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ORIGINAL ARTICLE

Morphometric analysis of oral submucous fibrosis and its correlation with histological staging and clinical severity of trismus

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Abstract *Aim:* To quantify the histopathological changes in oral submucous fibrosis morphometrically and to correlate those findings with the histological grading and clinical severity of trismus.

Methods: A total of hundred histological sections of oral submucous fibrosis were analysed morphometrically by using interactive image analysis system (Image Pro-Plus,V 6.0). Histological staging and the severity of trismus were then compared with the morphometry results. ANOVA and Pearson's chi square tests were applied using the software SPSS V. 13.0 for statistical analysis.

Results: The thickness of the epithelium and subepithelial collagen showed no statistically significant differences between the different stages (p value > 0.05). However, blood vessel density, mean blood vessel area and mean diameter of the vessels were indirectly proportional to the histological stages (p value < 0.001). Histological stages directly correlate the frequency of trismus, but the severity of trismus showed relation neither with the staging nor with the degree of collagenization, measured morphometrically (p value > 0.05).

Conclusions: The thickness of the epithelium and subepithelial collagen should not be included in the histological staging criteria of oral submucous fibrosis. Probably the degree of hyalinization of

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collagen fibres and involvement of muscle fibres are more important in causing trismus, rather than a simple increase in the subepithelial collagen thickness.

1. Introduction

Oral submucous fibrosis (OSF) is an irreversible precancerous condition of the upper aerodigestive tract.[1–3] With progression, patient develops trismus clinically. The alkaloid, arecoline released from the areca nut appears to increase the deposition of collagen, the major extracellular matrix molecule in the subepithelial zone.[4,5] This accumulation increases with the severity of the disease.[6,7]

In the present study the biopsies of lesions from clinically diagnosed [palpable bands on the buccal mucosa] OSF were analysed morphometrically and findings were correlated with the histological staging as well as with the clinical severity of trismus – to rationalize whether those pathological changes have any practical implication in disease progression and also to find out any relation between the clinical severity of OSF and its histological stages. There are limited studies in the literature in which the pathological changes in OSF were quantified morphometrically.[8,9]

2. Materials and methods

2.1. Ethical consideration

This study has been conducted in full accordance with ethical principles and has been independently reviewed and approved by the ethics board. All the subjects gave written consent. We preserved patient anonymity and did not use any personal information or photograph/s of any patient.

2.2. Patients and specimens

The study was conducted in the Department of Oncopathology, Medical College, Kolkata and Department of Pathology, Midnapore Medical College during the period of July, 2010 to June, 2012. On clinical suspicion of having OSF (palpable white bands in the buccal cavity), the patients were biopsied by the concerned surgeon after taking written consent from the representative site particularly from the periphery of the lesion to compare it with the unremarkable areas. Tissues were first sent to the Oncopathology Department where histopathological examination of the specimens is performed. Cases bearing adequate sampling indicated the presence of epithelial layer, subepithelial zone including the muscle layer considered for further evaluation. Histological staging of OSF was done. Before making the diagnosis, the staging criteria of OSF were circulated among the pathologists to minimize subjective error. We used the criteria proposed by Pindborg and Sirsat (1966),[10] who described four consecutive stages based upon sections stained with haematoxylin and eosin.

2.2.1. Very early stage

Characterized by fine collagen dispersed with marked oedema, prominent firbroelastic response dilated and congested blood vessels and inflammatory cells (mainly polymorphs and eosinophils).

2.2.2. Early stage

Early hyalinization in juxta epithelial area with thickened separate bundles of collagen and clumps of young fibroblasts in moderate number.

2.2.3. Moderately advanced stage

In this stage collagen is moderately hyalinized, the amorphous change starting from the juxta-epithelial basement membrane. Occasionally thickened collagen bundles are seen separated by slightly residual oedema. The fibroblast response is less marked. Blood vessels are either normal or constricted as, a result of increased surrounding fibrous tissue. Inflammatory exudates consist of lymphocytes, plasma cells and occasional eosinophils.

2.2.4. Advanced stage

Collagen becomes completely hyalinized and seen as smooth sheets with no separate bundles discernible. Oedema is absent. Hyalinized areas are devoid of fibroblasts, although their elongated cells or vestigial nuclei were seen at rare intervals along the fibre bundles. Blood vessels are completely obliterated or narrowed. Lymphocytes and plasma cells are variably present.

Then the slides were sent to Department of Pathology of Midnapore Medical College for unbiased second opinion as well as morphometric analysis. If we found any discrepancy in diagnosis between these two departments those cases were rejected. Fortunately only three cases were rejected on this ground. Thus a total number of hundred histological sections of OSF were staged and simultaneously analysed morphometrically by using interactive image analysis system (Image Pro-Plus, V 6.0). Paraffin embedded sections of 3–4 μm thickness were stained routinely with Haematoxylin/Eosin, Van Gieson's picric acid and acid fuchsin stain and Masson's Trichrome stains in the Onco-pathology department. The latter two special stains impart different colours to the different connective tissue elements and made the morphometric analysis easy as well as less erroneous by highlighting the area of interest (e.g. endothelial cells, collagen fibres etc.).

2.3. Image analysis

Photomicrographs of OSF cases were captured with the help of a camera fitted onto the microscope and directly displayed on the computer monitor. Scanner (4X) was used for measurement of epithelial thickness and collagen thickness, low power objective (10X) for number of endothelial cells, area of blood vessels and the high power objective (40X) for measuring vessel diameter. Before proceeding for morphometry these captured photomicrographs were adjusted according to their magnification with the help of the software. Then the areas of interest were selected and analysis of desired parameters was performed.