receptors (PRRs) that interact with the specific molecular structures on microorganisms called microbe associated molecular patterns (MAMPs) to signal immune responses. Innate immune response is nonspecific and results in excessive host tissue damage without effective antigenic clearance.[7] Adaptive immunity is slower and reliant on complex interactions between antigen-presenting cells and T and B lymphocytes, cytotoxic T cells and antibodies.[8] The immune response to pathogenic microorganisms involves the combination at the molecular, cellular, and organ level of elements often categorized as being part of the innate immune system or the adaptive immune system. However, Periodontal pathogens have developed mechanisms to inhibit and evade cell-mediated and humoral immune responses.[9] To stop the progression of periodontal disease, multiple approaches including host immune modulation and pathogen-specific approaches could be used.

Vaccines:

Vaccination is a process that induces specific immune resistance to a bacterial or viral infection. It is the development of immunity or resistance to infection, after a secondary response (booster) that is adequate to consider the individual immune to a subsequent infection.[10] More than 200 years ago, Edward Jenner showed a new outlook in preventive medicine with the introduction of Small Pox vaccine and its successful use to eradicate the epidemic. Advances in Microbiological and Biochemical methodologies led to revolutionary era that ultimately resulted in the formulations of vaccines against serious epidemic and endemic diseases affecting humans, including Tuberculosis, Tetanus, Typhoid, Cholera and Plague.[11]

The prime step in vaccine development is identification of an antigenic component from various organisms that can provide immune protection.

Vaccines may be prepared by:[12]

1.Killing the organism using formalin-called inactivated or killed vaccine.

2.Using only antigenic part of the disease causing organism, like the capsule, the flagella, the part of the protein cell wall-acellular vaccines.

3.By weakening a live microorganism by aging it or altering growth condition-attenuated vaccines.

4.Toxoids are vaccines from toxins, which are adsorbed onto aluminum salts to decrease their harmful effects and is administered with an ‘‘adjuvant’’ which can have effects on antigen delivery, immune modulatory cytokines, and antigen-presenting cells.[13]

Types of Vaccination:

Active Immunization:

Here an individual immune system is stimulated by administrating killed or live attenuated products derived from micro-organisms.

Passive immunization:

Here, the antibodies formed in one individual are transferred to another.

DNA vaccination:
Here, DNA plasmids encoding genes required for antigen production are transferred to an individual.[14]
Characteristics of an effective vaccines are- Safety, Protectivity, Sustained protection, production of neutralizing antibodies, Stimulation of protective t-cells. Practical considerations like cost-effectiveness, biological stability, access, minimum contraindications and side effects are also significant.[15]

Periodontal vaccines:
What is the prerequisite for periodontal vaccines?
Periodontal pathogens associated with periodontitis predominantly are gram-negative, anaerobic bacteria- *P. gingivalis, A. actinomycetemcomitans, T. denticola and T. forsythia* etc. Various immunization approaches both as active and passive immunization, against periodontal pathogens have been explored either using the whole organism or specific virulence factors.[16] Periodontal vaccines can be helpful in decreasing the incidence of Periodontal diseases. Periodontal diseases result in higher systemic levels of inflammatory markers which causes systemic changes leading to various systemic conditions. The subgingival biofilm associated bacteria are found to exacerbate diabetes and cardiovascular disease and it has been suggested that immunotherapy for periodontal disease may be considered as a second indication for controlling atherosclerosis.[17] Another group of patients that would benefit from the use of such a vaccine are immunocompromised patients affected by the disease through various acquired or congenital causes.[18] Periodontal treatment lays a monetary load on the individuals suffering from it. Availability of vaccine for preventing periodontal disease would be of great help for those individuals. So, the development of periodontal vaccines is of supreme importance in the management of periodontitis.

History:
In the early 20th century, 3 periodontal vaccines were employed. Pure cultures of streptococcus and other organisms, Autogenous vaccines and Stock vaccines- Vancott's vaccine, Goldenberg's vaccine and Inava endocarp vaccine.[19]

Strategies employed for development of Periodontal Vaccines:
Several immunization approaches against periodontitis have been tested, both as active and passive immunization. Besides, the target antigens have progressed from the whole organism in the past to current approaches wherein the specific virulence factors aim to confer immunity against colonization or the virulent activity of putative periodontal pathogens. Socranskyet al, analyzed 13,261 plaque samples using whole genomic DNA probes and checkerboard DNA–DNA hybridization and proposed five color-coded 'complexes' based on the extent of its presence in periodontitis-affected sites. They suggested that the organisms of the Red complex, namely *P.gingivalis T.denticola* and *T. forsythus,* are the predominant disease-associated organisms.[20]

The current arrival of advanced molecular diagnostic techniques, such as Polymerase Chain Reaction (PCR), viruses like herpesvirus and their interaction with the periodontal pathogenic bacteria have also been an emphasis of studies. [21-24] The new additions to this outwardly ever-growing list of implicated bacteria are *Desulfovibrio fairfieldensis*[25] and *Methanogenic archaea*.[26] Thus, the development of a vaccine has become more exciting, taking into consideration, the recent developments in the etiology and pathogenic mechanisms involved.

Mechanism of action:
Active immunization:
Various target organisms for vaccine preparation have been tried, of which *P.gingivalis* and *A. actinomycetemcomitans* are of major importance owing to their omnipresent role in the pathogenesis of periodontal disease. *P. gingivalis* is a gram-negative, non-spore/forming, nonmotile, assacharolytic, obligate anaerobic coccobacillus. The virulence factors of *P. gingivalis* which have been used as subunits for the development of active immunization.[27] It is known to survive in a hostile environment by successfully evading host antimicrobial defenses by utilizing a variety of virulence factors, such as cysteine proteases, gingipains, fimbriae, lectin-like adhesins, capsular polysaccharide, lipopolysaccharide, outer membrane , heat shock protein and the release of toxic metabolic products.[28] Apart from the whole cell, all these factors havebeen tested as target antigen for immunization that can produce functional protection against periodontal tissue destruction induced by the organism.

Outer membrane protein:
It was seen that transcutaneous injection of 40 kDa of outer membrane protein (OMP) inhibits coaggregation of outer membrane protein (OMP) inhibits co-aggregation of *P. gingivalis* with *Streptococcus gondonii.* This also can be used

for vaccine development for passive immunization. Polyclonal anti-40 kDa. OMP antibody exhibited potentially protective, complement-mediated bactericidal effect.[29]

'Whole cell' as a target antigen- It was one of the first approaches tried in various animal models. Perssonet al. reported that active immunization of non-human primate, *Macaca fascicularis*, with killed *P. gingivalis* whole cell conjugated with syntex adjuvant formulation-M, inhibits the progression of periodontal tissue destruction.[30] Page also confirmed the similar results employing the same animal model.[31] These studies showed that only humoral immune response was elicited that lasted for a short period and no cell-mediated immune response was triggered that could provide immune memory and thus provide long-term protection. Hence, there have been minimal advances, with respect to vaccine development, by means of this approach.

Gingipains- Gingipains is the specific term used to describe a host of cysteine proteases that impart major pathogenic capability to *P.gingivalis.* It was Coined by Travis and Colleague. These are cysteine proteinases which cleave synthetic and natural substrates after arginine or lysine residues and are referred to as arginine gingipain (RgpA and RgpB) and lysine gingipain (Kgp) respectively.[32] Kgp is most potent fibrinogen degrading enzyme of 3 gingipains in human plasma and involved in bleeding tendency at diseased gingival. They are expressed on the outer membrane of *P. gingivalis*. Rgp and Kgp are key determinants in the growth and virulence of *P. gingivalis*. Gingipains vaccines are mainly DNA vaccines. DNA vaccines induce both humoral and cellular immunity. They possess a hemagglutinin domain that plays an essential role in the adherence of microorganism to erythrocytes, while the catalytic domain present in RgpA, RgpBand Kgp plays an important role in the evasion of the host defense system by extensively degrading immunoglobulins, complement proteins and by disturbing the functions of neutrophils [33-34]. This activity also degrades C3-derived opsonin, thus rendering *P. gingivalis* resistant to phagocytosis [35]. Thus, a vaccine targeting this virulence factor may provide protection against both invasive and noninvasive strains of *P.gingivalis.* Other reports also suggested that immunization with the RgpA-Kgp proteinase–adhesin complexes of *P. gingivalis* protected against periodontal bone loss by inducing a high titer of serum IgG_{2a} response in the rat periodontitis model, similar to that seen in animals immunized with formalin-killed *P. gingivalis* whole cells [36]. So, this approach seems to be an essential way forward to achieving the goal of 'successful' vaccination.

Fimbriae as target antigens- Fimbriae from *P.gingivalis* play an important role in adhesion to oral tissue and are highly immunogenic. Electron microscopy studies of *P.gingivalis* have shown that it possesses at least three types of fimbriae [37]. It consists of major fimbriae (product of fimA gene) and other antigenically distinct fimbriae, designated as minor fimbriae, composed of a protein of 67 kDa [38]. Another distinct fimbrial structure, Pg-II (72-kDa protein) has also been detected by immunoelectron microscopy.[39] Although both major and minor fimbriae partake in the pathogenic process, major studies have been taken on in averting thepathogenic effects of major fimbriae. In a study on rats, it was observed that when rats were parenterally immunized with highly purified 43-kDa fimbrial(Fim) protein, the induced FimA-specific antibodies in serum and saliva gave 100% protection against *P. gingivalis* induced alveolar bone loss.[40] On the contrary, a study demonstrated that rabbits immunized with 43-kDa fimbrillin polymer of *P.gingivalis* did not show evidence of any protection against all the strains of *P.gingivalis*, suggesting that opsonic target sites are not shared across serotypes or five types of *P. gingivalis* fimbriae [41] These results suggest that the fimbrial protein of *P. gingivalis* could be used as a target antigen to yield an effective periodontal vaccine.

Hemagglutinins- Non-fimbrial adhesion hemagglutinin B (HagB) is a potential vaccine candidate. Rats immunized subcutaneously with recombinant HagB were protected against periodontal bone loss induced by *P.gingivalis* strain ATCC 33277. Human antibody against hemagglutinin should be ideal for practical use in immunotherapy.[42]

GroEL heat shock protein - Heat shock proteins have significant role in inflammatory mechanism and autoimmune diseases. Rats immunized with *P. gingivalis* HSP60 showed decrease in bone loss induced by infection with multiple periodontal bacteria. Significant association between HSP90 concentration and microbial colonization has been observed.[43]

Synthetic peptides- These require synthesis of linear and branched polymers of 3-10 amino acids based on the known sequences of microbial antigens. Such peptides are weakly immunogenic by themselves and need to be coupled to large proteins to induce antibody response. Genco 1992 found that synthetic peptides based on the protein structure of fibrillin inhibit the adhesion of *P. gingivalis* to saliva-coated hydroxyapatite crystals in vitro.

Passive immunization:
Passive immunization is short lived because host does not respond to immunization and protection lasts only as long as injected antibody persists. Antigens are injected into vector that produce antibodies. These antibodies when inoculated into host bring about passive immunization.
Passive immunization against *Porphyromonas gingivalis*- Developing monoclonal anti- bodies against the colonization factor of *P. gingivalis* could also be a potential target for immunotherapy. Two major colonization factors of *P. gingivalis* are coaggregation factor (outer membrane proteins [OMPs]) and hemagglutinins, both are involved in the adsorption, colonization and penetration of bacteria into host cells. [44-45]

Antibodies against coaggregation factor- There are several mechanisms by which *P.gingivalis* can cause disease, but perhaps the most important step is coaggregation between *P. gingivalis* and *Actinomyces viscosus. P. gingivalis* can also adhere to *Streptococcus gordonii*, mediated by the specific OMP on the cell surface and in extracellular vesicles.[46] This coaggregation contributes to the formation and maturation of biofilm, which is mainly implicated in causation periodontal disease.[47] *Actinomyces naeslundii* has also been implicated in coaggregation with *P. gingivalis*. Based on this fact, a panel of monoclonal antibody (mAb) was prepared by immunizing mice with purified r40-kDa OMP, which revealed that several mAbs specifically inhibited coaggregation of *A. naeslundii* with several strains of *P. gingivalis*.[48] A genomic analysis of *P. gingivalis* was conducted and it was reported that recombinant OMP antigens PG32 and PG33 (both known to play an important role in bacterial growth, coaggregation with other bacteria and transcription) are potential vaccine candidates.[49] Hence, OMPs have been reported to be highly immunogenic due to the easy access to host cells, it can be crucial in the development of a highly effective vaccine against *P. gingivalis* infection.

Immunization against *Aggregatibacter actinomycetemcomitans*- After *P. gingivalis*, *A. actinomycetemcomitansis* considered significant pathogen in human periodontal disease, especially in aggressive forms. In a study, it was demonstrated that sub-cutaneous and intranasal immunization of mice with capsular serotype b-specific polysaccharide antigen (SPA) produced a specific antibody, which efficiently opsonized *A. actinomycetemcomitans* serotype b, suggesting that antibodies to the SPA of the organism might have a protective role.[50] Furthermore, when mice were immunized with antisurface associated material from *A. actinomycetemcomitans* (anti-SAM-Aa), it was found to result in rapid healing of the primary lesions and a rise in protective antibody levels by acting as an opsonin against challenge with live *A. actinomycetemcomitans*.[51] However, astonishingly only a few studies have been conducted on developing vaccines targeting *A. actinomycetemcomitans*. Even though preliminary studies are promising, further research has to be carried out to depict a potential antigen for developing vaccine against *A. actinomycetemcomitans*.

Plantibodies- Apart from various microorganisms, plants are being increasingly used for the production of recombinant immune-therapeutic agents owing their high efficiency and low cost and the fact that they do not initiate an immunological reaction when administered orally. Molecular biological techniques to express bacterial or viral antigens in plants which could be used as orally administrated vaccines. Ma (2000) characterized a secretory IgG antibody against *streptococcus mutans*, produced in transgenic plants.[52] These studies are promising in that the plantibodies can be used to prevent specific microbial colonization in the oral cavity. Additional studies must be carried out to test the efficacy of plantibodies in eliminating periodontopathic bacteria.

Immunization targeting antecedent plaque microbes-In human plaque, *Fusobacterium nucleatum* colonizes prior to *P. gingivalis* and high levels of *F. nucleatum* have been demonstrated in association with *P. gingivalis*, as well as other bacteria associated with periodontal disease.[53] In a recent report, it was demonstrated that when mice were immunized with *F. nucleatum* prior to *P. gingivalis*, there was a significantly increased IgG_{2a} (Th1) response to *P. gingivalis*.[54] Besides, the inhibition of neutrophil phagocytosis of immune serum opsonized *P. gingivalis* was modulated by the presence of anti-*F.nucleatum* antibody.

Genetic immunization:
By the early 1990's, Scientists had initiated to study new approaches for the production of vaccines. This involves genetic engineering or recombinant DNA technology.

Plasmid vaccines- DNA does not have the ability to grow, whereas plasmids have the ability to grow. With this ability of the plasmids, they are fused with the DNA of a particular pathogen of interest and inoculated in an animal for the production of antibodies. This is then transferred to the host for immunization. Disadvantage- It may lead to oncogenesis.

Live, viral vector vaccines- A variety of infectious but non-disease causing DNA or RNA viruses or bacteria have been engineered to express the proteins of a disease-producing organism. The vector enters the body cells where the proteins are generated and then induce humoral or cellular immune responses.[55] Methods of DNA vaccine administration- Intranasal, Intramuscular and Gene gun. Breivik and Rook conducted a series of immunization trails in wistr rats employing various routes of administration. They reported that the subcutaneous injection of killed *Mycobacterium vaccae*, SRL 172, prophylactically [56], and therapeutically [57], and also oral administration of killed M. vaccae, SRP299 [58], diminished the Th2 response in wistar rats, thus reducing periodontitis induced bone loss.

Host modulation:

Pathogen-triggered host response is the foremost culprit in tissue destruction. Therefore, the focus of the immunization approach has shifted from targeting specific microbes to modulating the host innate immune response by altering specific immune cell functions. Excess inflammatory mediators as a result of host immune response can be blocked to prevent inflammation induced bone loss. It was demonstrated that immunization with formalin killed *P. gingivalis* blocked bone loss by depressing prostaglandin E2 levels, a major component involved in bone resorption.[59] Vaccines consisting of antigen alone are often not very effective in inducing the anticipated immune responses. So, adjuvants are commonly used to enhance the host response to the vaccine antigen. Yang et al. demonstrated that intranasal immunization of rHagB and monophosphoryl lipid (MPL) A, a nontoxic derivative of lipid A region of lipopolysaccharide, acts as mucosal adjuvant and potentates the response to rHagB.[60] There was an increase in the serum IgG1 antibody activity and amplified Th2 response. So, Host modulation could render satisfactory protection against pathogenic agents and formulating an immunization approach in this way could be possible in future.

Barriers in periodontal vaccine development:

As Periodontal disease is a multifactorial disease, eradication of certain bacteria will not stop the onset and progression of disease. Several Problems like maintaining adequate levels of antibodies for long time, generating T-cell mediated response needs to be overcome. The few comparations between the conventional animal models and human beings, and incidence of toxic reactions to inactivated whole cell vaccines add to our worries. Also, the functional differences between the antibodies produced by the infected host and that produced after immunization is to be borne in mind, as the former is usually present but is unsuccessful in resolving the disease. Hence, a vaccine that can create functionally viable antibodies will be the most desirable.

Future immunization approaches:

An effective periodontal vaccine for humans will probably require multiple bacterial species responsible for disease pathogenesis to be targeted.[61] The immunization approach could include introduction of nonpathogenic engineered microorganisms that could competitively and permanently adsorb on to the epitopes in the biofilm and eventually prevent coaggregation and maturation of biofilm. However, studies to validate such a possibility are yet to be carried out. Recent dawn of Nanotechnology opens an entire range of nanospheresand liposomes for controlled release of protein or nucleic acid for the particular delivery of vaccine in adequate amount. Also, delivery routes, likeoral drops, nasal spray, dermal patch and subcutaneous or intramuscular injections, are to be effectively studied in order to determine the most effective route of administration. Local drug delivery of the active ingredient is also a feasible choice.

Conclusion:-

The most critical step in developing a successful immunization approach against periodontal disease would be the identification of the individuals prior to the initiation of disease process. Several immunization approaches have been tested targeting virulence factors as antigens or by various forms of host modulation to modify the response against the pathogens. Many research groups focusing on vaccine development in the past and at present have developed effective immunization for animals and the same may be developed for human use soon.

References:-

1. Sharma DC, Prasad SB, Karthikeyan BV. Vaccination against periodontitis: The saga continues. Expert Rev Vaccines 2007;6:579-90.
2. Takei N, Carranza K. Text book of clinical Periodontology. 9th Ed. Saunders; 2005.
3. Newmann, Takei, Klokkevold, Carranza. Text book of clinical Periodontology. 10th Ed. Saunders; 2007.
4. Slots J, Ting M. Actinobacilus actinomycetemcomitans in human periodontal disease: occurrence and treatment. Periodontol. 2000 1999;20:82-121.

5. Gemmell E, Marshall RI, Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. Periodontol. 2000 1997;14:112-43.
6. Gianobile WV, Beikler T, Kinney JS. Saliva as a diagnostic tool for periodontal disease. Periodontol. 2000 2000;50:52–64.
7. Lamont RJ, Jenkinson HF. Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis. MicrobiolMolBiol Rev 1998;62:1244–63.
8. Gemmell E, Yamasaki K, Seymour GJ. The role of T cells in periodontal disease: homeostasis and autoimmunity. Periodontol. 2000 2007;43:14–40.
9. Kornman KS, Page RC, Tonetti MS. The host response to the microbial challenge in periodontitis. Periodontol. 2000 1997;14:33–53.
10. Kudyar N, Dani N, Mahale S. Periodontal vaccine: A dream or reality. J Indian Soc Periodontol 2011;15:115-20.
11. PlayfairJ, Roitt I, Brostoff J, Male D. Vaccination. Immunology 1998;19:1-10.
12. Choi JI, Seymour GJ. Vaccines against periodontitis: a forward looking review. J Periodontal Implant Sci 2010;40:153–63.
13. Verma JN, Rao M, Amselem S, Krzych U, Alving CR, Green SJ, et al. Adjuvant effects of liposomes containing lipid A: enhancement of liposomal antigen presentation and recruitment of macrophages. Infect Immun 1992;60:2438–44.
14. Reid R and Roberts F. Textbook of pathology illustrated 6th Ed. Churchill Livingstone; 2005.
15. Nikhil S, Nitin K. Periodontal vaccine: a new paradigm for prevention of periodontal diseases. J Oral Health Comm Dent 2010;4:23–8.
16. Happy D, Hadge P, Khopade S, Sayyed J, Sable S. Periodontal Vaccines. Journal of Dental & Allied Sciences 2013;2:21-3.
17. Kumar A, Sharnamma B, Dutt P, Gupta G. Periodontal vaccine-A boon in periodontics. IOSR J Dent Med Sci 2014;13:54-9.
18. Moritz AJ, Cappelli D, Lantz MS, Holt SC, Ebersole JL. Immunization with Porphyromonas gingivalis cysteine protease: effects on experimental gingivitis and ligature induced periodontitis in Macaca fascicularis. J. Periodontol 1998;69:686–97.
19. George VT, George AK, John S, Thomas A. Periodontal vaccine: A therapeutic modality on the horizon? Saudi J Dent Res 2015 Jul 1;6:73-8.
20. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J. Clin. Periodontol 1998;25:134–44.
21. Parra B, Slots J. Detection of human viruses in periodontal pockets using polymerase chain reaction. Oral Microbiol. Immunol 1996;11:289–93.
22. Contreras A, Umeda M, Chen C, Bakker I, Morrison LJ, Slots J. Relationship between herpes viruses and adult periodontitis and periodontopathic bacteria. J. Periodontol 1999;70:478–84.
23. Kubar A, Saygun I, Yapar M, Ozdemir A, Slots J. Real-time PCR quantification of cytomegalovirus in aggressive periodontitis lesions using Taq Man technology. J. Periodontal Res 2004;39:81–6.
24. Ling LJ, Ho CC, Wu CY, Chen YT, Hung SL. Association between human herpes viruses and the severity of periodontitis. J. Periodontol 2004;75:1479–85.
25. Loubinoux J, Bisson-Boutelliez C, Miller N, Le Faou AE. Isolation of the provisionally named *Desulfovibrio fairfieldensis* from human periodontal pockets. Oral Microbiol. Immunol 2002;17:321–23.
26. Lepp PW, Brinig MM, Ouverney CC, Palm K, Armitage GC, Relman DA. Methanogenic archaea and human periodontal disease. Proc. Natl Acad. Sci 2004;101:6176–81.
27. Nail BS, Paul VD and Staurt DG. Antigens of bacteria associated with periodontitis. Periodontol. 2000 2004;35:101-34.
28. Sundqvist G. Pathogenicity and virulence of black-pigmented Gram-negative anaerobes. FEMS Immunol. Med. Microbiol 1993;6:125–37.
29. Katoh M, Saito S, Takiguchi H and Abiko Y. Bactericidal activity of monoclonal antibody against a recombinant 40-kDa outer membrane protein of Porphyromonas gingivalis. J periodontal 2000;71:368-75.
30. Persson GR, Engel D, Whitney C, Darveau R, Weinberg A, Brunsvold M. Immunization against Porphyromonas gingivalis inhibits progression of experimental periodontitis in nonhuman primates. Infect. Immun 1994;62:1026-31.
31. Page RC. Vaccination and periodontitis: myth or reality. J. Int. Acad. Periodontol. 2000;2:31–43.
32. Marawar PP and Devkar N. Gingipains:The virulence factor P.Gingivalis.J Indian SocPeriodontol 2004;7:95-9.
33. Kadowaki T, Yoneda M, Okamoto K, Maeda K, Yamamoto K. Purification and characterization of a novel

arginine- specific cysteine protease (argingipain) involved in the pathogenesis of periodontal disease from the culture supernatant of Porphyromonas gingivalis J. Biol. Chem 1994;269:21371–78.
34. Imamura T. The role of gingipains in the pathogenesis of periodontal disease.J. Periodontol. 2003;74:111–18.
35. Discipio RG, Daffern PJ, Kawahara M, Pike R, Travis J, Hugli TE. Activation of complement component C5 by cysteine proteases from Porphyromonas gingivalis. Prioroxidation of C5 augments proteinase digestion of C5. Immunology 1996;87:660–67.
36. Rajapakse PS, O'Brien-Simpson NM, Slakeski N, Hoffmann B, Reynolds EC. Immunization with the RgpA-Kgp proteinase-adhesin complexes of Porphyromonas gingivalis protects against periodontal bone loss in the rat periodontitis model.Infect.Immun 2002;70:2480–86.
37. Lamont RJ, Jenkinson HF. Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis.Microbiol. Mol. Biol. Rev 1998;62:1244–63.
38. Hamada N, Sojar HT, Cho MI, GencoRJ. Isolation and characterization of a minor fimbria from Porphyromonas gingivalis. Infect. Immun 1996;64:4788-94.
39. Ogawa T, Yasuda K, Yamada K, Mori H, Ochiai K, Hasegawa M. Immunochemical characterization and epitope mapping of a novel fimbrial protein (Pg-II fimbria) of Porphyromonas gingivalis. FEMS Immunol. Med. Microbiol 1995;11:247–55.
40. Evans RT, Klausen B, Sojar HT, BediGS, Sfintescu C, Ramamurthy NS. Immunization with Porphyromonas (Bacteroides) gingivalis fimbriae protects against periodontal destruction. Infect. Immun 1992;60:2926–35.
41. Fan Q, Sim ST, Sojar H, Genco R, Page RC. Fimbriae of Porphyromonas gingivalis induce opsonic antibodies that significantly enhance phagocytosis and killing by human polymorphonuclear leukocytes. Oral Microbiol. Immunol 2001;16:144-52.
42. Kaizuka K, Hosogi Y, Hayakawa M, Shibata Y, Abiko Y. Human monoclonal antibody inhibits Porphyromonas gingivalis hemagglutinin activity. J Periodontol 2003;74:38- 43.
43. Lopatin DE, Shelburne CE, Van Poperin N, Kowalski CJ, Bagramian RA. Humoral immunity to stress proteins and periodontal disease. J Periodontol 1999;70:1185- 93.
44. Slots J, Gibbons RJ. Attachment of Bacteroides melaninogenicus subspecies asaccharolyticus to oral surfaces and its possible role in colonization of the mouth and of periodontal pockets. Infect.Immun 1978;19:254–64.
45. Mouton C, Bouchard D, Deslauriers M, Lamonde L. Immunochemical identification and preliminary characterization of a nonfimbrial hemagglutinating adhesin of Bacteroidesgingivalis. Infect. Immun 1989;57:566–73.
46. Ellen RP, Grove DA. Bacteroides gingivalis vesicles bind to and aggregate Actinomyces viscosus. Infect. Immun 1989;57:1618–20.
47. Gibbons RJ, Nygaard M. Interbacterial aggregation of plaque bacteria.Arch. Oral Biol 1970;15:1397–1400.
48. Saito S, Hiratsuka K, Hayakawa M, Takiguchi H, AbikoY. Inhibition of a Porphyromonas gingivalis colonization factor between Actinomyces viscous ATCC19246 by monoclonal antibodies against recombinant 40-kDa outer membrane protein. Gen.Pharmacol 1997;28:675–80.
49. Ross BC, Czajkowski L, Hocking D. Identification of vaccine candidate antigens from a genomic analysis of Porphyromonas gingivalis. Vaccine 2001;19:4135–42.
50. Takamatsu-Matsushita N, Yamaguchi N, Kawasaki M, Yamashita Y, TakeharaT, Koga T. Immunogenicity of Actinobacillus actinomycetemcomitans serotype b-specific polysaccharide-protein conjugate. Oral Microbiol. Immunol 1996;11:220–25.
51. Herminajeng E, Asmara W, Yuswanto, BaridI, Sosroseno W. Protective humoral immunity induced by surface associated material from Actinobacillus actinomycetemcomitans in mice. Microbes Infect 2001;3:997–1003.
52. Chargelegue D, Vine ND, van Dolleweerd CJ, Drake PM, Ma JK. A murine monoclonal antibody produced in transgenic plants with plant-specific glycans is not immunogenic in mice. Transgenic res 2000;9:187-94.
53. Tanner AC, Socransky SS, Goodson JM. Microbiotaof periodontal pockets losing crestal alveolar bone.J. Periodontal Res 1984;19:279–91.
54. Gemmell E, Bird PS, Ford PJ. Modulation of the antibody response by Porphyromonas gingivalis and Fusobacterium nucleatum in a mouse model. Oral Microbiol. Immunol 2004;19:247–51.
55. Barry MA, Johnston SA. Biological features of genetic immunization. Vaccine 1997;15:788-91.
56. Breivik T, Rook GA. Prevaccination with SRL172 (heat-killed Mycobacterium vaccae) inhibits experimental periodontal disease in wistar rats. Clin. Exp. Immunol 2000;120:463–67.
57. Breivik T, Rook GA. Treatment with SRL172 (heat-killed Mycobacterium vaccae) inhibits progression of established experimental periodontal disease in wistar rats. J. Periodontal Res2002;37:210–14.
58. Breivik T, Rook GA. Oral treatment with SRP299 (killed Mycobacterium vaccae) inhibits experimental periodontal disease in wistar rats.J.Clin.Periodontol 2003;30:931–36.

59. Roberts FA, Laura S, Lukehart SA, Mancl LA, Persson R, Page RC. Periodontitis vaccine decreases local prostaglandin E2 levels in a primate model. Infect. Immun 2004;72:1166–68.
60. Yang QB, Martin M, Michalek SM, Katz J. Mechanisms of monophosphoryl lipid A augmentation of host responses to recombinant HagB from Porphyromonas gingivalis. Infect. Immun 2002;70:3557–65.
61. Myneni SR, Brocavich K, H Wang H. Biological strategies for the prevention of periodontal disease: Probiotcs and vaccines. Periodontol. 2000 2020;84:161-75.