of oral cancer tissues were reported by Pillai *et al.* (1982) whereas elevated levels of serum IgA and IgE in OC and reduced IgM levels in oral leukosis were reported by Scully (1982). Increases in serum IgA, IgD and IgE in carcinomas of the oral cavity, uterine cervix and breast were reported by Vijayakumar *et al.* (1986). No attempts were made by previous investigators to study the changes, if any, in the immunoglobulin concentrations in the tissues of premalignant lesions of the oral cavity. Care was taken to see that all the subjects selected for the study were free from allergy, asthma, liver involvement or any other infections. The age and sex distribution of the subjects are given in Table I.

Tissue extracts were prepared as described by Pillai *et al.* (1982). In short, tissue samples, obtained by punch biopsy or from the operating room and subjected to histopathological analysis, were washed several times in phosphate buffered saline (PBS) (to rule out the possibility of saliva contamination as assessed by the absence of alpha-amylase activity), homogenized in 1 :4 w/v PBS (0.01 M, pH 7.4) and centrifuged at 20,000 g for 30 min. The supernatant was used for the study (saline extract). Punch biopsy specimens from the unaffected areas of the oral cavity of the patients and specimens from the plastic surgery department were processed similarly and served as normal control. The precipitate was suspended in 3 M KCl and kept overnight at 4°C. The supernatant was collected by centrifugation as above, dialyzed against several changes of normal saline, centrifuged and the clear supernatant collected (KCl extract). The saline and KCl extracts were concentrated individually by lyophilization to the same volume and the Ig contents of the extracts were estimated by the immunodiffusion technique of Mancini *et al.* (1965), using the test kits from Kallestad Labs, Inc., USA. The protein content of the extract was estimated by the method of Lowry *et al.* (1951).

The results of the investigations are given in Table II. IgG was present in all the samples, whereas IgA was absent from the normal samples. IgM could be detected only in OSMF and OC. There are significantly elevated levels of IgG and IgA in OSMF and OC, the elevation being more predominant in OC. Immunoglobulins were not detected in the KCl extracts of any sample.

The present observation indicates that Igs are present in detectable quantities in OC and OSMF. We have previously reported the presence of a tumor-associated antigen (TAA) in OC (Abdulal Khader *et al.*, 1981). Antibodies against TAA might become attached to the tumor cells and this may be one reason for the presence of higher quantities of antibodies in OC tissues. The presence of tumor-bound immunoglobulins were reported earlier by Mitz (1973) and Donval *et al.* (1976). The biological significance of this is not fully understood, but may possibly form the blocking factors or be beneficial to the host, as reported by Ran *et al.* (1976), Haskill *et al.* (1977) and Vijayakumar *et al.* (1986).

Changes in Ig fractions in patients with OSMF was reported as early as 1975, by Phatak and Gosavi. Recently we also observed increases in serum levels of IgA, IgD and IgE fractions in OSMF patients (Ankathil *et al.*, 1985). Vijayakumar *et al.* (1986) reported similar changes in the sera of patients with OC. On the other hand, cell mediated immune responses were reported to be depressed in OC (Vijayakumar and Vasudevan, 1985) and in OSMF patients (Ankathil *et al.*, 1985 ; Vijayakumar *et al.*, 1986).

In the present investigation, Igs were detected only in the saline extract and not in the KCl extract. 3 M KCl is commonly employed to extract proteins which are bound tightly to membranes. This report suggests that the Igs are present either in the soluble cytoplasmic component or in the surface of the cells loosely attached to other membrane proteins. It is not known whether the Igs are produced locally or they come from the serum.

An association between Herpes simplex virus and oral cancer is reported by Kumari *et al.* (1984). If virus infection is the cause of OC, viral antigens may be expected to be present on the tumor cell surface and this will in turn induce the production of antitumor antibodies. This could explain the presence of Igs in OC tissue extracts, but not in OSMF tissue extracts. The earlier reports on OSMF indicate that this may be an intermediary stage in the conversion or transformation stage of a normal cell into a malignant one. We propose that the presence of Igs in OSMF biopsies may be considered as yet another criteria of precancerous lesions △

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LETTER TO THE EDITOR

Quantitation of tissue immunoglobulins in premalignant and malignant lesions of the oral cavity.

Sir,

Oral submucous fibrosis (OSMF) is a precancerous lesion reported mainly among Indians, characterized by stomatitis and vesiculization in early stages followed by stiffening of certain areas of the oral mucosa with difficulty in opening the mouth and swallowing (Paymaster, 1956). Pindborg *et al.* (1964) reported 1.2 % incidence in this area. Pindborg and Zachariah (1965) found OSMF in 40 out of 100 oral cancer (OC) patients. A very high incidence of OC is reported in Kerala (Padmanabhan and Vasudevan, 1982). So it is reasonable to assume that the high incidence of OSMF may be related to the high incidence of oral cancer (OC) observed in Kerala.

Pathological processes produce tissue damage with a concomitant release of proteins into the circulation. Many of these proteins are shared by many tissues and others are specific for a given tissue or organ and therefore their detection can be used to pinpoint the tissue of their origin (Ruddon, 1978). The quantitative estimation of these proteins will provide a means for detecting tumor growth before it produces clinical manifestations and thereby allow for earlier and more effective treatment.

Immunoglobulin (Ig) levels, which are used as a parameter to assess humoral immunity, continues to be an area of intensive research. A rise in serum IgG in OSMF was reported by Phatak and Gosavi (1975), whereas Abrol (1975) reported a rise in salivary IgG. Ankathil *et al.* (1985) reported immunological alterations in patients with OSMF. Vijayakumar *et al.* (1986) reported biochemical and immunological derangements in OSMF. The presence of IgG and IgA in the extracts

Subjects	Male	Age years Mean ± S.D.	Female	Age years Mean ± S.D.
Normal controls (n = 50)	25	36.2 ± 6.2	25	37.3 ± 7.2
Oral leukoplakia (n = 45)	25	39.0 ± 8.2	20	35.6 ± 9.1
Submucous fibrosis (n = 50)	20	40.5 ± 7.6	30	33.9 ± 8.6
Oral cancer (n = 50)	33	47.4 ± 11.2	17	49.6 ± 9.3

Table I. Age and sex distributions.

Tissue	Protein content (mg/ml)	mg/ml of protein		
		IgG	IgA	IgM
Normal controls	8.8 ± 1.3	Trace	Nil	Nil
Oral leukoplakia	9.2 ± 1.7	Trace	Trace	Nil
OSMF	10.7 ± 1.5	0.0598 ± 0.011	0.0234 ± 0.005	Trace
Oral cancer	12.8*± 2.1	0.117**± 0.025	0.0391*± 0.006	Trace

All values are means ± S.D.
* $p < 0.01$ and ** $p < 0.001$ compared to OSMF.

Table II. Tissue immunoglobulin levels in saline extract.
All values are means ± S.D. ; * : $p < 0.01$ and ** : $p < 0.001$ compared to OSMF.