The emerging role of epigenetics in the pathogenesis of periodontitis - A review

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PC Srinivasan

ABSTRACT

Periodontitis is a chronic inflammatory disease affecting the supporting structures of the teeth. It is a complex disease with multifactorial etiology. Numerous studies have examined the role of the genetic factors in the etiology of periodontitis. Epigenetics is the study of the mitotically and meiotically heritable changes in the gene function that cannot be explained by changes in the DNA sequence. Studies have demonstrated that epigenetic alterations contribute to a number of diseases like cancer, metabolic and autoimmune disorders. An understanding of the epigenetic mechanisms helps to develop novel therapeutic aids which target the specific epigenetic sites. This article attempts to shed light on the role of epigenetic alterations in the pathogenesis of periodontitis. The role of the bacteria-induced epigenetic alterations in the host cell, the alterations in the cytokine profile and the role of the environmental factors like smoking on the epigenome are reviewed. Technological advances have enabled us to analyse and quantify the epigenetic changes on a large scale. Drugs which specifically target the epigenetic mechanisms may be used as valuable adjuncts to conventional periodontal therapy leading the way to personalized and preventive regimes.

Keywords: Periodontitis, inflammation, epigenetic mechanisms, pathogenesis, epigenome, DNA methyltransferase.

INTRODUCTION

Periodontitis is one of the most common oral diseases in adult populations and is characterized by inflammation and destruction of the tooth supporting structures, ultimately leading to tooth loss in severe cases.[1-2] Although periodontitis has a microbial etiology, its progression is multidimensional and can be influenced by several factors such as systemic diseases, environmental factors, and genetic factors.[3] The individual variability in the susceptibility to periodontitis and in the response to the therapy as well can be attributed to intrinsic factors such as genetic[4] and epigenetic factors.[5] Epigenetics is the study of alterations in gene regulation not caused by changes in the DNA sequence. The term "Epigenetics" was coined by Conrad Waddington, who defined it as "the branch of biology that studies the causal interaction between genes and their product, which bring the phenotype into being."[6] The Greek prefix 'epi' in epigenetics means 'on top of' or 'in addition to' genetics.[7]

Prathiba Chichurakanahalli Srinivasan: Professor, Periodontics, NSVK SV Dental College, Bangalore, Karnataka, India.
Cell: 984 548 5963, E-mail: csprati@yahoo.com

ACRONYMS

ChIP: chromatin immuno-precipitation
COX-2: Cyclooxygenase-2
CpG: Cytosine-phosphodiester-Guanine
DAM: DNA adenine methyltransferase
DNMT1, DNMT3, and DNMT3b: DNA methyltransferases
EBV: Epstein-Barr virus
GEC's: gingival epithelial cells
HAT's: histone acetlytransferases
HDAC's: histone deacetylases
HDACi: Histone deacetylase inhibitors
HIV: human immunodeficiency virus
HPDL: human periodontal ligament
IFNG: interferon gamma gene
IRAK-1: receptor associated kinase 1
KSHV: Kaposi sarcoma-associated herpes virus
LPS: lipopolysaccharide
MMP-2: matrix metalloproteinase-2
MBDs: methyl-CpG-binding domain proteins
mi-RNA: Micro-RNA's
THP1: monocytic leukemia cell line
NF-Kappa B: nuclear factor kappa-B
SAM: S-adenosyl-L-methionine
SCFA: short chain fatty acid
siRNA's: short-interfering RNA's
STAT: signal transducers and activators of transcription
TAB2: TAK-1 binding protein-2
TLRs: Toll-like receptors
RNA's: Transfer RNAs (tRNA's), ribosomal RNAs (rRNA's), micro-RNA's (mi-RNA's
TRAF-6: tumour necrosis factor receptor-associated factor
TSG: tumour-suppressor genes

EPIGENETIC MECHANISMS

Important epigenetic mechanisms include DNA methylation, post-transcriptional histone modifications (methylation, acetylation, ubiquitylation, and phosphorylation) that affect chromatin structure, RNA associated gene silencing and chromosome inactivation.[8]

A. DNA Methylation:

DNA methylation is the covalent transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the 5th carbon atom of the cytosine residue in the Cytosine-phosphodiester-Guanine (CpG) dinucleotides. The methylation process occurs mostly in regions containing a high frequency of CpG dinucleotides, called "CpG islands" in the promoter region of a gene, and is associated with gene silencing in most cases.[9] The process of DNA methylation is catalysed by a family of closely related DNA methyltransferases (DNMT1, DNMT3, and DNMT3b).[10] The methyl groups block the binding of the transcription factors to DNA. This transcriptional repression leads to "gene silencing."[11] The exposed methylation sites allow for interaction with methyl-binding proteins, such as methyl-CpG-binding domain proteins (MBDs).[12] Additionally, these proteins are instrumental in assembling histone deacetylases (HDAC's) and thus influence chromatin condensation. HDAC's are enzymes which remove the acetyl group from histones. Thus the DNA gets wrapped more tightly around the histones.[13] The closed chromatin configuration leads to gene silencing.[14] Studies in the literature have shown that hypomethylation of DNA is associated with chromosomal instability and activation of transposable elements in human cancers.[15] Thus, abnormal methylation patterns can lead to the development of diseases.

Histone modification

A nucleosome is the basic unit of the chromatin. It comprises DNA wrapped around an eight-member histone complex which consists of two copies each of H2A, H2B, H3, and H4. This composition provides a rigid structure to the chromatin. Histones have unstructured N-terminal tails, which undergo post-translational modifications including acetylation, methylation, ubiquitination, glycosylation, citrullination, ADP-ribosylation, carbonylation and sumoylation at certain positions.[16] Post-translational modifications such as acetylation and methylation of conserved lysine residues on the amino terminal tail domains have been reported.[17] Enzymes such as methyltransferases, demethylases, histone acetlytransferases (HAT's), and HDAC's either write or erase these modifications.[18] Acetylation of core histones results in an "open" chromatin structure that facilitates gene transcription.[19] Conversely, histone deacetylases remove the acetyl groups, causing the chromatin to become more condensed and thus gene transcription is repressed.[20] Some histone marks, such as H3K27ac (H3 acetylated at lysine 27) and H3K9ac (H3 acetylated at lysine 9) are associated with active transcription of genes, whereas others like H3K27me3 (H3 methylated at lysine 27) and H4K27me3 (H4 methylated at lysine 27), are responsible for repression of gene activity. Thus acetylation is associated with activation of the gene, whereas methylation leads to silencing of gene.[21] To summarize, post-translational histone modifications are powerful epigenetic mechanisms. These modifications can alter the chromatin structure. The chromatin is either open or condensed, thus regulating the gene transcription. This article mainly focuses on the acetylation of histones, the histone deacetylase inhibitors, their implications for the pathogenesis and therapeutic management of, periodontitis.

B. NON-CODING RNA:

The non-coding RNA's do not encode for a protein, but they are functionally relevant RNA molecules. These include transfer RNAs (tRNA's), ribosomal RNAs (rRNA's), micro-RNA's (mi-RNA's), and short-interfering RNA's (siRNA's).[22] Studies have reported that these non-coding RNA's play a pivotal role in the development of oral cancer, specific syndromes, and exert influences on the immune mechanisms in the oral cavity.[23,24] Micro-RNA's and short-interfering RNA's have been shown to regulate gene expression without altering the DNA sequence. Micro-RNA's have been shown to negatively regulate the expression of their target genes at the post-transcriptional level, thus leading to "gene silencing".[20,25] This article attempts to review the role of micro-RNA's in the pathogenesis of periodontitis.

As our understanding of the pathogenesis of periodontal disease continues to grow, additional potential mechanisms linking the microbial biofilm to the disease process are being described.[26] This review focuses on the intriguing role of epigenetic alterations in the pathogenesis of periodontitis.

The role of epigenetic mechanisms in periodontitis.

Upon a microbial attack, the host mounts an immune inflammatory response. Recent studies have demonstrated that bacteria can affect the chromatin structure and transcriptional program of host cells by influencing diverse epigenetic mechanisms.[27] Epigenetic events determine gene expression and selective activation or inactivation of genes. These events modulate the production of inflammatory mediators, expression of cytokines and thus contribute to the pathogenesis of various infectious and inflammatory diseases.[28]

The role of microbial plaque-induced epigenetic changes in the pathogenesis of periodontitis.

The oral biofilm is a complex structure. Using whole genomic probes and Checkerboard DNA-DNA hybridization methodology, Sockransky *et al* analysed 13, 261 plaque samples. About 40 different bacterial species were determined in the subgingival plaque and these bacteria were found to exist in complexes. Five major complexes (red, orange, yellow, green and purple complexes) were consistently observed. Of these, the red complex, comprised of *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola* is strikingly associated to the clinical measures of periodontal disease such as destruction-pocket depth and bleeding on probing. The orange complex consists of microbes like *Fusobacterium nucleatum*, *Campylobacter rectus*, *Campylobacter showae etc*. Plaque formation begins by early colonizers (*streptococcus* species) attaching to the pellicle-coated tooth structure. The early colonizers alter the local micro-environment and make it conducive for the intermediate (orange complex bacteria) and the late colonizers (red complex) to establish themselves and thrive in the area. Thus the plaque mass undergoes maturation.[3]

Studies in the literature point to the fact that putative periodontal pathogens like *Porphyromonas gingivalis* and *Campylobacter rectus* can induce epigenetic alterations in the gingival cells and tissues.[29] The microbe-induced epigenetic alterations with the resultant disruption of the host innate immune mechanisms is a vital step in the disease progression.[30]

Plaque accumulation in the dento-gingival area elicits a host immune response in the gingival epithelium. The cells of the gingival epithelium make use of a myriad of signalling pathways to modulate the innate immune response to the various microorganisms.[31] The toll-like receptors (TLRs) enable the gingival epithelial cells (GEC's) to recognize the pathogen-associated molecular patterns. The gingival epithelial cells then produce antimicrobial peptides such as human beta defensins and chemokines that activate the adaptive immune response.[32-34] Yin and Chung demonstrated that *Porphyromonas gingivalis* perked up the expression of antimicrobial proteins human beta defensin and CC chemokine ligand 20 (CCL20). The gingival epithelial cells treated with this microbe showed decreased expression of histone deacetylase 1 and 2 (HDAC 1, HDAC 2), and DNA methyltransferase 1 (DNMT1). *P. gingivalis* also induced increased methylation of the promoter region of six genes, including the immune regulator CD276, elastase 2, toll-like receptor-2 (TLR2), interleukin-12 A(IL-12A), and two putative tumour-suppressor genes (TSG). The levels of the activating histone modification H3K4me3 were found to be reduced in GEC's incubated with *Porphyromonas gingivalis*. *Fusobacterium nucleatum*, a non-pathogen, did not induce such alterations in the GEC's. These findings corroborate the fact that *P.gingivalis* is capable of suppressing gene transcription.[31] The lipopolysaccharide (LPS) produced by Porphyromonas ginigivalis induces signalling of the toll-like receptor (TLR). The study demonstrated that the TLR signalling has far-flung effects on the epigenetic profiles of genes which respond to the TLR.[35,36] Keratinocytes in the gingival epithelium when exposed to *P.gingivalis* LPS showed decreased expression of DNA methyltransferase 1 (DNMT1), Histone deacetylase 1 and 2 (HDAC1 & 2), the key enzymes involved in epigenetic mechanisms.[37] Riggs *et al*. and Sealy *et al*. in their study demonstrated that butyric acid, a major short chain fatty acid produced by *P.gingivalis*, is a histone deacetylase inhibitor (HDACi).[38,39] Thus, butyrate, a short chain fatty acid (SCFA) produced by *P.gingivalis* induces acetylation of histones in the cells of the periodontium.[40-42]

Recent evidence has also pointed to another interesting aspect in the bacteria-induced epigenetic alterations. This butyrate, an HDAC inhibitor produced by *P.gingivalis*, causes reactivation of human immunodeficiency virus (HIV) and Epstein-Barr virus (EBV).[43-45] Morris *et al*. in their study showed that *P.gingivalis* metabolites like butyric acid enhanced the replication of Kaposi sarcoma-associated herpes virus (KSHV). The bacterial supernatant contained HDAC inhibition properties. This increased the global acetylation of H3 and H4 leading to the reactivation and replication of KSHV.[42] Thus periodontal microbes like *P.gingivalis* and other opportunistic bacteria associated with the state of immunosuppression in HIV-positive individuals may collectively contribute to AIDS progression by reactivating the latent virus through HDAC inhibition.[46,47] Oxidative stress is an imbalance between the production of a reactive oxygen species and the antioxidant defense, leading to tissue damage.[48] *P.gingivalis* induces oxidative stress, a process which is central to epigenetic modifications.[49,50]

Additional studies by Yin *et al*.[51] and Chung *et al*.[52] have demonstrated that epigenetic changes in *P.gingivalis*-stimulated dendritic cells and GECs resulted in lower levels of cytokines and chemokines secreted by these cells. Uehara *et al*. reported that LPS derived from *P.gingivalis* inhibits osteoblastic cell differentiation. DNA hypermethylation was involved in the inhibitory effect of LPS on osteoblastic differentiation of fibroblasts derived from human periodontal ligament (HPDL).[53]

Campylobacter rectus, another putative periodontal pathogen may also induce epigenetic alterations in human cells. Bobetsis demonstrated that *C. rectus* downregulated the expression of insulin-like growth factor 2 (Igf2) gene via hypermethylation of Igf2 promoter in murine placenta.[54] The reduced placental growth and foetal growth as a result of these epigenetic alterations may be involved in preterm births associated with *C. rectus* infection in humans.[55] A study conducted by Miao *et al*. observed that *Treponema denticola*, another periodontal microbe, upregulated the matrix metalloproteinase-2 (MMP-2) gene. There was hypomethylation in the promoter region of the MMP-2 gene. MMP-2 is responsible for the matrix degradation and bone resorption in periodontitis. The authors suggested that adherence/internalization of *T. denticola* into the periodontal ligament cells may have contributed to the epigenetic alterations.[56] Wu *et al*. suggested that DNA adenine methyltransferase (DAM) may regulate the genes required for the invasion process of *Actinobacillus actinomycetemcomitans*. Inactivation of DAM alters the virulence properties of this microbe.[57]

The role of microbe-induced epigenetic alterations in Herpesvirus Periodontitis.

Though periodontitis is a highly prevalent, chronic infectious disease afflicting the tooth-supporting structures, the etiology is still poorly understood.[58] The major clinical characteristics of this enigmatic disease are perplexing. Findings such as the bilateral symmetrical pattern of the disease, spontaneous remission, why periodontitis affects only few teeth, with neighbouring teeth exhibiting much less attachment loss etc., cannot be explained solely on the basis of a microbial etiology. The co-infection of viruses with the microbes and their synergistic effect on the tissues has been suggested and may offer explanations for the confounding aspects of the disease.[59]

A number of studies have shown that herpes viruses, including Epstein-Barr Virus (EBV), Human Cytomegalovirus (HCMV) and Herpes Simplex Virus-1 (HSV-1) are detected in high numbers in patients with periodontal and endodontic disease. They act synergistically with the periodontal bacteria.[60-65] The herpes viruses may impair the local host defences and thus increase the pathogenic potential of the bacteria. The bacteria in turn may increase the virulence of the herpes viruses.[66] The microbe-induced epigenetic modification of the viral genome may explain the link between viruses and bacteria in the pathogenesis pathway. Butyrate, produced by *P.gingivalis*, is a HDAC inhibitor. Bacteria-induced HDAC inhibition may reactivate the latent EBV and HIV virus.[43,44,45] Reactivation of latent HIV virus through HDAC inhibition by *P.gingivalis* may contribute to AIDS progression.[46,47]

To conclude, studies strongly indicate that microbe-induced epigenetic alterations in the host cells and the viral genome may play an important role in the disease pathogenesis. These alterations have far-reaching implications. They can affect the prognostic and therapeutic outcomes of the disease. For example, butyrate, a metabolite of *P.gingivalis* can reactivate the latent HIV, EBV, and KSHV, thus leading to progression of virus-associated diseases. Bacteria and viruses have a synergistic action, thus increasing the

pathogenicity. The use of anti-viral drugs as adjunct to conventional periodontal therapy may provide beneficial results and is a subject for further research.

Epigenetic changes and cytokines

Inflammation is the central component in the pathogenesis of periodontitis. Epigenetic alterations may have an effect on the cytokine profile, and thus can determine the outcome of the disease.

Epigenetic mechanisms have been evaluated in some cytokine genes. In periodontal disease, there is an overexpression of pro-inflammatory cytokines (IL1, IL4, IL6 and IL-10).[67-69] Epigenetic events such as hypomethylation and histone acetylation are associated with the inappropriate over-transcription of genes.[70] Gomez *et al*. observed hypomethylation in the gene of cytokine interleukin-6 (IL-6) in the tissues of individuals with periodontitis, leading to an overexpression of this cytokine in the inflamed tissues.[71] IL-6 is a key cytokine involved in bone resorption and has been detected in high levels on patients with periodontal disease.[71,72] Babel *et al*. in their study showed overexpression of cytokine IL-6 in the inflamed tissues of subjects with chronic periodontitis.[73]

Interestingly, the overexpression of IL-6 might have an influence on the epigenetic changes in the cells. Studies conducted by Hodge *et al*., Stenvinkel *et al*., and Hodge *et al*., suggested that the overexpression of IL-6 might exert an epigenetic influence in the cells by regulating the DNMT gene or by maintaining it's methylation status.[74-76] Long-standing persistent inflammation and bacterial infection may cause DNA methylation which in turn inactivates the suppressors of cytokine signalling and may thus contribute to exaggerated cytokine signalling.[71,75] Wehbe *et al*. suggested that the over-expression of IL-6 may influence the expression and activity of DNMT, demethylases or histone expression, which participates in regulation of gene methylation.[77] A similar study by Hmadcha *et al*. showed that interleukin-1 beta causes activation of DNA methyltransferase and thus markedly supresses the genes which code for the interleukin.[78]

In periodontitis, the inflammatory response involves upregulation of transcription factors-nuclear factor kappa-B (NF-Kappa B) and signal transducers and activators of transcription (STAT).[20] Oliveira *et al*. in their study evaluated the methylation status of DNA in the promoter region of interleukin-8 (IL-8, a chemokine) in gingival and oral mucosal cells, leukocytes in blood from healthy individuals, smokers and non-smoker subjects with chronic periodontitis. The methylation status was co-related with mRNA levels of IL-8. The study revealed that there was a higher percentage of hypomethylation of IL-8 gene in chronic periodontitis subjects in the DNA of oral mucosal cells.[79]

Cyclooxygenase-2 (COX-2) is an enzyme governing the production of prostaglandins that promote pain and inflammation. Zhang *et al*. evaluated the epigenetic changes in the promoter region of Prostaglandin synthase 2, the gene encoding for COX-2 in chronic periodontitis patients. The results revealed a hyper-methylation status of the gene and lower levels of COX-2 transcription in inflamed gingival biopsy cells.[80] A study by Andia *et al*. in subjects with generalized aggressive periodontitis evaluated the DNA methylation status in the promoter region of IL-8 gene in oral and GEC's. The authors reported a hypomethylated status in oral and GEC's of these subjects.[81] Zhang *et al*. evaluated the presence of epigenetic modifications in the promoter region of interferon gamma (IFNG) gene in gingival biopsy of chronic periodontitis subjects. Their study reported a significant hypomethylation and increased IFNG transcription.[82] Corroborating this evidence, White *et al*, in their study, suggested that the expression of IFNG is regulated by the status of methylation in its promoter region.[83] Sullivan *et al*. demonstrated that epigenetic alterations in the gene which codes for tumour necrosis factor alpha (TNF-α) actively regulates its expression and is present both consecutively and in response to acute stimulation of cells from the myeloid lineage.[84] Another recent study by Zhang *et al*. showed that the Tumour necrosis factor alpha (TNF-α) promoter was hypermethylated at two CpG sites, resulting in decreased expression. Reversing the methylation by treatment with a demethylating agent *in vitro*, caused increased expression of TNF-α, indicating that the methylation indeed regulated the expression.[85] De Souza *et al*., stated that variations in DNA methylation between healthy and periodontitis cases are higher in genes related to the immune-inflammatory process. DNA methylation must be modulating the chromatin regions, and consequently modulating the mRNA transcription of immune inflammatory genes associated with periodontitis. Thus DNA methylation can have an impact on the prognosis of the disease.[86]

Studies have revealed that in addition to DNA methylation, other epigenetic changes such as histone modifications also play a pivotal role in the pathogenesis of periodontitis. Gemmell *et al*. stated that the nature of the lymphocytic response determines the destructive periodontitis lesion. The progression from gingivitis to periodontitis is characterized by a transition from the Th1 to the Th2 subset of the T lymphocyte.[87] Changes in the chromatin structure occur through epigenetic mechanisms like histone modification, DNA methylation and generation of DNAse I hypersensitive sites. These epigenetic modifications occur during the process of differentiation of the naïve T cells into the various lineages, thus determining the destructive characteristics of the lesions.[88] Cantley *et al*. in their experimental mouse model study demonstrated that treatment by histone deacetylase inhibitors (HDACi) efficiently suppressed periodontal bone loss.[89]

The role of Micro-RNA's (mi-RNA) in the pathogenesis of periodontitis have only been recently reviewed. Comparison of the mi RNA profiles in the healthy and the periodontitis tissues revealed that the mi RNA levels were increased in the latter group.[90-92] In a recent study, Ogata *et al*. used mi-RNA microarray profiling and real time PCR analysis to determine the micro-RNA expression in the inflamed and healthy periodontal tissues. The results of their study revealed that the three most overexpressed miRNA's were hsa-miR-150, hsa-miR-223, and hsa-miR-200b, and the three most under expressed mi-RNA's were hsa-miR-379, hsa-miR-199a-5p, and hsa-miR-214. The overexpressed mi-RNA's are associated with inflammatory disease, organismal injury, abnormalities, urological disease, and cancer.[93] Micro-RNA's have been implicated in controlling the TLR pathway which connects the innate and the adaptive pathways of the immune response. In their experimentally induced periodontitis in apolipoprotein E-deficient (ApoE-/-) mice, Nahid *et al*. reported that polymicrobial infection with periodontal pathogens like *P.gingivalis*, *T. denticola*,

T. forsythia enhanced the levels of miR-146a. In the same study, human monocytic leukemia cell line (THP1) cultures were stimulated with a combination of periodontal pathogens. The results revealed that there was an increase in the mi-RNA levels in a time-dependent manner and this co-related with the downregulation of adaptor kinases IL-1 receptor associated kinase 1 (IRAK-1), tumour necrosis factor receptor-associated factor (TRAF-6), and TNF-α production by these cells. The authors concluded that the elevated levels of miR-146a downregulates IRAK-1 and TRAF-6. This may have been the reason for endotoxin tolerance. Hence, miR-146a may represent a target for therapeutic intervention.[94] Similar results were obtained in the study of Xie *et al*. miRNA-146 inhibited the secretion of pro-inflammatory cytokine through IL-1 receptor-associated kinase 1 in human gingival fibroblasts.[95] Ceppi *et al*. examined the levels of miR-155 in monocyte-derived dendritic cells exposed to endotoxin lipopolysaccharide. An elevated level of mi-R-155 was demonstrated and it was shown to downregulate TAK-1 binding protein-2 (TAB2), which plays an important role in IL-1 signalling pathways. The authors concluded that this negative feedback loop helped modulate the inflammatory responses of dendritic cells.96 Perri *et al*. in their study determined the expression profile of micro-RNA in obese individuals with and without periodontitis. They found that there was upregulated expression of several mi-RNA's in the inflamed gingival tissues of their patients with periodontitis and obesity. The findings suggest that inflamed periodontal tissues and obesity may share the inflammatory mi-RNA targets.97 Abnormal micro-RNA expression has been implicated in several inflammatory diseases and cancer. The micro-RNA targets may be amenable to intervention by drugs which target the specific epigenetic sites.

Tout ensemble, experimental studies have shed light on the impact of altered epigenetic patterns on the cytokine profile in patients with periodontitis. Long-standing chronic inflammation and bacterial infection may have an effect on the enzymes involved in the epigenetic mechanisms. The alterations in the cytokine profile may affect the prognostic outcome of the disease.

Role of the environmental factors in the patho-epigenetics of periodontitis.

Periodontitis is a chronic inflammatory disease. Microbial plaque, the primary etiological agent, is essential to cause the disease in a susceptible host. There are several other factors like nutrition, toxic components in the environment, tobacco smoke, alcohol and different infectious agents that can affect the disease outcome. These factors can induce epigenetic alterations in the host cells.[98,99] Studies have observed associations between smoking and global DNA methylation, linked with poor prognosis in lung cancer.[100,101] A study conducted by Oliveira *et al*.,[79] revealed that there was a higher percentage of hypomethylation of IL-8 gene in the DNA of oral mucosal cells of subjects with chronic periodontitis. But there was no difference in the methylation status of IL-8 promoter in smokers and non-smoker subjects with periodontitis in this study. Smoking is an important risk factor for periodontal disease. Further studies evaluating the methylation in smokers with periodontal disease may be of interest.

Studies have elicited the role of diet and nutritional influences on the pathogenesis of periodontitis.[102,103] Okano *et al*.[104] stated that folate deficiency during pregnancy leads to a lack of S-adenosylmethionine, a substrate required for the enzyme DNMT (DNA methyltransferase) to methylate CpG residues during embryonic development. Increase in the methylation rate in older individuals leads to gene silencing and could thereby contribute to the development of chronic diseases.[105] Ohi *et al*.[106] in their study demonstrated hypermethylation of CpG in the promoter of the collagen-alpha 1 gene in the aged periodontal ligament.

The pathogenesis of periodontitis is complex. The above mentioned studies point to the multiple confounding factors that might play a role in the epigenetic mechanisms and thereby affect the disease outcome.

Epigenetic therapy in the management of periodontitis : personalized periodontal therapy.

Epigenetic changes occur more frequently than the genetic changes and are rendered reversible by treatment with pharmacological agents.[107] Research in the use of pharmaceutical agents targeting the "epigenetic sites" is ongoing. Histone deacetylase inhibitors and DNA methyltransferase inhibitors have been in the vanguard of these approaches. Histone deacetylase inhibitors help in supressing bone resorption by osteoclasts.[89] The deacetylase inhibitors help in promoting osteoblast maturation.[108] In their study on *P.gingivalis* - induced experimental periodontitis in mice, Cantley *et al*. evaluated the bone volume changes after administering novel compounds targeting both Class I & II HDACs (1179.4b) and MS-275, which targets specifically the Class I HDAC. The results of the study revealed that Class I and II histone deacetylase inhibitor, 1179.b, significantly reduced *P.gingivalis*- induced bone loss.[109]

The above mentioned studies suggest that agents targeting the specific "epigenetic sites" can be considered as useful adjuncts in the management of periodontal disease.

Methods for analysing epigenetic mechanisms.

Technological advances have enabled the analysis of epigenetic analysis on a large scale.[21]

DNA methylation can be detected and quantified by the following techniques:

a. Bisulphite conversion.-In this technique sodium bisulphite modification of DNA enables the conversion of unmethylated cytosines to uracil, while the methylated cytosines remain unchanged.[110]
b. Global DNA Methylation Analysis-High Performance Liquid Chromatography (HPLC) is a classical method to quantify global DNA methylation.[111]
c. Gene-specific methylation analysis-can be characterized as either "candidate gene" or "genome-wide" approach.

The candidate gene approaches can be further divided into "sensitive" and "quantitative" approaches.

Methods for genome-wide analysis:

a. Microarray-based genome-wide analysis. Three main classes of microarray methods have been developed to map the 5-methylcytosine patterns in genomes :

1. Methods which enrich the highly methylated regions using an antibody specific for 5-methylcytosine or methyl-binding proteins.
2. Methods based on bisulphite modification.
3. Methods using methylation-sensitive restriction enzymes.[112]

Methylated DNA Immuno-precipitation-MeDIP

The DNA is immuno-precipitated using antimethylcytosine antibody and this immuno-precipitated DNA is hybridized to microarrays.[113]

Analysis of histone modifications:

The histone modification signals can be captured by chromatin immuno-precipitation (ChIP), in which an antibody is used to enrich DNA fragments from modification sites. ChIP-chip, ChIP-PET, ChIP-SAGE are some of the several ChIP based techniques.[114-116] Ultrahigh-throughput sequencing technologies such as Illumina/Solexa sequencing has enabled the use of a new technique called ChIP-seq. ChiP-seq is becoming one of the main approaches due to its high coverage, high resolution and low cost. In ChIP-seq, the sequence of one end of a ChIP-enriched DNA fragment is read, and it is followed by mapping the short read, called tag, to the genome assembly in order to find the genome location of the fragment.[117-119]

Thus technological advances have enabled geneticists to analyse and quantify the epigenetic modifications on a large scale. The use of bioinformatics to compute the epigenetic alterations is very exciting and promising. These data are of enormous use in research and can be used in the development of drugs that target the epigenetic sites with greater specificity.

CONCLUSION

Periodontitis is a chronic inflammatory disease involving the supporting structures of the tooth. It is a polymicrobial infectious disease. The host tissue mounts an immune inflammatory response to combat the bacterial attack. A number of studies reveal the role of epigenetic mechanisms in the pathogenesis of periodontitis. Bacteria cause epigenetic alterations in the gingival cells and tissues. These epigenetic changes can cause "silencing/shut down" of genes involved in local defences and so the chances of survival of the microbes in the local microenvironment is significantly enhanced. They may also cause rapid re-establishment of virulent flora, thereby giving rise to refractory/resistant forms of periodontal disease.[26] This emphasizes the importance of thorough surgical debridement and complete removal of infected granulation tissue which may act as a reservoir of bacteria. It is also logical to use antimicrobials as adjuncts to non-surgical/surgical periodontal therapy to eliminate the microbes which have tissue-invasive properties. Studies have also revealed that epigenetic changes in the cytokine genes have a crucial role to play in the pathogenesis of this inflammatory disease. However it needs to be ascertained whether these epigenetic alterations lead to increased susceptibility to the disease or whether they are a consequence of the long-standing chronic inflammatory response.[51] It is a "chicken or egg" scenario which is most perplexing. Periodontitis has a complex multifactorial etiology. Though microbial plaque is the primary etiologic factor, systemic diseases like diabetes, age and environmental factors such as smoking may affect the disease outcome. These confounding factors also have a role to play on the epigenome. Hence studies evaluating the epigenetic events in the pathogenesis of periodontitis should be viewed with caution. Geneticists have made terrific progress and have developed drugs that target the "epigenetic sites." These drugs can be used as valuable adjuncts to conventional periodontal therapy. This type of therapeutic approach is showing great promise in the treatment of other diseases affected by aberrant epigenetic marks like cancer, lupus, and asthma, neurological disorders like Huntington's chorea, Alzheimer's disease and diabetes. The challenge with this approach is to specifically target the epigenetic marks which have negatively influenced the gene, leaving alone the beneficial ones that help maintain health. It has always been thought that "our genes are set in stone" and are beyond our influence. The concept that the epigenome can be altered by pharmacologic intervention is very profound and empowering. Technological advances have enabled analysis and quantification of the epigenetic changes and have been instrumental in the development of drugs which target the epigenetic sites with greater specificity.

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