immune cells from the innate and adaptive responses, which may be a potential mechanism by which smoking exacerbates periodontal breakdown.

Altered Cytokine Production

Smoking induced functional changes in the main immune cells (neutrophils, macrophages, natural killer cells, mast and dentritic cells, eosinophils and B and T lymphocytes) indicate multiple intracellular signaling pathways as possible mechanisms to explain the effects of smoking on cytokine production in periodontal tissues. Nicotine per se stimulates the production of interleukin-6 and interleukin-8 and the association of high doses of nicotine with lipopolysaccharide synergistically up-regulates the production of these mediators[34].Giannopoulou et al using multiple linear regression analysis demonstrated that smoking was associated with increased levels of interleukin-4, interleukin-6 and interleukin-8 in gingival crevicular fluid[35].

The salivary levels of biomarkers like prostaglandin E_2, lactoferrin, albumin, aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase are found to be significantly lower in current smokers compared to non-smokers suggesting a possible role of smoking in suppressing the host-defense system[17]. Smoking subjects with periodontitis exhibit decreased amounts of proinflammatory cytokines (interlekin 1α, interleukin-6 and interleukin-12), chemokines (interleukin-8, monocyte chemoattractant protein-1, macrophage inflammatory protein-1 and RANTES) and regulators of T-cells and natural killer cells (interleukin-7 and interleukin-15) than non-smokers, suggesting an immunosuppressive effect of smoking on periodontal tissues which may contribute to the high susceptibility to periodontitis[36]. Also the levels of interleukin 1β in the gingival crevicular fluid are reduced in periodontally diseased sites in smokers, but are enhanced in smokers who are periodontally healthy. This suggests the possibility of an imbalance in cytokine production that may affect the pathogenesis of periodontal disaease in smokers.

The molecular intracellular signalling of cytokine production as a mechanism which might be related to the modulation of inflammation by smoking involves in addition to activation of nuclear factor kappa B, a number of transcription factors, including GATA, PAX5 and Smad ¾, which also have been implicated in smoking related inflammation[37]. Smoking –induced cytokine expression thus seems to be a complex and multistep system with participation and cooperation of multiple signalling pathways.

Fibroblast, Osteoblast & Periodontal Cell Function

Cigarette smoke condensate affects the proliferation of human gingival fibroblasts and increases the collagen degrading ability of these cells by changing the production and localization of matrix metalloproteinase and its inhibitors[38]. Nicotine has been shown to negatively regulate the differentiation and mineralization of murine periodontal ligament cells, as the expression of extracellular matrix and osteoblastic transcription factor genes were reduced in these cells treated with nicotine[39]. Periodontal ligament cells treated with various concentrations of cigarette smoke extracts showed reduced survival and altered expression of molecules involved in the structural integrity of the ligament[40]. Supplementation of osteoblast culture with nicotine and/or lipopolysaccharide increase the expression of matrix metalloproteinases 1,2 and 3 and tissue type plasminogen activator and decrease the expression of tissue inhibitor of matrix metalloproteinases 1,3 and 4[41]. Nicotine has also been shown to suppress the expression of bone sialoprotein in rat osteoblast like cells[42]. A lower serum concentration of osteoprotegrin and a higher ratio of receptor activator of RANKL/osteoprotegrin were observed in smokers than in non-smokers[43].

Tissue Degrading Enzymes

Increased release and diminished inhibition of tissue degrading enzymes such as collagenases and serene proteinases are essential for destruction of periodontal tissues. Smoking causes an elevation in the circulating levels of certain enzymes like myeloperoxidase, lysozyme, human neutrophil lipocalin and matrix metalloproteinases[44].

Studies have demonstrated that smokers with chronic periodontitis had significantly higher serum concentrations of myeloperoxidase and elastase and lower concentrations of tissue inhibitor of matrix metalloproteinases compared to non-smokers. However these differences were not seen in periodontally healthy individuals, regardless of smoking status. The matrix metalloproteinase 9/ tissue inhibitor of matrix metalloproteinase -1 ratio was also seen to be higher in smokers with periodontitis but not in periodontally healthy smokers. In addition to the elevated serum concentrations, tissue degrading enzymes have also been shown to be elevated in gingival crevicular fluid and in periodontal tissues.

In-vitro studies show that smoke and smoke components have an activating effect on leukocytes. Nicotine decreased chemotaxis and phagocytosis of neutrophils in a dose dependent manner while it increased degranulation and generation of eicosanoids on these cells. A decreased respiratory burst and increased degranulation in neutrophils are seen in smokers indicating a decreased ability to kill bacteria by the production of reactive oxygen species which contributes to increased release of tissue degrading enzymes and consequently tissue destruction[45].

Oxidative Stress

There is a relationship between oxidative stress, periodontitis and smoking related periodontitis. The possible mechanism may be an oxidant-anti oxidant imbalance which can cause progressive damage of the periodontal tissues. Polymorphonuclear cells in the infected sites produce reactive oxygen species (eg:- hydrogen peroxide and hydroxyl radical) when activated by inflammatory mediators and generate oxidative stress[46]. In addition to this, neutrophils exposed to tobacco smoke display elevated destructive oxidative burst products, with the release of superoxide and hydrogen peroxide, which also may cause oxidative damage in several tissues[47].Systemic oxidative stress is associated with a decrease of total IgG and smoking is found to be an effect modifier of this association[48].

Up-regulation of RAGE

Another potential mechanism by which smoking may affect periodontal diseases is through the up-regulation of the receptor of advanced glycation end-products (RAGE). The biological function of RAGE is dependent on the presence of its various ligands (e.g. advanced glycation end products, S100-calcium binding protein, high-mobility group protein 1 etc) [49].

Smokers express a higher level of RAGE and the cells treated with nornicotine present a time-dependent increase in RAGE expression, compared to non-smokers[50]. These findings suggest that RAGE might be associated with periodontal disease related to smoking.

Osteoclast Activation

RANKL and osteoprotegerin are important modifiers of alveolar bone resorption. RANKL initiates osteoclast differentiation by activating the osteoclast progenitors and regulates the activity of mature osteoclasts. Osteoprotegerin inhibits osteoclast differentiation by binding to RANKL and blocking the RANK/RANKL interaction. Smokers have decreased levels of osteoprotegerin. Since there are contradictory reports on the levels of osteoprotegerin in the serum of smokers, this potential mechanism for the detrimental effect of smoking needs further investigation.

Reactive Oxygen Species

Another possible mechanism by which smoking affects periodontal tissues is by the generation of reactive oxygen species. It is suggested that one puff of cigarette contains up to 10^{17} oxidant molecules. These molecules are important for intracellular bacterial killing but may also cause destruction of extracellular tissues. The tissue destruction can be direct ie via increased oxidative stress or indirect, by inducing a pro inflammatory state[51].

The literature regarding the effect of smoking on the generation of reactive oxygen species is inconclusive. Most studies investigating the effects of smoking have shown that smoking causes a reduction in the generation of reactive oxygen species[45].However, some recent animal and cinical studies have shown an increased generation of reactive oxygen species[52,53]. Moreover, several studies have shown a systemic imbalance between oxidant-antioxidant levels as a result of decreased plasma levels of circulating antioxidants. Such reduction in antioxidants combined with increased numbers of activated leukocytes could contribute to periodontal tissue destruction.

Gene Smoking Interaction

It has been suggested that susceptibility to periodontal disease has a strong genetic component[54]. However, the possibility of a gene-environment interaction between a specific genotype and smoking has not been explored for periodontitis. Some studies have shown an additive effect of a specific genotype and smoking. These studies investigated a possible composite effect of interleukin 1β C (3953/4) T polymorphism and smoking. The original study on this polymorphism indicated that smoking attenuated the effect of the genotype, while later studies showed an additive effect[55,56]. A recent meta-analysis showed a significant association between the same polymorphism and chronic periodontitis[57]. It has been shown that the combined effect of being genotype positive and smoking increased the risk of tooth loss by 7.7 fold[58] and the likelihood of having periodontitis from 2.4 to 4.5[59]. However there was no increase in risk among non-smokers who were genotype positive. Another study showed that smokers positive for the high ligand - binding genotype of the Fcγ-receptor (Fcγ RIIa-H/H131) have more periodontitis than smokers not positive for this genotype and non-smokers positive for the genotype[60].

Role of Nicotine

The exact role of nicotine on periodontal inflammation is still unclear. Some in-vitro studies have shown that application of nicotine to various types of cultured cells induced an inflammatory response that could be of importance for the initiation and progression of periodontitis[61]. Animal studies have shown that systemic administration of nicotine increased alveolar bone loss in rats with ligature induced periodontal inflammation[62]. Another study showed that smoke extract increased the respiratory burst in neutrophils, where as nicotine and cotinine alone had no effect[53].

5. Effect of Passive Smoking

The effect of passive smoking on periodontal health has not been extensively studied. A study using the data from NHANES III reported that the odds of having periodontitis was 1.6 higher among individuals exposed to passive smoking after adjusting for sociodemographic factors, diabetes and dental care. Another study showed that passive and active smoking increased the likelihood of having periodontitis by 2.9- fold and 4.9- fold respectively after adjusting for other lifestyle factors. Exposure to passive smoke was associated with elevation of interleukin 1-β, albumin and aspartate aminotransferase levels in saliva[63]. Taken together, the literature seems to indicate a relationship between passive smoking and destructive periodontal diseases. Further longitudinal studies are necessary to find out the role of passive smoking on immunological changes in the periodontium.

6. Conclusion

Although there are several possible mechanisms which could clearly explain the higher prevalence of periodontal disease and reduced healing seen in smokers, there is no clear evidence that points to one particular mechanism as being of greater importance. The response to smoking is likely to be mediated through a number of pathways, including a shift towards a more pathogenic subgingival flora, reduced microcirculation, dysfunction of neutrophils, production of proinflammatory cytokines and increased levels of pathogenic T-cells. Evidence indicates that smoking is an important risk factor for periodontal disease as well as for many other chronic diseases and events in

humans, including heart disease, stroke and cancer. Hence it is imperative for dentists to actively encourage their patients to engage in smoking cessation activities, which is a major key in improving their oral health as well as their overall health.

Conflict of Interest

There are no conflicts of interest or source of funding for this article.

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