<u>Review Article</u>

Oral Malodor-A Menace To Periodontist

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Abstract

Oral malodor significantly affects our daily social life. It affects a large population and is a psychological setback to those suffering from it. This common disease has been really ignored for too long by periodontists, even though the most common cause is related to subgingival microbiota and coating of the tongue. It has been proved that a link exists between oral malodor and periodontal disease. Similar processes exist for the development of oral malodor and the progression of periodontitis. Compounds associated with oral malodor like volatile sulfur compounds (VSC's) are mainly produced by the gram negative, anaerobic bacteria associated with periodontitis. These VSC may further contribute to the etiology and pathogenesis of periodontal disease, so the purpose of this review article was to correlate the relationship between oral malodor and adult periodontitis.

<u>Key words:</u> halitosis; oral malodor; periodontitis; volatile sulfur compounds.

Introduction

Halitosis is a general term which is used to describe the unpleasant breath. It can have an oral or a non oral cause. Oral malodor refers to the unpleasant breath originating from the oral cavity.[1] According to the small number of epidemiological data available, around 25% of the population suffers from at least occasional halitosis and around 6% suffers from permanent halitosis.[2]

Oral production of malodorous substances is mostly associated with the degradation of protein by the bacteria present in the oral cavity especially the tongue. Putrefaction is the term which is used to describe the process of protein hydrolysis and the catabolism of resulting amino acids which produces the unpleasant smelling volatiles. Excessive amount of the oral malodor is usually associated with the increased oral alkalinity.[1]

Oral malodor involves the mixture of variable odourous volatiles like methanethiol, dimethylsulfide, n-tetradecanol, hydrogen sulfide, ndodecanol, indole, pyridine, phenol, diphenylamine and others.[3] But it is now agreed that the sulfur containing volatiles are the central elements for the production of oral malodor and their levels correlate with the intensity of the oral malodor. Other volatiles function as modifiers.[1]

Classification of halitosis:[2]

I) Real halitosis:
Distinct foetor exceeding the socially tolerable level.

A) Physiological:
It is temporarily occurring and has its origin in the oral cavity, in the absence of any specific illness or pathological process. The source of the smell is usually the dorsal part of the tongue.

B) Pathological:
<u>Intra-oral</u>: Caused by pathological process within the oral cavity and by a coating of the tongue, modified by pathological conditions (e.g. paradontopathy, xerostomia)

<u>Extra-oral cause:</u> malodor from the ENT area (e.g. nasal, Paranasal, laryngeal), from the respiratory tract and the Upper digestive tract and malodor due to other general illnesses (e.g. diabetes, cirrhosis of the liver, uraemia).

II) Pseudo halitosis:
Patient complains of halitosis although nobody else notices it. The situation can be improved by enlightening the patient through the use of literature and discussing the results of the examination.

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III) Halitophobia: Patient complains of halitosis although nobody else notices it. Enlightening the patient by using literature and discussing the results of the examination can not convince the patient that no malodor exists.

Clinical relationship between oral malodor and periodontal disease:

It was proposed McNamara et al (1972)[4] that gram negative bacteria were responsible for the production of malodor. *F.nucleatum*, a gram negative microorganism associated with periodontitis can catabolize cysteine and methionine and produce the sulfur volatiles which contribute to oral malodor. It was demonstrated[5] that the desulfuration of cysteine was initiated by cysteine desulfhydrase and this process generates pyruvate, ammonia and hydrogen sulfide. Similiarly the hydrolysis products of methionine are ?-ketobutyrate, ammonia and methyl mercaptan. Grenier and Maynard showed that *F.nucleatum* also produced[6] of higher levels of butyrate in culture filterates as compared to the similar filterates from the black pigmented *Bacteroides.*[1]

Tonzetich and McBride examined the strains[7] of B. melaninogenicus for their ability to produce volatile sulfur compounds (VSC).[1] *Treponema denticola, Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedius,* and *Bacteroides loescheii* produced higher amounts of sulfides than any other groups. Other bacterial species recovered from the periodontal pockets such as *Enterobacteriaceae, Bacteroides forsythus, Centipede periodontii, Eikenella corrodens, Fusobacterium periodonticum* etc also had a high capability to generate VSC in vitro.[3]

Solis-Gaffer et al examined[8] eight prominent Gram positive and four Gram negative bacteria for their ability to produce VSC, but Gram negative, anaerobic bacteria are mainly involved. The development of increasing numbers of anaerobic bacteria and a zone of anaerobiosis would necessarily depend upon the oxygen levels, plaque thickness, and rate of oxygen utilization by the bacteria in the outer plaque layers. Basically, thicker plaque and lower salivary oxygen level are the keys to the development of larger zones of anaerobiosis and the lower redox conditions conducive to oral malodor.[1]

The investigations have shown that dietary fermentable carbohydrates and salivary proteins, peptides, and amino acids readily stimulate oxygen consumption by the oral bacteria. Reduced salivary flow rate or the stagnation of saliva was found to be one of the contributory factors for the bacterial shift and the production of malodor. A typical odor is produced when the saliva is alkaline and the opposite is seen if the saliva is slightly acidic. An acidic pH prevents the formation of odourous metabolic end products by bringing about the inactivation of the enzymes involved in the putrefaction of amino acid.[3] As the plaque thickens the number of gram negative microorganisms also increases which further coincides with the rise in the severity of gingivitis or periodontitis. Plaque[9] which is present closest to the gingiva is at a lower oxidation-reduction potential than the thinner and younger plaque more coronally located on the tooth surface.[1]

Plaque is composed of cells, mostly bacterial and the outermost layer, referred to as the material alba usually contains the desquamated epithelial cells and blood cell elements.[10] These cells and their debris are good sources of VSC for odor production. The innermost region is the acquired pellicle, which is derived mainly from the salivary glycoprotein and bacterial cell wall[11] and produces lesser amount of sulfur containing amino acids. The oxygen entering the outermost regions of plaque from saliva is removed by its resident bacteria and this depletion makes it possible for the reduced conditions and the more Gram negative types of flora[12] needed for malodor formation[4] to develop in the innermost plaque regions. The bacteria which are present on the oral soft tissues and on the teeth are able to produce a favorable reduced condition for the conversion of readily available disulfide in the sulfoproteins of oral epithelial cells to cysteine and eventually to thiol containing volatiles.[1]

The periodontal pocket is an ideal environment for the production of VSC. Rizzo in 1967 measured the production of hydrogen sulfide in periodontal pockets semi quantitatively by placing filter paper strips impegranated with lead acetate. If the paper turned black or brown after insertion the result was consid-

ered to be positive and this positive correlation was found between the depth of periodontal pocket and the amount of hydrogen sulfide present.[13]

Similarly the concentration[14] of VSC were higher in patients with probing depth greater than 4 mm and that the ratio of methyl mercaptan to hydrogen sulfide significantly increased in periodontal disease. Studies conducted by Coil & Tonzetich (1992)[15] concluded that periodontitis patients with inflamed pockets showed a significantly higher amount of sulfide than the patients with the non inflamed periodontal pockets.

In various studies conducted by Sulser et al. (1939) and Berg et al (1947) it was found out that[16,17] it was found out that the saliva collected from the individuals with periodontitis putrified more rapidly than the saliva from healthy individuals and this saliva also had a more offensive malodor. It was[18] analyzed that a positive correlation was present between the gingival index, GCF volume and hydrogen sufide production.

Morita & Wang (2001) demonstrated in one of their studies that the amount[19] of VSC production was more in the individuals who showed radiographic bone loss and the results also correlated the other parameters like pocket probing depth, clinical attachment level and bleeding on probing. Furthermore studies conducted by Tonzetich J & Coil JM[20] showed that the routine periodontal therapy decreased the baseline values of odour associated compounds to near-normal levels.

Effect of VSC in Periodontal Disease:

Periodontal disease is generally considered to be a non-specific inflammatory response to factors produced by dental plaque microorganisms. The process is characterized by complex alterations in gingival tissues and several stages of progression from the early to advanced lesion formation. Since the VSC are produced by degradation of protein and are markedly reduced in absence of disease, it is unlikely that they are responsible for initiation of the disease process. However, once an initial lesion is established, the potentiation of the response is modified by the multiple factors which were not associated with the original etiology.[21]

The by-products of bacterial metabolism induce a change in the structure of crevicular epithelium to permit an increase in accessibility of microbial substances to the underlying connective tissue layer, where they produce the destructive inflammatory reactions. So the tissue barrier should be maintained such that the toxic substances such as endotoxins and bacterial dextrans are incapable of causing inflammation.[22]

Both the junctional and the sulcular epithelium appear to be crucial sites with regard to the development of periodontal disease. Oral sulcular epithelium is a weak barrier to antigenic substances as

attested by the numerous *in vivo* and *in vitro* studies because of its morphology and the constant exposure to mechanical stress and inflammatory agents.[22]

The initiation of the periodontal disease appears to be associated with the breakdown of the integrity of epithelial basement membrane.[23] The onset of periodontal disease follows the initial damage to the tissue barrier[24].

The mucosal specimens show three compartments and the permeability is regulated by extacellular matrix, so the thiols are believed to have penetrated the surface epithelium and cross the underlying basal membrane. To induce an increase in permeability of these tissues, it appears that the VSC also react with the basal membrane or intercellular matrices and altered their permeability.[22]

Thiols may also increase the permeability by the following mechanism: Once synthesized intracellulary, proteoglycans and glycoproteins are secreted and are held in an aggregate state[25] through disulfide bridges in the extracellular matrix. The VSC may induce de-aggregation of proteoglycans by cleaving disulfide bonds, thereby inducing an increase in permeability of oral mucosa. This is supported by findings that the blocking of cysteine residues results in blocking of proteoglycan aggregation.[26]

Furthermore, the de-aggregation of the matrix can be induced by the disulfide-cleaving agents such as dithiothreitol. So, it may be concluded that the peri-

odontal disease which comprises of the loss of inter-cellular substances in the oral sulcular epithelium may be aggravated by the ability of VSC to further increase the permeability which promotes the penetration of antigenic substances such as endotoxins through the tissue barrier.[22]

Hydrogen sulfide and methyl mercaptan, derived through putrefactive activity of micro-organisms, are believed to be toxic to oral tissues. They comprise 90% of VSC content of mouth air and have been shown to increase with the severity of periodontal disease.[22] Thiol groups have been shown to play an important regulatory role in a number of complex biological systems. Since hydrogen sulfide and methyl mercaptan have free thiol functional groups they can bind covalently to epithelial components in salivary sediment.[27]

The thiol group present in methyl mercaptan and hydrogen sulfide can also alter the cellular metabolism by interaction with the thiol sensitive intracellular regulatory processes. Its effects were evaluated using human gingival fibroblast cultures and viable porcine non-keratinized oral mucosal tissues. A significant reduction on the total protein was found out and this change was irreversible for at least 24hrs. It mainly occured due to the oxidation and methiolation of cellular protein. The results also showed that extended periods of exposure to thiols cause greater reduction of protein, approximately 34% at 48 hr and 25% at 24hr. Part of the effect was found to be related to reductions in collagenous proteins.[27]

Although both methyl mercaptan and hydrogen sulfide (H_2S) inhibit protein synthesis, mercaptan produced greater inhibition and appears to have more destructive potential. This difference between the two agents may reflect the nature in the reaction with the protein. The interaction with H_2S would form a complex which could still contain exposed free thiols. Methyl mercaptan on the other hand acts as an alkylating agent and is potentially more damaging than H_2S.[21]

Slab gel electrophoresis of proline labeled fibroblast medium indicates that synthesis of collagen is inhibited. Processing of procollagen and changes in gene expression was also seen. This change was found to be irreversible.[27] This was consistent with the changes in the gingival tissue observed during the initial stages of inflammation, there is rapid loss of 60-70% collagen and there is also a drastic reduction in acid soluble and insoluble collagen, the more mature and more cross linked forms of the molecule. Thiols can interact with intact collagen molecules and therefore significantly affect the structure of collagen molecules and can alter the intracellular and extracellular degradation. Initial binding of sulfur radical from VSC into salivary proteins as well as type I collagen, was also noticed.[21]

Volatile thiols can both bind and solubilize type I collagen *in vitro* thereby altering its structure or synthesizing of abnormal collagens such as type I trimer and making it more susceptible to enzymatic degradation.[21]

This reduction of protein and collagen is apparent within 24 hrs following exposure of cultures to VSC and persists following the removal of the etiological agent.[28]

It has been found out that DNA synthesis was also supressed by VSC by 44.1% at the 24 to 26 hr peak of DNA synthesis which may be related to membrane effects or inhibition of intacellular enzymes. Mercaptan non-specifically methiolates the proteins. It was a true inhibition and not a shift in peak of maximum DNA synthesis as the shape and location of time-course curves of control and test systems was very much similar. The same study also showed that the proline transport was inhibited which mainly occurs due to the interaction with the cell membranes resulting in alteration of membrane permeability and cell viability.[27]

Methyl mercaptan when used alone or in combination with interleukin-1 or lipopolysaccharide, can significantly enhance the human gingival fibroblasts to secrete prostaglandin *E2*, cAMP and procollagenase. These secreted substances may contribute to the increased production of collagenase and tissue destruction in periodontal disease[29]. Claesson et al.[30] reported that polumorphonuclear leucocytes (PMNs) were able to kill bacteria even in the presence of sulfide. However, sulfides may annihilate the opsonization of C3bi and affect its ability to fight bacteria .[31] VSC can also induce periodontal tissue destruction through variable pathways directly or indirectly. It is known that when cysteine and methionine are desul-

furated by the various microorganisms, they produce H_2S, pyruvate, NH_3, and CH_3CH, which are toxic to the periodontal cells. When the porcine epithelial tissues were treated with methyl mercaptan, the tissues demonstrated extensive impaired and dead cells .[27] These findings suggest that VSC are directly toxic to epithelial cells and can facilitate the bacterial invasion into underlying connective tissue.[3] Once into the connective tissue, these products can be expected to induce the inflammatory reactions. NH_3 can readily penetrate the membrane and cells and raise the intracellular pH. This changes the rate of enzyme reactions, the solubility of intracellular proteins, permeability of membranes, and cell vacuolization, disruption of lysosomes, and secretory vesicles.[1]

The other volatile fatty acids are also produced by oral bacteria during amino acid degradation which produce malodor and periodontal tissue destruction; these include the propionate, butyrate, lactate, and acetate.[1] The culture fluid and aqueous extracts of human plaque incubated *in vitro* for their effects on cultured mammalian fibroblasts. The test fluids showed propionate and butyrate which were found to be inhibitory. Similar findings were made in tests with cultured human gingival cells, thus identifying butyrate and propionate as potential toxic plaque products. Levine[33] anticipated the same results.

When Singer et al[32] applied solutions of either propionate or butyrate twice daily to the gingiva of beagle dogs to see if these acid anions affected gingival inflammation, sodium propionate and sodium butyrate both caused an increase in gingival inflammation within a period of several days. Saline solutions tested in the same way on control group of animals had no effect. It was thus concluded that propionate and butyrate at concentrations generated in dental plaque could induce gingival inflammation in the beagle dogs and presumably in humans.[32]

Conclusion:

Except for a few investigators, the subject of oral malodor has been largely ignored by the academic dental research community. Part of this can be attributed to the reluctance to work with the complexity inherent to mixed rather than pure cultures. Part may also be the result of few investigators recognizing that oral malodor manifestation reflects basically the same processes in the development of gingivitis/periodontitis. But, recently along with the growing public and media interest in oral malodor, dental professionals are becoming more aware of the patients concern and need.

The data in this manuscript indicates a link between oral malodor and periodontal disease. The VSC in periodontal pockets might be used as a predictor of periodontal disease, these can be used for assessing the active disease sites, quantitatively monitoring of therapy response, and prediction of future disease sites. The information also indicates that the compounds associated with oral malodor can mimic a number of effects linked with periodontal disease and they may prove to be an important etiologic factor in the pathogenesis of the periodontal disease.

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