

Oral submucous fibrosis: etiology, pathogenesis, and future research

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Oral submucous fibrosis (OSMF), a precancerous condition of the oral cavity, has been studied by a number of workers in the field. The available epidemiological data showed a clear-cut geographical and ethnic predisposition, which suggested that certain customs/habits prevalent among the population groups in south-east Asia might be possible etiological factors. However, none of these customs was shown to be causally linked and the association in many cases was 'casual'. This led some workers to consider the importance of systemic predisposition, in addition to the effects of local factors on the oral mucosa. More research is needed to elucidate this problem.

Introduction

In 1952, Schwartz (1) described five Indian women from Kenya with a condition of the oral mucosa including the palate and pillars of the fauces, which he called "atrophia idiopathica (tropica) mucosae oris". Later it was termed oral submucous fibrosis (OSMF) (2); other names are "diffuse oral submucous fibrosis", "idiopathic scleroderma of the mouth", "idiopathic palatal fibrosis", "sclerosing stomatitis" and "juxta-epithelial fibrosis" (3).

Submucous fibrosis is an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx (4). Occasionally it is preceded by and/or associated with vesicle formation (5) and is always associated with a juxta-epithelial inflammatory reaction followed by progressive hyalinization of the lamina propria (6). The later subepithelial and submucosal myofibrosis leads to stiffness of the oral mucosa and deeper tissues with progressive limitation in opening of the mouth and protrusion of the tongue, thus causing difficulty in eating, swallowing and phonation (7). Epithelial atrophy is marked in advanced stages of the disease.

Apparent divergencies in these characteristics between groups of patients in different studies raised the question whether OSMF should be considered as one, or more than one disease. Although the evidence that it predisposes to cancer is not yet absolutely conclusive, it is highly probable that this rela-

tionship exists. The WHO definition (8) for an oral precancerous condition—a generalized pathological state of the oral mucosa associated with a significantly increased risk of cancer—accords well with the characteristics of OSMF.

Geographical distribution and prevalence

Numerous published reports on OSMF allow an informed appraisal of its geographical distribution (Table 1), together with data on the percentage prevalence. A community-based epidemiological survey in three areas of India (north and south) recorded the following prevalences of OSMF: 0.36% in Ernakulam, Kerala, and 0.04% in Srikakulam district of Andhra Pradesh (both in the south), and 0.16% in Bhavnagar, Gujarat (in the north) (9). An epidemiological assessment of the prevalence of OSMF among Indian villagers based on baseline data recorded a prevalence of 0.2% ($n = 10\ 071$) in Gujarat, 0.4% ($n = 10\ 287$) in Kerala, 0.04% ($n = 10\ 169$) in Andhra Pradesh, and $\leq 0.07\%$ ($n = 20\ 388$) in Bihar. The prevalence among 101 761 villagers in the state of Maharashtra (central India) was 0.03% (10).

A hospital-based survey among patients attending the four dental colleges (in Lucknow and Bombay in the north, and Bangalore and Trivandrum in the south) recorded percentage prevalences of 0.51, 0.50, 0.18 and 1.22, respectively (11). In a 10-year follow-up study of oral precancer, Gupta et al. (9) calculated the incidence rates of OSMF in Ernakulam: 8 for men and 19 for women per 100 000. Variations in the prevalence figures are common between different studies, probably because of differences in the clinical criteria for diagnosis. While some investigators adhered to the earlier signs and symptoms

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Table 1: Distribution of OSMF according to geographic location, age, sex and percentage prevalence in various investigations

Investigator and year of study	Place/ethnic group	Percentage prevalence	Percentage of women	Age (years)
Schwartz, 1952	Kenya, E. Africa/Indians	?	100	?
Joshi, 1953	India/Indians	?	54	10-65
Lal, 1953	India/Indians	?	?	?
Su, 1954	Taiwan/Chinese	?	?	30-40
Desa, 1957	India/Indians	?	53	10-55
Sharan, 1959	India/Indians	?	Males predominant	12-62
Rao, 1962	India/Indians	?	63	12-64
Sirsat et al., 1962	India/Indians	?	53	10-58
Shear et al., 1967	S. Africa/Indians	0.5	100	18-53
Pindborg et al., 1968	India/Indians	0-0.4	47	20-89
Pindborg et al., 1980	India/Indians	?	73	15-55
Lay et al., 1982	Bilugyum/Burmese	0.1	80	30-68
Pindborg et al., 1984	Hainan Island/Chinese	?	100	?
Hardie, 1987	Canada/Indians	?	33.3	25-56
Seedat et al., 1988	Durban/Indians	3.4	?	?
Rajendran et al., 1992 ^a	India/Indians	0.27	58.8	10-79

^a *Bulletin of the World Health Organization*, 1992, **70**: 783-789.

such as pain, history of vesicles and ulcers, and blanching of the mucosa for diagnosis of OSMF (12), others looked for fibrous bands as the diagnostic criterion. In Durban, Seedat & van Wyk (13) found 186 betel-nut chewers in a random stratified sample of 2058 subjects older than 10 years, of whom 70 (38%) exhibited features of early and established OSMF (prevalence rate, 3.4%). According to Pindborg (14), if fibrous bands only was the criterion for diagnosis, the prevalence rate would have been about 1.6%.

Most examples of the disease are found in India, especially in the southern states (3, 15), although cases have been reported in China (Province of Taiwan) (16), China (Hainan Island) (17), Malaysia (16, 18), South Africa (Natal) (14), Papua-New Guinea (19), Sri Lanka (14), Myanmar (Burma) (20), the United Kingdom (21), and Canada (22). Sporadic cases among non-Asians have also been reported (23). No relationship to any community or religious group has been suggested, but an ethnic basis is indicated because OSMF is found mostly in Asians or Asians settled in other countries. The relative contribution of environmental and genetic factors to OSMF will be indicated by the degree to which future investigations show that ethnic Indian settlers manifest the condition and also retain, outside India, the social and cultural habits which may predispose

to it. The term "ethnic Indian settlers" is used here until a more precise indication, such as south Indian (mostly Dravidian) and north Indian (mostly Indo-Aryan and Mongolian), becomes possible (24). Prevalence, by sex, varies widely in the different published studies (Table 1). The general female preponderance may be related to the deficiency of iron and vitamin B complex among many Indian women (see below, etiology).

Diagnosis

Clinical criteria

Various investigators have correlated the salient clinical and histological features of this condition (3, 7, 11, 26-41). The onset is insidious over a 2 to 5-year period (3). The prodromal symptoms include a burning sensation in the mouth when consuming spicy food, appearance of blisters especially in the palate (6), ulcerations or recurrent generalized inflammation of the oral mucosa, excessive salivation, defective gustatory sensation, and dryness of the mouth (3). There are periods of exacerbation manifested by the appearance of small vesicles in the cheek and palate. The intervals between such exacerbations vary from three months to one year. Focal vascular dilatations manifest clinically as petechiae in the early

stages of the disease (12). This may be part of a vascular response due to hypersensitivity of the mucosa towards some external irritant like chilli (30) or areca nut (21). Petechiae were observed in about 22% of OSMF cases, mostly on the tongue followed by the labial and buccal mucosa with no sign of blood dyscrasias or systemic disorders; histologically they revealed a slightly atrophic epithelium with numerous dilated and blood-filled capillaries juxta-epithelially (31).

As the disease progresses, the oral mucosa becomes blanched and slightly opaque, and white fibrous bands appear (3). The buccal mucosa and lips may be affected at an early stage although it was thought that the palate and the faucial pillars are the areas involved first (2). The oral mucosa is involved symmetrically and the fibrous bands in the buccal mucosa run in a vertical direction (3, 11). The density of the fibrous deposit varies from a slight whitish area on the soft palate causing no symptoms to a dense fibrosis causing fixation and shortening or even deviation of the uvula and soft palate. The fibrous tissue in the faucial pillars varies from a slight submucosal accumulation in both pillars to a dense fibrosis extending deep into the pillars with strangulation of the tonsils (2). It is this dense fibrosis involving the tissues around the pterygomandibular raphe that causes varying degrees of trismus (15).

The exact site and extent of the fibrosis and its role in the causation of trismus are determined by several factors. For example, the anatomical and physiological integrity of the underlying musculature is vital for the degree of mouth opening. Based on electron microscopical observations El Labben et al. (40) reported muscle degeneration in OSMF, the extent of which may significantly affect the already existing trismus in these patients. Equally important is the involvement of the pterygomandibular raphe, a site commonly reported to accentuate the extent of trismus. Another factor is the duration of the disease in the affected individuals, which depends on the subjective evaluation of signs and symptoms. Current views of a protracted and insidious onset of the disease and its very slow progression make any sort of objective diagnostic criterion difficult, at least in the earlier stages.

A factor which seems to be overlooked by many investigators while recording the extent of mouth opening is the acuteness of oral symptoms (persistent/recurrent stomatitis and glossitis) at the time of recording. Most investigators agree that in OSMF the patient experiences a protracted period of stomatitis and/or glossitis with remissions and exacerbations (3, 11), which must be taken into consideration, together with the age of the patient and the extent and site of fibrosis, when recording the extent of trismus.

Sometimes the fibrosis spreads to the pharynx and down to the pyriform fossae. Upon palpation, a circular band can be felt around the entire rima oris, and these changes are quite marked in the lower lip (27). All observers have noted impairment of tongue movement in patients with advanced OSMF, but only some have registered an atrophy of the tongue papillae (12). With progressing fibrosis, patients complain of stiffening of certain areas of the mucosa leading to difficulty in opening the mouth, inability to whistle or blow out a candle, and difficulty in swallowing (27). When the fibrosis involves the pharynx, the patient may experience referred pain in the ear (3). Millard (34) mentioned a nasal voice as one of the later signs in some patients.

Is OSMF a precancerous condition of the oral cavity?

The precancerous nature of OSMF was first postulated by Paymaster (25), who described the development of a slow-growing squamous cell carcinoma in one third of OSMF cases seen in the Tata Memorial Hospital, Bombay. This precancerous potential was also emphasized by other authors (9, 42-46), based on clinical and epidemiological grounds. The frequency of malignant change in patients with OSMF ranges from 3% to 6%. In a 10-year follow-up study in Ernakulam district, Kerala, Gupta et al. (9) reported malignant transformation in 2.3% of patients with OSMF. Utilizing this material and additional material from the same area, with a 15-year follow-up, Pindborg et al. (43) demonstrated a malignant transformation rate of 4.5%. From the same area and patient group, 66 patients with OSMF were followed up for a period of 17 years by Murti et al. (44), who recorded a malignant transformation rate of 7.6%. With a longer follow-up of the same group, the malignant transformation rates could increase further.

Surveys in various cancer hospitals in India reveal a 15-20% frequency of oral cancer among all cancers (47). The finding of a high frequency of OSMF among oral cancer patients in India (e.g., 40 among 100 oral cancer patients) has strengthened the postulated link between the two.

Pathology

Structural and microstructural changes

An evaluation of the epithelial changes in the different grades of OSMF shows that increase in the clinical severity of the disease may be accompanied by epithelial hyperplasia or atrophy, which is associated with an increased tendency for keratinizing metaplasia. The epithelial atrophy reported by Pindborg

and associates (3) is one of the marked changes in OSMF, which contrasts with the predominantly hyperplastic epithelium reported by Sirsat & Khanolkar (35) and by Wahi and associates (7). This disparity may be due to the selection of cases and also to the sites of biopsy in the various studies. Wahi et al. (7) correlated the type of keratinizing metaplasia with the site of the lesion and the habits of the patients. Lesions involving the palate showed predominantly orthokeratosis and those of the buccal mucosa, parakeratosis. The high mitotic count in parakeratotic epithelia, which is more common with OSMF, and the association with parakeratotic leukoplakia (43) predispose to carcinoma.

A useful histological grading in conjunction with the clinical progression of the disease was proposed by Pindborg et al. (3). It is still not clear whether the epithelial atrophy, as reported by various workers, is the aftermath of heavy fibrosis in the underlying connective tissue or is a result of malnutrition. At least a few hold the view that the epithelium has become stretched and thinned by the changes in the underlying connective tissue. Whatever the cause, it has been stated that atrophic changes in the mucosa predispose to malignant changes in the epithelium (42, 46).

Subepithelial changes

It is generally agreed that the pathological alteration in OSMF begins in the lamina propria and the epithelium responds only secondarily to it (43, 46). On the basis of the histopathological appearances of stained (H & E) sections, the surgical specimens from OSMF can be grouped into four clearly definable stages (6): very early, early, moderately advanced, and advanced. These stages are based not only on the amounts and nature of the subepithelial collagen, but also on the following criteria taken together: (a) presence or absence of oedema, (b) physical state of the mucosal collagen, (c) overall fibroblastic response (number of cells and age of individual cells), (d) state of the blood vessels, and (e) predominant cell type in the inflammatory exudate. Except for cases which begin with vesicles, the changes in OSMF start in the connective tissue (3). The histological demonstration of subepithelial vesicles in OSMF should encourage further studies on a possible allergic relationship (6).

A vascular response due to inflammation, apart from the connective tissue repair process, has been very commonly found in OSMF (12, 26). Normal, dilated and constricted blood vessels have been seen, often in combination, in the same section (27, 31). Apparent narrowing of the smaller vessels appears first in the upper mucosa and spreads gradually to the larger, deeper vessels (27). Persistent dilatation

has also been seen in many moderately advanced and advanced biopsies. A rise in mast cells occurs in the earlier stages of the tissue reaction, but in the more advanced stages the counts are similar to those seen in normal mucosa, or even lower (48).

The inflammatory cells seen are mainly lymphocytes and plasma cells (6, 26, 27, 31). The presence together of large numbers of lymphocytes and fibroblasts, as well as plasma cells in moderate numbers, suggests the importance of a sustained lymphocytic infiltration in the maintenance of the tissue reaction in OSMF (6). Using standard connective tissue staining methods, Hamner et al. (29) demonstrated abnormal juxta-epithelial connective tissue, indicating a probable alteration in the collagen. As the disease progressed, the connective tissue lost its fibrillar staining pattern and became amorphous. This staining probably indicates biochemical changes which may account for the atypical appearance of the overlying epithelium. In a transmission electron microscopical study from England of an Indian patient, Binnie et al. (36) found that the normal pattern of uniformly sized collagen fibrils, gathered together in bundles, was replaced by many fine (immature) fibrils in an interfibrillar matrix.

Relevance of epithelial-mesenchymal interactions in OSMF

The epithelium depends on the underlying connective tissue for its nutritive supply; with changes such as inflammation in the connective tissue, the epithelial cells seem to respond in a characteristic manner (49-51). The pathogenic mechanism generally in OSMF starts in the connective tissue, the epithelium responding secondarily (43, 46). A persistent juxta-epithelial inflammatory response is characteristically seen in OSMF. The hyperplastic epithelial response, noticed during the early and moderately advanced stages of OSMF, may be a reaction to this. An alternative explanation for the epithelial hyperplasia is an adaptive response to local irritants to provide a greater degree of protection to the underlying tissues. However, a hyperplastic oral epithelium resulting from mild mechanical abrasion or chemical treatment has shown increased permeability to water or horseradish peroxidase (52, 53). This reduced barrier function of the hyperplastic epithelium may be related to an increased widening of the intercellular spaces and an increased turnover rate of this tissue.

Serum-derived antibodies provide a further basis for an increase in mucosal permeability (52). Although serum-derived IgG retards the penetration of its corresponding antigen, it nevertheless can cause impairment of the mucosal barrier through immune complex formation or an increased absorption of antigen into epithelial cells. Ultrastructural

studies of small intestinal mucosa have indicated increased antigen absorption into epithelial cells after parenteral immunization (52).

Thus, the epithelial response in OSMF is secondary to progressive changes in the connective tissue; there are also changes in the epithelium *per se*. The hyperplastic epithelial response and later atrophy probably reduces the barrier function of the mucosa to local irritants. Circulating immune complexes and serum antibodies in OSMF probably help to accentuate the already existing pathological change in the oral mucosa (54, 55).

Etiology

The etiology of OSMF is unknown. Most of the ideas proposed have been derived from existing clinical and epidemiological data. Considering the percentages of female patients in Table 1, it may be asked whether OSMF is due to overindulgence by females in the study populations in such habits as tobacco/areca nut chewing. Most studies on OSMF have emphasized only the role of irritant substances acting locally on the oral mucosa. An equally important second aspect which needs to be considered is the pre-conditioning of the oral mucosa by a prolonged, chronic deficiency of iron and/or vitamin B complex. Such conditions are much more commonly seen among Indian females than males, which may explain the higher incidence of OSMF among females. Other fibrotic diseases related to the basal lamina and involving underlying muscles are known, e.g., endomyocardial fibrosis.

The various hypotheses put forward so far suggest a multifactorial origin for this condition. Alongside the role of local irritants such as capsaicin (30), tobacco (25), areca nut (14, 16, 21, 62, 63), pungent and spicy foods (3), and alcohol (7), an underlying systemic predisposition is likely because of the geographical and ethnic distribution of OSMF. Among the systemic factors, the main ones incriminated are chronic iron and vitamin B-complex deficiency, anaemia (64), and a genetic predisposition to the disease (63).

Local factors

The pathogenesis of OSMF was at first linked with the continuous and prolonged action of mild irritants on the oral mucosa. Sirsat & Khanolkar (30) verified this by applying capsaicin, the active irritant in chillies (*Capsicum annum*, which is used to spice food), to rat palates and found only a limited connective tissue response in the unimpaired animal. In protein-depleted or vitamin B-deficient animals, however, the response was more widespread and extensive.

Other local irritants like tobacco have also been suggested (42), but certain features of the condition suggest an allergic origin. The custom among Indians of chewing "pan" (betel leaf with tobacco powder and other ingredients) has led to the assumption that this habit is the cause of submucous fibrosis. On the other hand, vast numbers indulging in this habit have come to no harm, while many afflicted with the condition have never used "pan" (5, 11). Based on their animal experiments, Sirsat & Khanolkar (30) believed that tobacco chewing is not the causative agent in OSMF although allowance should be given to the composite nature of "pan". Whether the effect of tobacco-derived products on the oral mucosa is synergistic with the other ingredients of pan or antagonistic has yet to be elucidated. Various forms of tobacco induce lesions in the oral mucosa which differ in morphology, reversibility (on discontinuation of the habit), and malignant potential. The complex constituents diffusing from tobacco mixtures, whether smoked or chewed either alone or in combination, can have mutagenic and carcinogenic effects.

The role of other local factors (alcohol, oral sepsis and infections (bacterial, fungal and viral)) cannot be appraised at present in the absence of pertinent literature, but it is unlikely to be significant. Recently several epidemiological studies have focused on the habit of areca nut chewing, the frequency of which in populations affected by OSMF ranged from 34% to 100% (15). This has been reported to be higher among OSMF patients than in the general population (44). In a study of patients in China (Province of Taiwan), Shiau & Kwan (16) found that 43% of OSMF patients chewed areca nut alone, compared with 0% of 100 OSMF-free, control subjects. Further, in a study of 100 000 villagers in India (Maharashtra), 4.2% of females who chewed areca nut and did not use tobacco suffered from OSMF (9). Thus chewing of areca nut may be an important factor in the etiology of OSMF.

The possible involvement of areca nut was amplified recently by Sinor et al. (45) who computed the relative risk and found, in a bivariate analysis, the effect of frequency and duration of chewing to be multiplicative. In this study, as elsewhere, the areca nut was used in a mixture, either as *mawa* (containing mainly areca nut, some tobacco and a little lime) or in betel quid. The relative risk of areca nut chewing *per se* was 29.9; the overall relative risk for the composite chewing habit was 109.6. Further work is needed to confirm areca nut as the most important etiologic factor in OSMF.

Autoimmunity

There is clinical and experimental evidence on OSMF to support an autoimmune etiology, e.g., the

high incidence of anti-nuclear antibodies together with autoantibodies to gastric parietal cells, thyroid microsomes, reticular and smooth muscle noticed in this disease (63). The increased frequency of HLA haplotypic pairs A10/DR3, B8/DR3 and A10/B8 in OSMF and scleroderma suggests an MHC-mediated immunological derangement operating in this disease (63). There is increasing evidence that immunoregulatory aberrations are primarily involved in the pathogenesis of autoimmune diseases. In man, evidence is accumulating that immune response genes are associated with the HLA complex, indicating a relation between autoimmunity and HLA type.

Biological studies on individuals and tissues from cases of OSMF

(a) Blood chemistry and haematological variations

It has been established that deficiencies of vitamin B₁₂, folate and iron can affect the integrity of the oral mucosa (64). Significant haematological abnormalities have been reported in OSMF, including an increased blood sedimentation rate (ESR), anaemia and eosinophilia (3), increased gammaglobulin, a decrease in serum iron ($P < 0.05$), and an increase in total iron-binding capacity ($P < 0.05$). The percentage saturation of transferrin also decreased ($P < 0.001$) and a significant reduction in total serum iron ($P < 0.01$) and in albumin ($P < 0.01$) was found (64). Thus, iron-deficiency anaemia appears to be one of the causes and not the effect of the disorder. A rise in serum mucoproteins, mucopolysaccharides and anti-streptolysin titre O (measured in Todd's units) has also been reported (49).

Desa (28), the only investigator to report on the culture of vesicular fluid, found no specific organism. As this work was carried out more than thirty years ago and no details of the type(s) of culture procedures used are given, modern techniques could lead to the isolation of specific organisms, including viruses, in these early vesicles.

It is known that widespread iso-enzymatic modifications may be present in apparently unaffected regions of tumour-bearing organs and that such modifications are especially pronounced in organs with simultaneous or interval tumours (66), indicating the presence of a malignant potential. A significant depression of the lactate dehydrogenase iso-enzyme ratio (LDH IV/LDH II) is reported at the tissue level in OSMF. A significant reduction in the serum copper and zinc ratio is also reported (67).

(b) Lectins as cytochemical markers

In recent years there has been much interest in cell surface carbohydrates. These vary during differentiation and maturation and show marked changes dur-

ing malignant transformation (56). Among these the A, B, H and related blood group system antigens have been demonstrated in the epithelial cells of the oral mucosa, in salivary secretions and in salivary glands (57). A change in the distribution of blood-group-related antigens has furthermore been described in pre-malignant and malignant epithelial lesions (58). Lectins are proteins or glycoproteins mainly of plant origin, the interactions of which can be inhibited or reversed by simple sugars. Lectins have two or more binding sites and can agglutinate cells by binding specific carbohydrate molecules on cell surfaces. The diagnostic and prognostic values of lectin immunohistochemistry on OSMF biopsies have not yet been explored in detail (59). Work is currently underway in our laboratory on the use of lectins as cytochemical markers for the early detection of tissue dysplasia in OSMF.

(c) Cytogenetics

Chromosomal instability has long been associated with the neoplastic process (60), and the quantitative assay of sister chromatid exchange (SCE) provides an easy, rapid and sensitive method for studying chromosome/DNA instability and its subsequent repair processes. An attempt was made to investigate SCE levels in the peripheral blood of patients with OSMF and to correlate them with the habit of different forms of tobacco/areca nut usage. These values were significantly higher compared with the SCE values observed in normal controls. The increase in frequencies of SCEs observed in patients with OSMF may be attributed to the genotoxic effect of the constituents of betel quid. The role of areca nut alkaloids in this regard may be significant.

AgNOR. Silver-binding nucleolar organizer region proteins (AgNORs) were counted in sections from formalin-fixed, paraffin-embedded tissue blocks of OSMF. It was found that the pooled mean AgNOR in clinically advanced OSMF was higher than in moderately advanced cases (61). Counting of AgNORs may be a promising predictor of the biological behaviour of OSMF.

(d) Immunological studies

No significant alterations in the serum levels of total haemolytic complement (CH50) and the fractions (C3 and C4) is reported in OSMF (68). Studies show decreases in high-affinity rosette forming cells (HARFC) and increases in serum levels of IgA, IgD and IgE (54). These studies indicate that the role of altered and foreign tissue antigenic determinants deserve further study. The increased predisposition of OSMF mucosa to the development of oral carcinoma is of interest (33, 35) and the circulating immune complexes (CIC) and the immunoglobulin

contents were found to be elevated significantly in both OSMF and oral cancer (55).

Management

Reduction or even elimination of the habit of areca nut chewing is an important preventive measure. At least in the early stages of OSMF, it could probably slow the progress of the disease. To improve current treatment regimens for OSMF, the following strategies have been proposed.

(a) Nutritional support

Supplementary diets administered to OSMF patients are mainly for high protein and calories and for vitamin B complex and other vitamins and minerals. These are commonly employed in combination with other more specific therapeutic agents like ingestion of iodinated salt and/or local applications (see below).

(b) Immunomodulatory drugs

Local and systemic application of glucocorticoids and placental extract are commonly used. By opposing the action of soluble factors released by sensitized lymphocytes following activation by specific antigens, glucocorticoids act as immunosuppressive agents (69). These also prevent or suppress inflammatory reactions, thereby preventing fibrosis by decreasing fibroblastic proliferation and deposition of collagen.

(c) Physiotherapy

Physiotherapeutic measures such as forceful mouth opening and heat therapy have been tried. The former has been almost discarded owing to the poor results and the fact that it may accentuate the fibrosis. Heat has been commonly used and the results have been described as satisfactory (69). It can be in the form of hot rinses, lukewarm water, or selective deep heating therapies like short-wave and microwave diathermy. The latter avoids the inadvertent heating of the superficial facial tissues like skin and adipose tissue. Microwave diathermy seems superior to short wave, because selective heating of the juxta-epithelial connective tissue is possible, thereby limiting the area treated (70, 71).

(d) Local drug delivery

Local injections of dexamethasone, hyaluronidase and placental extract have been tried. *In vitro*, collagen from patients with OSMF, in contrast to normal collagen, is attacked rapidly by hyaluronidase (72). By breaking down hyaluronic acid, hyaluronidase lowers the viscosity of the intercellular cement sub-

stances and also decreases collagen formation. The effects of steroids and hyaluronidase are thought to be responsible for the satisfactory results obtained in OSMF patients who have severe limitation in mouth-opening. Chymotrypsin, an endopeptidase, hydrolyses the ester and peptide bonds, thus acting as a proteolytic and anti-inflammatory agent (69).

Placental extracts (aqueous solution of human placenta) in the form of local injections as well as in parenteral form have been tried with varied results (69). They can be separated into four different fractions: aqueous extract, lipid extract, immune gammaglobulins, and tissue coagulants. Only the aqueous extract of placenta acts as a biogenous stimulator—by accelerating cellular metabolism (through the pituitary-adrenal cortical axis), assisting in the absorption of exudate, stimulating the regenerative process, and increasing the physiological actions of organs. The other actions of placental extract are an anti-inflammatory and significant analgesic effect, increase in blood circulation and tissue vascularity, arrest of tissue growth stagnation, metabolic degenerative conditions, and lowered immunity response factors. Placental extract has been found to contain between 50 and 100 King-Armstrong units of alkaline-phosphatase; it has been used as a local nutrient (73, 74).

(e) Combined therapy

Significantly better results have been obtained by giving local injections of chymotrypsin, hyaluronidase and dexamethasone together than with one drug alone or a combination of dexamethasone with either chymotrypsin or hyaluronidase (69). Combined therapy with nyliadin hydrochloride (a peripheral vasodilator), vitamins D, E and B complex, iodine, placental extract, local and systemic corticosteroids, and physiotherapy claims a success rate of 62% in OSMF (73).

Evaluation of the merits and disadvantages of individual items in treatment is not possible owing to the use of combined treatment protocols, which is unavoidable at present because of the empirical nature of each approach.

(f) Surgical management

Teeth needing extraction should be dealt with before the commencement of any treatment in the management of OSMF patients. This may help to alleviate undue effects on the already inflamed and atrophied mucosa.

Surgical measures such as forcing the mouth open and cutting the fibrotic bands under anaesthesia have resulted in more fibrosis and disability. Submucosal resection of fibrotic bands and replacement with a partial-thickness skin or mucosal graft have

also been attempted; modification of this surgical procedure by carrying out a bilateral temporalis myotomy seems more promising. A new treatment regimen composed of surgical excision of the fibrotic bands with submucosal placement of fresh human placental grafts, followed by local injections of dexamethasone was recommended recently for advanced cases (69). The rationale for using placental grafts in OSMF is that they have both a hormonal and a mechanical effect; the biogenic stimulant effect is because the placenta is a homograft that is immunologically competent and rich in steroids, proteins, chorionic gonadotrophins, estrogens and progesterones. The grafts are easily mouldable and undergo total absorption only after prolonged periods, thus mechanically preventing fibrosis (69).

Future perspectives

Further clinical and laboratory studies of OSMF patients and tissues are required, including comparison with other fibrotic disorders. The frequency of factors apparently specific for OSMF and other disorders with generalized fibrosis (as in progressive systemic sclerosis) or more localized fibrosis (as in morphea and Dupuytren's contracture) should be studied in OSMF patients and their near relatives, as well as members of the same ethnic group and other population groups. Studies of patients should include the recording of apparently related habits and nutritional status, particularly with reference to iron and vitamin B complex and the testing of the immune status, including autoimmune damage. In this regard, Desa (28) found "localised fibrinoid degeneration" in earlier cases of OSMF and suggested a relationship with "collagen diseases". However, the use of the term "fibrinoid" is now limited to fibrinoid degeneration of the walls of blood vessels, and the terms "collagen diseases" and "connective tissue disorders" have recently been largely discarded because the crucial element was not collagen *per se*.

A search for factors pointing to disorders of the microvasculature, including increased levels of factors VIII and of increased circulating platelet aggregates, would be worthwhile. The frequency and titre of antibodies directed against collagen types I and IV and against laminin, as found in progressive systemic sclerosis, would be of interest. Investigations on tissues from patients should include the metabolism of fibroblasts cultured from lesions and from "unaffected" tissues and their response to cytokine activation (75).

Studies of genetic predisposition should include genetic analysis of probands and their families. Examples of monozygotic twins, where one or both are affected should be sought in order to define

better the involvement of genetic factors. In situations where twin pairs are not available, information on the nature and degree of genetic contribution to the development of OSMF can be obtained from family studies (76). In this regard the process of linkage analysis represents a major benefit for progress in DNA biology. Through it the segregation pattern of the disease gene(s) is compared with that of a detectable genetic marker (76). The histocompatibility antigen (HLA complex) status of patients, including the frequency of individual class II (D region) alleles, should be further investigated. The remark by Canniff et al. (63) that "OSMF is a chronic autoimmune disease initiated by constituents of betel nut" needs further explanation. In the first instance, OSMF has been reported in people who never resorted to any form of areca nut habits (19), and further it lacks experimental support. The assumption that it is an autoimmune disease seems logical in the light of the HLA haplotypic linkage (linkage disequilibrium). But the overemphasis on areca nut initiating this autoimmunity needs further examination. Experimental evidence is still lacking to show that there is an autoimmune response directed to the mucosa in OSMF. Neither the presence of an altered mucosal antigen nor the localization of immune complexes anywhere in the mucosa has been demonstrated. In what way does areca nut alter the mucosa? It is commonly believed that the pathogenic mechanism in OSMF begins in the connective tissue and the epithelium responds secondarily to it. Does the areca nut exert the antigenic alteration in the connective tissue or the epithelium in the first instance? If it does, where precisely does it act and what is the mechanism of alteration? An immune-complex-mediated reaction at the basal lamina zone in OSMF seems unlikely owing to the integrity of this layer even in the advanced stages of the disease (37).

The effect of areca nut alkaloids and/or tannins on the oral mucosa may be secondary to the initial immunologically mediated tissue alteration. They may act as a potential carcinogen on an already weakened and atrophic oral epithelium, which is itself the result of an altered connective tissue interaction. Permeability studies directed to normal and atrophied oral mucosa may throw further light on this subject. As already mentioned, till sufficient evidence is available to postulate that either the epithelium or the components of the connective tissue act as targets for an immune-complex-mediated attack, it is safer to exclude the role of areca nut or any other extraneous agent in the initiation of this condition.

The potential for the development of squamous cell carcinoma in areas of OSMF should be further studied by investigation of epithelial cell membrane carbohydrates, antigenic determinants including

blood group antigens, and mutations in oncogenes. It is apparent that in many cases of oral carcinoma, some genetic change (point mutation, deletion, translocation of a gene, amplification or mutation of an oncogene) is required for the initiation of malignant change in a cell (77, 78). The amplification of *myc* or *ras* oncogenes was detectable in more than half the specimens of 23 squamous cell carcinomas in Indian patients, but not in their peripheral blood cells, whereas *ras* mutations were infrequent in other examples of oral malignancies in the United Kingdom (79). If this is not due to genetic factors which is unlikely, it may point to the role of betel quid chewing in these mutations. Very recently, employing the NIH3T3 cell transfection assay, an effort was made to detect transforming genes in DNA from squamous cell carcinomas of the head and neck (80). They were able to produce primary and secondary transformants containing the human *K-ras* oncogene. This study points to the possibility that head and neck cancers may possess activated *ras* oncogenes more often than is normally expected.

Controlled studies of different regimens in the management of OSMF are needed. They will not be easy to organize because of the number of items in current management protocols, but they should greatly increase our understanding of OSMF. This major health problem is no longer confined to Asia, because emigrants from these high-risk regions now reside in many parts of the world.

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Résumé

Fibrose sous-muqueuse de la bouche: étiologie, pathogénie et recherches futures

En 1952, Schwartz décrivait chez cinq femmes indiennes du Kenya une affection de la muqueuse buccale, touchant également le pharynx, qu'il

nommait "atrophia idiopathica (tropica) mucosae oris"; cette affection a été par la suite appelée fibrose sous-muqueuse buccale. La fibrose sous-épithéliale, sous-muqueuse, et la myofibrose qui caractérisent cette maladie conduisent à une raideur de la muqueuse buccale et des tissus profonds, entraînant une limitation progressive de l'ouverture de la bouche et de la protrusion de la langue, d'où des difficultés de mastication, de déglutition et d'élocution. La définition OMS pour les affections précancéreuses de la bouche—état pathologique généralisé de la muqueuse buccale associé à un risque significativement accru de cancer—est compatible avec les caractéristiques de cette pathologie.

De nombreuses observations de cas ayant été publiées, il est possible de dresser un tableau de la distribution géographique de la maladie. Celle-ci s'observe principalement chez les Indiens et autres groupes ethniques de l'Asie du Sud-Est. Des cas sporadiques ont été enregistrés ailleurs. La prévalence de la maladie dans différentes régions de l'Inde, où elle est endémique, va de 0 à 0,4%. La répartition selon le sexe varie largement selon les études publiées: la prépondérance générale des cas féminins peut être liée à une carence en fer et en complexe vitaminique B, qui touche de nombreuses Indiennes. La maladie a été observée chez des sujets âgés de 4 à 87 ans, l'incidence maximale se situant chez les 35–54 ans.

Dans la fibrose sous-muqueuse buccale, le processus pathologique commence dans la lamina propria et entraîne une réponse secondaire au niveau de l'épithélium. D'après l'aspect histopathologique des coupes colorées (H et E), les pièces opératoires peuvent être classées en quatre stades bien définis: très précoce, précoce, modérément avancé et avancé. Ces stades sont basés non seulement sur la quantité et la nature du collagène sous-épithélial, mais également sur l'épaisseur et la nature de l'épithélium sus-jacent. On observe typiquement une réponse inflammatoire juxta-épithéliale. La réponse épithéliale hyperplasique observée au cours des stades précoce et modérément avancé pourrait être une réaction à cette inflammation.

L'étiologie de la fibrose sous-muqueuse buccale est inconnue. La plupart des hypothèses reposent sur les données cliniques et épidémiologiques. A côté du rôle d'irritants locaux tels que le piment, le tabac et la noix d'arec, il est probable, d'après la répartition géographique et ethnique de la maladie, qu'il existe une prédisposition générale. Les principaux facteurs généraux incriminés sont la carence chronique en fer et en complexe

vitaminique B, l'anémie, et une prédisposition génétique. Ces dernières années, plusieurs études épidémiologiques ont souligné le rôle étiologique de la noix d'arec dans la fibrose. Diverses données cliniques et expérimentales plaident en faveur d'une étiologie auto-immune, notamment la présence d'anticorps antinucléaires associée à celle d'auto-anticorps dirigés contre les cellules pariétales de l'estomac, les microsomes thyroïdiens, la réticuline et les muscles lisses.

Pour améliorer le traitement de la fibrose sous-muqueuse buccale, les stratégies suivantes ont été proposées: soutien nutritionnel, immunomodulateurs, physiothérapie, administration locale de médicaments, polychimiothérapie, et traitement chirurgical.

Il est nécessaire d'entreprendre de nouvelles études cliniques et biologiques chez les sujets atteints de fibrose sous-muqueuse buccale, et de comparer cette affection à d'autres fibroses. L'étude de la prédisposition génétique devra comporter une analyse génétique des sujets atteints et de leurs familles. On recherchera des exemples de jumeaux homozygotes, dont un ou les deux sont atteints, afin de mieux cerner la participation des facteurs génétiques.

Des études contrôlées comparant les différentes modalités de prise en charge de la fibrose sous-muqueuse buccale sont nécessaires. Elles seront certainement difficiles à organiser en raison de la complexité des protocoles actuels de prise en charge, mais aideront grandement à mieux comprendre la pathogénie de cette maladie.

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