Original Article

A study to estimate the serum IgA and salivary IgA levels in patients with oral leukoplakia and oral squamous cell carcinoma

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Abstract

Context: The increasing death rate because of oral cancer is mainly due to its late diagnosis. Tumour markers are often detected in abnormal amounts in blood, urine or saliva of patients with certain types of cancer. Diagnosing cancer through human saliva has advantages such as low invasiveness, minimum cost and easy sample collection. We have used serum immunoglobulin A (IgA) and salivary IgA for our present study.

Aims: The aim of present study was to estimate serum and salivary IgA levels in oral leukoplakia and oral squamous cell carcinoma (OSCC) patients.

Settings and Design: The study included 40 patients; 10 in the control group, 15 cases with oral leukoplakia and 15 cases with OSCC.

Methods and Material: The blood samples and saliva were taken from clinically diagnosed oral leukoplakia and OSCC patients and were tested for IgA levels.

Statistical Analysis Used: The data were analysed using SPSS 16.0. The mean values were compared between the groups by using analysis of variance (ANOVA) followed by post-hoc test for group-wise comparison. *P* value ≤ 0.05 was considered significant.

Results: It was observed that the comparison of levels of serum IgA in control and leukoplakia group; control and OSCC group; leukoplakia and OSCC group were found to be statistically significant. Also, comparison between the levels of salivary IgA in control and OSCC group was found to be statistically significant.

Conclusion: It is suggested that the serum and salivary IgA levels could be a better adjuvant diagnostic marker along with routine markers in patients with premalignant and malignant lesions.

Keywords: Oral leukoplakia, oral squamous cell carcinoma, salivary IgA, serum IgA

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INTRODUCTION

Cancer is the sixth leading cause of death and oral cancer is the most commonly occurring malignancy, amounting to about 40% of all malignancies in India contrast to 2-4% in western countries.[1] A high morbidity rate, 5-year mortality rate and an increasing incidence in younger people have been reported in recent years.[2]

The increasing death rate due to oral cancer is not because it is difficult to diagnose but largely because of its late diagnosis.[3] Apart from serum and blood, saliva has been used to detect tumour markers in the recent past.[4] There are several added advantages of human saliva for disease diagnosis and prognosis, such as low invasiveness, minimum cost and easy sample collection with minimum discomfort to the patient/subject. Additionally, the handling of saliva during the diagnostic procedures is much easier than blood as it does not clot and there is no risk of exposure of the laboratory technician to blood-borne diseases.[5]

Serum immunoglobulin A (IgA) is the most abundant antibody isotype produced in the body which is the second dominant isotype in the blood after IgG. Recent work has shown that IgA can activate complement and will efficiently trigger cell-mediated events.[6] It has been observed that the carcinoma that occurs from the secreting mucous membrane has high levels of serum IgA. The high levels of serum IgA is said to be due to the secretory components of immunoglobulins which are present in the epithelial cells that diffuse in the circulation causing the rise in IgA levels.[7]

Salivary IgA can be considered an important first line of defence against many invading pathogens. The increased serum concentration of IgA might reflect T lymphocyte defect.[8]

The aim of the present study is to estimate serum and salivary IgA levels in patients with oral leukoplakia and oral squamous cell carcinoma (OSCC) in comparison with normal individuals. With the help of this study, an attempt is made to standardize the use of these two tumour markers which could be used as adjuvant diagnostic parameters.

SUBJECTS AND METHODS

A sample size of 40 cases was taken for the present study. The cases in the study included 10 in the control group, 15 with oral leukoplakia and 15 with OSCC. Clinical data of the patients were recorded by following a proper case history entered in a Performa. Before starting any further procedure, an informed written consent of the patients was taken in his/her own language. The blood samples and saliva were taken from clinically diagnosed oral leukoplakia and oral SCC patients. The blood and saliva samples were centrifuged (8000 rpm for 15 minutes), and serum and supernatant were separated respectively, which were further tested for IgA levels. Ethical committee approval was taken in year 2012.

The inclusion criteria for the selection of cases:

- 15 patients with oral leukoplakia (histopathologically diagnosed)
- 15 patients with OSCC (histopathologically diagnosed)
- Patients without any systemic disease
- Patients without any other lesion in the oral cavity

The exclusion criteria for the selection of cases:

- Patients with systemic disease or possible compromised immune system
- Patients with any other lesion in the oral cavity
- Patients above 75 years of age

The control group used for the study were 10 healthy individuals without any deleterious habit or any other oral lesion.

A case series analysis of the above-mentioned patients was done. Biopsies were taken from the same patients and evaluated histopathologically. Evaluation of the serum and salivary IgA levels were done using a highly sensitive two-site Enzyme Linked Immunoassay and for routine histopathology, haematoxylin and Eosin staining were used.

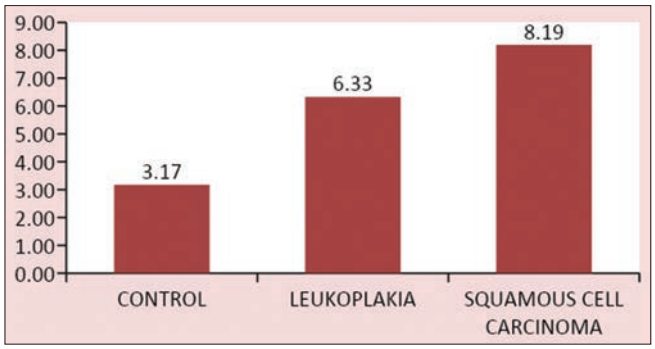
Statistical analysis

The obtained data were statistically analysed using SPSS version-16.0. Chi-square test was used to assess the association between the parameters analysis of variance (ANOVA) was used for testing the mean between more than two groups followed by post-hoc test for group-wise comparison. *P* Value < 0.05 was considered to be statistically significant.

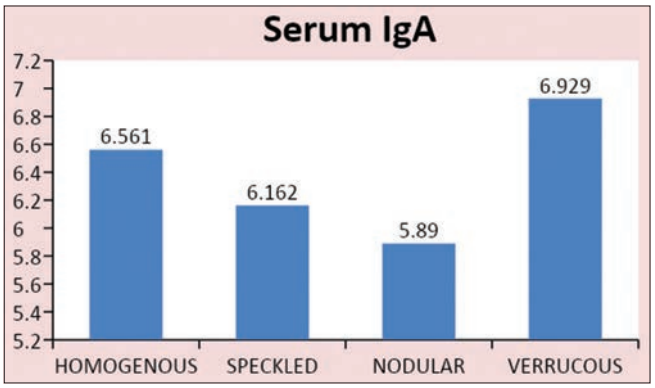
RESULTS

The mean values of the groups and subgroups are shown in Graphs 1–10. On comparing the levels of Serum IgA in control and leukoplakia group, control and squamous cell carcinoma group, leukoplakia and squamous cell carcinoma group the results were found to be statistically significant.

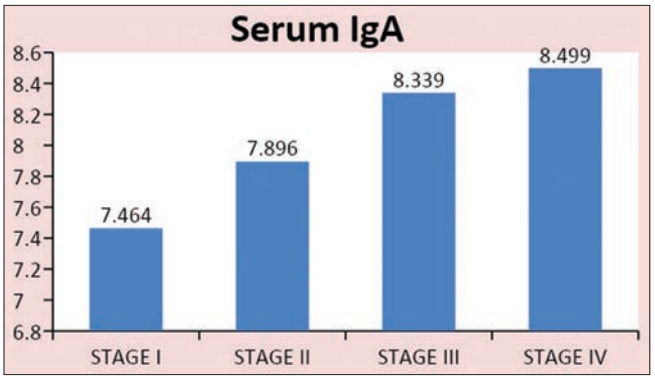
On comparing the levels of salivary IgA in control and squamous cell carcinoma group, the results were found to be statistically significant.



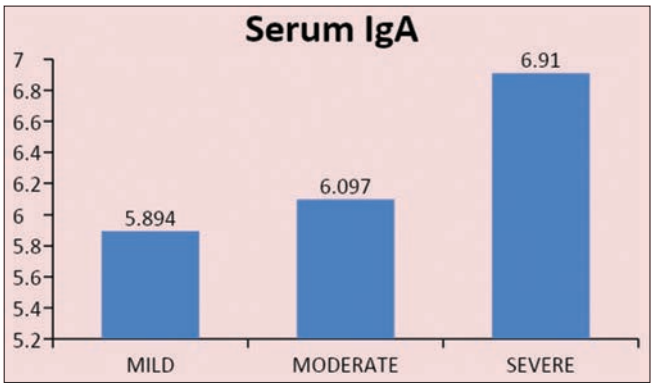
Graph 1: Comparison of mean values of serum IgA levels between the control, leukoplakia and OSCC group



Graph 2: Comparison of mean values of serum IgA levels between the clinical variants of leukoplakia



Graph 3: Comparison of mean values of serum IgA levels between the clinical stages in OSCC



Graph 4: Comparison of mean values of serum IgA levels between histopathological grades of epithelial dysplasia

DISCUSSION

Despite diagnostic and therapeutic progress, the overall prognosis of OSCC remains poor.[9] To ensure a better prognosis, an early diagnosis of OSCC is crucial followed by a timely institution of therapy.[10] So along with the gold standard histopathological diagnosis, reliable and time-tested biomarkers should be established which will serve as an adjuvant and early indicator of OSCC.

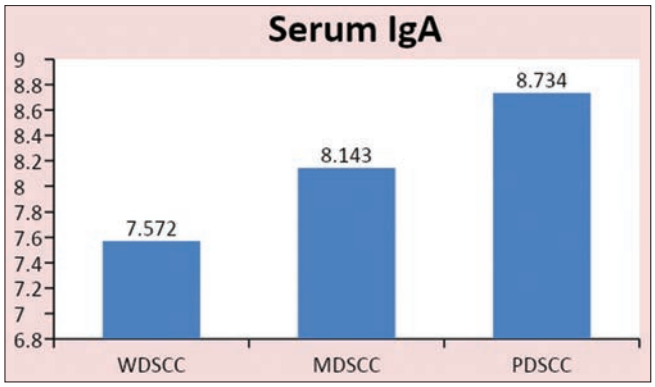
The concept of a two-step process of cancer development in the oral mucosa, that is, the initial presence of a precursor subsequently developing into cancer is well-established.[11] Oral leukoplakia is the best-known precursor lesion. Leukoplakia of the oral cavity are frequently encountered and have a potential to develop into OSCC.[12]

Currently, various tumour markers are being used for a wide range of cancer types. Measuring the number of specific immunoglobulins in the blood and saliva may be helpful in diagnosis of premalignant and malignant lesion.[13]

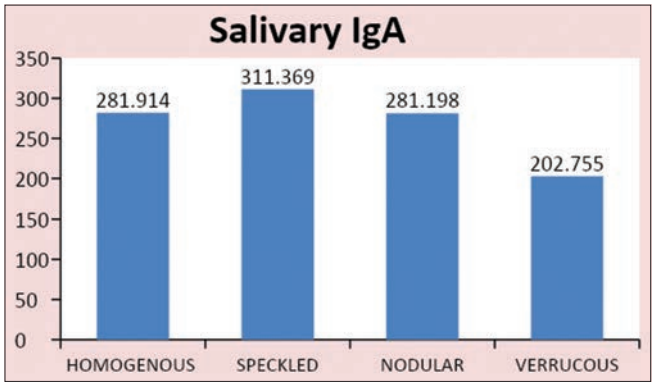
Apart from the serum, the immunoglobulins are normally found in other body fluids and tissues such as urine, spinal fluid, milk, saliva, tears, lymph nodes and spleen. IgA is the 2nd most common serum immunoglobulin and the major and predominant class of immunoglobulin in secretions like saliva, tears, colostrum and mucus. Because it is found predominantly in secretions, secretory IgA is important in local (mucosal) immunity.[14]

Serum IgA prevents the activation of the complement system and to inhibit phagocytosis, chemotaxis and antibody-dependent cellular cytotoxicity. The predominant role of serum IgA is the removal of antigenic substances without the generation of an inflammatory response. IgA can activate complement and will efficiently trigger cell-mediated events.[15] Secretory IgA (SIgA) is produced at the mucosal surfaces and is important in maintaining the local (mucosal) immunity which acts as the first line of defence against pathogens. Salivary IgA may also prevent the penetration of antigens in the oral mucosa.[16]

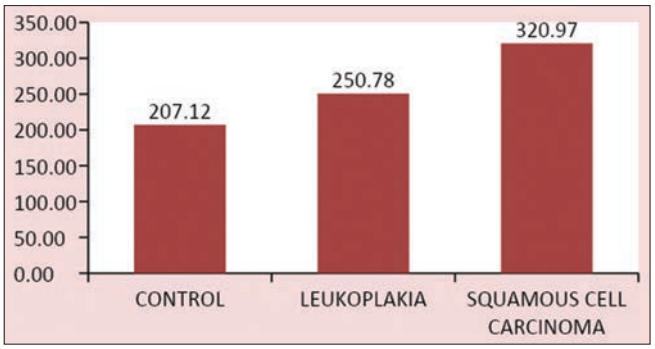
A total of 40 cases (15 with leukoplakia, 15 with squamous cell carcinoma and 10 controls) were examined during the course of the study. In our study, maximum numbers of cases (46.70%) were histologically graded as moderate epithelial dysplasia. It was seen that the mean value of serum IgA was the highest in case of severe epithelial dysplasia being 6.910 g/l followed by moderate



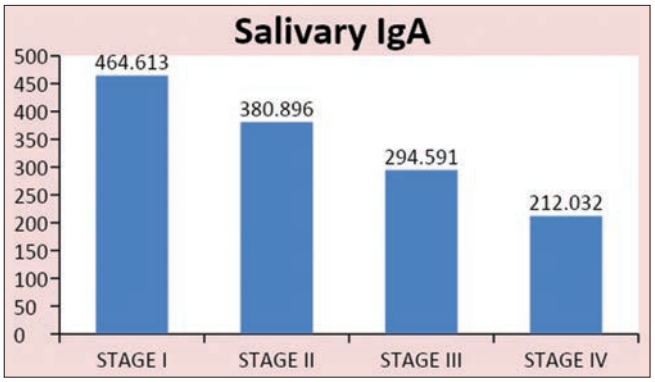
Graph 5: Comparison of mean values of serum IgA levels between histopathological grades of OSCC



Graph 7: Comparison of mean values of salivary IgA levels between the clinical variants of leukoplakia



Graph 6: Comparison of mean values of salivary IgA levels in control, leukoplakia and OSCC group



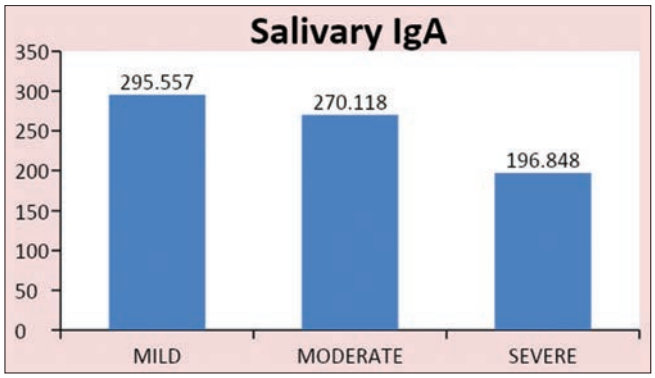
Graph 8: Comparison of mean values of salivary IgA levels between the clinical stages of OSCC

dysplasia (6.097 g/l) and mild dysplasia (5.894 g/l). Studies have not been done to correlate the levels of serum IgA with the different histological grades of epithelial dysplasia. As found in our study, the values of serum IgA were raised with the increasing grades of dysplasia.

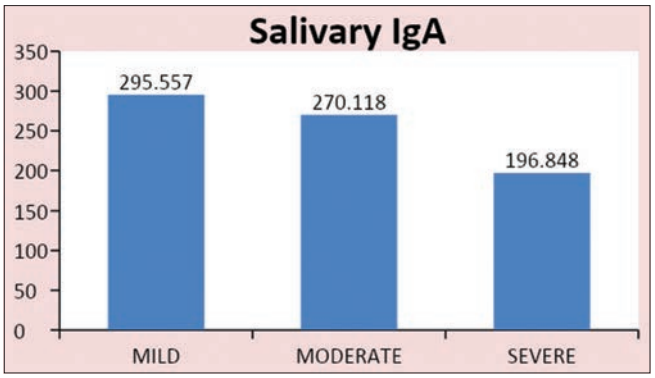
In our present study, six (40%) cases of OSCC were in Stage III, five (33.30%) were in Stage IV, three (20%) in Stage I and one (6.70%) in Stage II. On analysing the levels of serum IgA in patients of SCC according to the clinical staging, it was found that the mean value of serum IgA was increased from Stage I to Stage IV. The mean value in Stage I was 7.464 g/l and in Stage IV was 8.499 g/l. Khanna *et al.,*[6] Kohli GS *et al.*[17] and Parveen *et al.*[3] in their study also found a significant increase in serum IgA levels with the advanced clinical stage of malignancy. Schantz SP *et al.*[18] found the significant elevated levels of serum IgA in patients with lymph node metastasis when compared with patients who did not show metastasis. The increased serum IgA values in oral cancer cases may due the local immune response to the antigenic stimulation for the tumour. Khanna *et al.*[6] stated that the increased levels of serum IgA may be due to secretory components of immunoglobulins present in the epithelial cells which diffuse into the circulation causing an increase in serum IgA concentration. They also proposed that the extension and dissemination of the tumour beyond the confines of the primary site rather than local mass is the important factor responsible for serum immunoglobulin alterations.

The present study consisted of six (40%) cases with moderately differentiated SCC, five (33.30%) were poorly differentiated and four (26.70%) were well differentiated SCC. It was found that the mean value for serum IgA was the least in well-differentiated SCC (7.572 g/l), levels of serum IgA showed an increase in moderate SCC (8.143 g/l) and were highest in poorly differentiated SCC (8.7344 g/l). In this study, serum IgA levels were seen to be increased in patients with OSCC when the levels were compared with the control group. Our study also showed an increase in the levels of serum IgA with the increasing histopathological grade of SCC. Studies have not been done which showed the correlation of serum IgA levels with the histological grades of OSCC. (is this the objective of your study? If so, kindly change the title)

In the present study, when serum IgA levels were assessed between the three groups (leukoplakia, OSCC and the control), it was found that the mean serum IgA level for the control was 3.17 g/l, for leukoplakia was 6.33 g/l



Graph 9: Comparison of mean values of salivary IgA levels between histopathological grades of epithelial dysplasia



Graph 10: Comparison of mean values of salivary IgA levels between histopathological grades of OSCC

and for OSCC was 8.19 g/l. Serum IgA levels were significantly increased in patients with leukoplakia when levels were compared with the control group, which was statistically significant with a *P* value of <0.000. On comparison of levels of serum IgA in leukoplakia and in patients with OSCC, serum IgA levels were increased in patients with OSCC which was statistically significant with a *P* value of <0.003. In our study, the serum IgA levels were seen to be higher in OSCC when compared to the control group which is statistically significant with a *P* value of <0.000. Similar observations were made by Brown AM *et al*.,[19] Gupta SC *et al*.,[20] Scully C,[21] Khanna *et al*.,[6] Veltri RW *et al*.,[22] Kohli GS *et al*.,[17] Schantz SP *et al*.[18] Parveen *et al*.[3] and Lasisi TJ *et al*.[16] Whereas Neuchrist C *et al*.[23] found no significant differences between the levels of serum IgA in patients with OSCC and control group. Scully C *et al*.[21] stated that an increase in concentrations of serum IgA in patients with carcinoma of head and neck might reflect the T lymphocyte defect. Gupta SC stated that the rise in immunoglobulin A may due to defective lymphocyte function and alteration of thymus dependent (T cells) and non-thymus dependent (B cells) lymphocytes which might remove the suppressive action of T cells on the B cells leading to hyperactivity of B cells which causes hyperimmunoglobulinaemia. He also postulated that the mechanism of elevation of serum titres in epithelial cancer is due to reabsorption of secretory IgA back into the serum.[20] Kohli *et al*.[17] and Parveen *et al*.[3] stated that the increased serum immunoglobulins may reflect local infiltration of plasma cells adjacent to the neoplastic growth and superadded infection may further increase immunoglobulin levels. Increased levels of immunoglobulins with advanced stage of cancer are indicative of an adverse prognosis. Lasisi TJ *et al*.[16] proposed that the increased level of serum IgA was attributed to the secretory components of immunoglobulin present in the epithelial cells with the subsequent diffusion into the circulation.

In the present study, we also assessed salivary IgA levels, both in patients with premalignancy and malignancy. When the clinical variety of leukoplakia were compared, it was seen that the mean levels of salivary IgA was the highest in case of speckled leukoplakia being 311.369 μg/ml followed by homogenous leukoplakia (281.914 μg/ml), nodular leukoplakia (281.198 μg/ml) and verrucous leukoplakia (202.755 μg/ml). No such literature is found which evaluates the correlation between the clinical variety of leukoplakia and the salivary IgA levels.

In our study, maximum number of cases (46.70%) were histologically graded as moderate epithelial dysplasia. It was seen that the mean value of salivary IgA was the highest in case of mild epithelial dysplasia being (295.557 μg/ml) followed by moderate dysplasia (270.118 μg/ml) and severe dysplasia (196.848 μg/ml). Studies have not been carried out which show the correlation of salivary IgA levels with the histopathological grades of leukoplakia. The values of salivary IgA were reduced with the increasing grades of epithelial dysplasia.

In the present study, six (40%) cases of OSCC were in Stage III, five (33.30%) were in Stage IV, three (20%) in Stage I and one (6.70%) in Stage II. On analysing the levels of salivary IgA in patients of OSCC according to the clinical stages, it was found that the mean value of salivary IgA was decreased from Stage I to Stage IV. The mean value in Stage I was 464.613 μg/ml and in Stage IV was 212.032 μg/ml. The present study showed that the levels of salivary IgA were decreased with the increasing clinical stage of OSCC. Shpitzer T *et al*.,[24] showed the decreased secretory IgA levels when compared with the patients with OSCC. They stated that the decrease in the concentration of SIgA may be due to local/regional immune suppression. They proposed this may result either from a primary reduced antibacterial salivary capacity and/or an increased level of oral infections in the oral cavity of OSCC patients.

The present study consisted of 40% of the cases with moderately differentiated SCC, 33.30% were poorly differentiated and 26.70% were well-differentiated SCC. It was found that the mean value for salivary IgA was the least in poorly differentiated SCC (239.352 μg/ml), levels of salivary IgA were raised in moderately differentiated SCC (367.400 μg/ml) and was higher in well differentiated SCC (443.684 μg/ml). Studies have not been carried out which show the correlation of salivary IgA levels with the histological grades of OSCC.

In the present study, salivary IgA levels were compared between the control group, leukoplakia and OSCC. It was found that the mean value of salivary IgA was 320.97 μg/ml for the OSCC, 250.78 μg/ml for leukoplakia and 207.12 μg/ml for control. On comparison of levels of salivary IgA in control group and in patients with OSCC, salivary IgA levels were increased in patients with OSCC which was statistically significant with a *P* value of <0.049. This finding was consistent with Brown AM *et al*.,[19] Iglehart JD *et al*.,[25] Scully C,[21] Krasteva A *et al*.[26] and Patidar KA *et al*.[27] Lee YN,[28] Krasteva A *et al*.[26] and Sato K[29] found the increased levels of salivary IgA within the leukoplakia. Shpitzer T *et al*.,[24] showed the decreased secretory IgA levels when compared with the patients with OSCC. They stated that the decrease in the concentration of SIgA may be due to local/regional immune suppression. They proposed this may result either from a primary reduced antibacterial salivary capacity and/or an increased level of oral infections in the oral cavity of OSCC patients.

(any correlation done between serum IgA and salivary IgA?)

Our study did not show any correlation between serum IgA and salivary IgA. Saliva is a physiological body fluid whose composition is greatly affected by various factors. This could be one of the reasons for its inability to indicate the levels of serum IgA. In the present study, the increased titers of salivary IgA could be due to a possible local antibody response to tumour because transudation of serum IgA to saliva is responsible for only minute amount of total salivary IgA. This finding is in accordance with the study by Vinzenz *et al*.[30] which states no correlation between serum IgA levels and salivary IgA levels. This further suggests that extravascular transfer of IgA primarily depends on the mucosal status of the individual and necessarily not on the serum level.

CONCLUSION

In the present study, it was observed that the serum and salivary IgA levels were increased in patients with leukoplakia and OSCC when compared with the control group. Thus, the time calls for further studies with a larger sample size to establish the efficacy of Serum and salivary IgA levels in the early diagnosis of premalignant and malignant disorders.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Key messages

Serum and salivary IgA levels could be a better adjuvant diagnostic marker along with routine markers in patients with premalignant and malignant lesions.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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