

# Intestinal Parasites and Anaemic Status in Oral Submucous Fibrosis

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**Abstract:** The study was undertaken to assess the intestinal helminth load of patients with advanced OSF by stool examination for representative pathogens. The findings were correlated with the haematologic profile (anaemic status) of these patients. A statistically significant reduction in haemoglobin levels of test subjects compared to healthy controls was reported. ( $9.47 \pm 0.95 \text{ gm/dL}$  against  $11.70 \pm 0.72 \text{ gm/dL}$ ). This was in agreement with earlier haematological studies on OSF but the concurrent low intestinal parasite load recorded amongst them is difficult to be explained. (Chi-square 2,  $df=1$ ,  $p=0.15$  - not significant). We are lead to believe that intestinal parasites are not contributory, at least amongst the reported cases, in inducing and sustaining iron-deficiency state in the diseased individuals. The association does not seem to be simple and straight forward in the light of the confounders involved in the disease process.

## Introduction

Significant haematological abnormalities have been reported in oral submucous fibrosis (OSF). This includes an increased blood sedimentation rate (ESR), anaemia and eosinophilia, 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<sup>1</sup>. Thus iron-deficiency anaemia appears to be commonly associated with this disorder.

The immune-mediated expulsion of intestinal helminths from their natural host is widely known. This involves the mediation of cytokines like IL-4, IL-9, and IL-13 along with TNF- $\alpha$  and IFN- $\gamma$ . The levels of TNF- $\alpha$  ( $p=0.016$ ) and IFN- $\gamma$  ( $p=0.012$ ) significantly increased with age, suggesting a switch to a more chronic infection phenotype. The predominant parasite specific antibodies produced were IgG1, IgG4, IgA and IgE. The parasite specific IgE correlated positively with host age depicting its chronicity ( $p=0.010$ )<sup>2</sup>. These findings suggest a mixed cytokine response and an IgE associated level of protection in helminthic carriers. Immunologic studies on OSF showed a decrease in cell mediated immune response (CMI) and an increase in serum levels of IgA, IgD and IgE<sup>3</sup>. These studies indicate that the role of altered and foreign tissue antigenic determinants in OSF deserve further study. The circulating immune complexes (CIC) and the immunoglobulin content of which were found to be elevated significantly in both OSF and oral cancer<sup>4</sup>.

The background data available on the haematological and immunological fronts of OSF lead us to hypothesize a putative link between them and the pathogenesis of the disease. A confounder in this regard was the worm infestation status of these patients which was hitherto unexplored, and the clinical spectrum of its is envisaged to have similarities with the disease (haematologic and immunologic). A study was therefore undertaken to assess the intestinal helminthic load of patients with advanced OSF by stool examination for representative pathogens and correlate the findings with the haematologic profile (anaemic status) of these patients. The role, if any, of intestinal parasites in inducing anaemia in this group of patients was expected to be studied.

## Materials and Methods

**Patient selection :** Twenty five (25) cases (age 24 to 70 year 6 males & 19 females) of histologically confirmed OSF comprised the study

group. An equal number of age and sex matched disease free adults formed the control. The intestinal parasitic load was assessed by microscopic examination of the stool samples using the same technique for both test and control groups. For this, stool samples should be properly collected and preserved<sup>5</sup>. The examination was done to detect (a) adult worms, (b) ova and cysts, (c) larvae, (d) trophozoites and (e) cellular exudates such as WBCs, RBCs, macrophages and Charcot-Leyden crystals.

**Macroscopy and Microscopy :** Grossly the points noted were (a) consistency of stool samples, (b) presence of blood and mucous, (c) presence of round worms, thread worms or tape worm proglottids and (d) colour and odour of the stool.

The following techniques were used to detect microscopically the presence of worms, eggs or larvae, protozoan trophozoites and cysts. (a) saline wet mount, (b) iodine wet mount and (c) floatation procedure (saturated salt floatation)<sup>5</sup>. The egg count was done by direct smear count technique. ( $\text{no. of eggs/gram of faeces} = n/2 \times 1000$  where 'n' is the number of eggs). The following intestinal parasites were examined in the stool samples: (a) *Entamoeba histolytica*, (b) trichuriasis, (c) ascariasis *lumbricoides*, (d) *Ancylostoma duodenale*, (e) *Taenia solium* and (f) *Strongyloides stercoralis*.

## Results

Twenty five (25) clinically diagnosed, histologically confirmed OSF (advanced) cases (mean age  $49.72 \pm 11.41$  years, M:F=6:19) comprised the study group. An equal number (25) of age and sex matched, disease free adults (mean age  $50.12 \pm 11.16$  years, M:F=6:19) constituted the controls. The faecal parasite status and percentage distribution of were not significantly different ( $p=0.15$ ) amongst the patient and control groups; fecal helminths were not seen in 1 and 4 of the patient and control group respectively (out of 25 in each group). The distribution of mean haemoglobin levels among cases and controls is given in Table 1.

The types of parasites observed in patients & controls, are given in table 2 & Fig. 1. The results were analysed by the paired Students 't' test. A 'p' value  $< 0.05$  was considered to be statistically significant.

## Discussion

Iron deficiency anaemia appears to be one of the major and almost consistent haematological variables in OSF<sup>1</sup>. Whether this variability is causative, which is quite unlikely, may have a contributory effect in its progression. Effective management of these haematological deficiencies may help to alleviate the symptoms of this disorder by modulating the disease progression to an atrophic mucosa, which is more vulnerable to the effect of carcinogens.

**Table 1 : Distribution of Mean haemoglobin levels among case and control.**

	Group	N	Mean	S.D.
Haemoglobin (gm%)	Case	25	9.47	0.953
	Control	25	11.70	0.722

The mean haemoglobin level in the test cases is 9.47 with a standard deviation of 0.953 and the mean haemoglobin level amongst control is 11.70 with a standard deviation 0.722. This difference is found to be significant ( $t=9.33, p=.001$ ).

**Table 2 : The faecal parasite in test and control samples.**

Type of Parasite	Case	Control
Entamoeba Histolytica	-	-
Ascaris Lumbricoides	-	+
Ancylostoma Duodenale	+	+
Taenia Solium	-	-
Trichuriasis	-	-
Strongyloides stercoralis	-	-

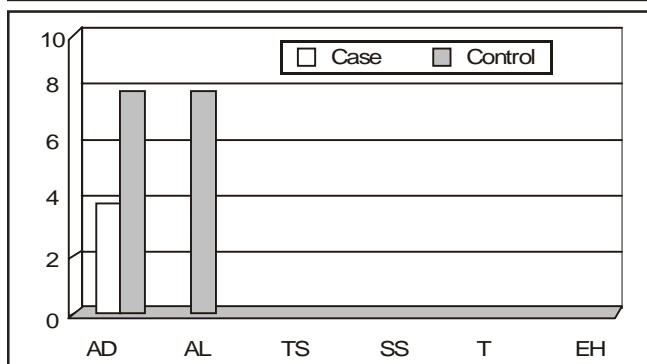


Fig. 1 : percentage distribution of parasitic load among test and control samples.

The high incidence of various intestinal parasitic infestation in tropical populations could be responsible for malnourishment and lack of haematopoietic nutrients thus contributing to anaemia. In OSF the type of anaemia reported was principally hypochromic, microcytic variant resultant to iron deficiency. This could be due to poor nutritional status of the cohort compared to control population and/or caused by chronic blood loss, due to parasitic infestations and other causes (blood loss anaemia).

Immunological responses are of great importance in parasitic diseases as a source of tissue damage. Significant immunological alterations were reported in OSF<sup>9</sup>. This includes elevated serum levels of IgA, IgD and IgE along with significant reduction in CMI response (by enumerating the high affinity rosette forming cells - HARFC). Eosinophilia is a characteristic phenomenon related to helminthic infestation. But eosinophilia in peripheral blood is not as frequently observed in helminthic infections that reside in the human gut (eg. hook worm, tape worm). Eosinophils have been demonstrated to play a significant role in host resistance to worm infections, as well as in protection against the tissue stages of these parasites. Although other cells of the immune system may contribute to acquired resistance,

the close association of eosinophilia and helminths points to a specific set of interactions and adaptations to helminthic infection. Tissue eosinophilia is frequently reported in OSF biopsies<sup>6</sup>.

Increase in absolute mast cell count with changes in the mast cell - histamine chain causing fibrosis of the submucosa was outlined earlier in OSF<sup>7</sup>. The mast cell degranulation product of histamine may have direct effects on fibroblasts which range from stimulation of proliferation to inhibition depending on the stage of the cell cycle of the fibroblasts<sup>8</sup>. We reported earlier<sup>9</sup> the infrequency of systemic fibrosis (visceral organ) in OSF even though a strong likelihood exist in its occurrence. The failure of visceral fibroblasts respond in the same manner to that of oral could be explained further in this context. Let us turn first to the fact that the mast cells may be heterogeneous in respect to tissue site of origin within a species and also with respect to the same site of origin between species<sup>10</sup>. The intestinal mucosal mast cells differ from the peripheral mucosal cells and other connective tissue mast cells by virtue of histochemical properties which reflect in their granular contents. It is therefore presumed that oral mucosal mast cells respond differently to the effect of allergenic stimulants by selective degranulation of its contents which could act upon the oral fibroblasts to synthesize excess amount of collagen. This explains rather vividly the localized nature of fibrosis in OSF and the failure to demonstrate similar fibrosis in visceral organs like liver.

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### APPEAL

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