Evaluation of Serum Immunoglobulin (IgG, IgM, IgA) in Oral Cancer Patients – A Case Control Study

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Keywords: IgG, IgM, IgA, Oral Cancer, Oral Squamous Cell Carcinoma, Serum Immunoglobulin, Tumour Marker

Posted Date: October 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-87055/v1>

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Abstract

Background: Very few studies have been done to evaluate level of serum immunoglobulins (IgG,IgM,IgA) in patients with Oral cancer as tumour markers.

Aims and Objectives: To estimate the serum immunoglobulins level (IgG, IgM, IgA) in oral cancer patients and its comparison with control groups and weather these values can be used to predict severity of disease or not.

Materials and Methods: 30 patients with different clinical stages oral cancer and 30 healthy controls were selected at random and subjected for analysis of serum IgG, IgA, IgM. Statistical methods employed were the mean, standard deviation and t-test.

Results: Significant elevated levels of all the immunoglobulins in oral cancer patients observed when compared with the control group and levels were increasing with clinical stages. ($p<0.05$).

Conclusions: All these immunoglobulins are indicative of tumour burden or transformation of malignancy in higher stage and might be employed as prognostic indicators in oral cancer.

Introduction

Cancer is Greek word 'Karkinos', meaning crab, denoting how carcinoma extends its claws like a crab into the adjacent tissues. Cancer is the second and third most leading cause of mortality in economically developed and developing countries respectively.^[1]

Oral squamous cell carcinoma (OSCC) is the most frequent malignancy in the mouth, accounting to 90–95% of all oral malignant lesions. It is an important cause of morbidity and mortality worldwide with an incidence rate that varies widely by geographic location.^[2, 3]

In India, cancer is the 6th leading cause for death and oral cancer accounts for 30–40% of all cancers in contrast to 2–4% in other western countries and is the most prevalent cancer in males and the third most prevalent in females. In India, there is a striking incidence of oral cancer where tobacco chewing with betel nuts and reverse smoking is practiced.^[2, 4] Tongue, lower lip and floor of the mouth are most affected sites. It affects mostly in the fifth to eighth decades of life, who is a tobacco chewer and/or a smoker.^[2]

There are multiple risk factors for the development of oral cancer. The high incidence is attributed to the habits of chewing betel leaf with arecanut with or without tobacco, betel quid chewing, smoking, alcohol, malnutrition etc.^[5] It is important to note the frequency per day and duration of the habit that patient had the habit in years which is called Habit Index. So, Habit Index = frequency per day x duration of habit in years.^[6]

The term tumour marker is applied to indicate the risk, presence, status, or future behaviour of the oral cancer. Immunoglobulins are proteins of animal origin endowed with known antibody activity and for certain other proteins related to them by chemical structure and have antigenic specificity. Based on physiochemical and antigenic differences, five classes of immunoglobulins have been recognized – IgG, IgA, IgM, IgD, and IgE.^[7]

Material And Methods

Present study was performed in patients attending dental OPD at tertiary care Institute of Gujarat for the duration of 2 years. Ethical approval was taken from the institutional ethical committee and written informed consent was taken from all the participants. Total 60 patients with age range from 18 to 70 years were included in the study. 30 patients (21 males and 9 females) with different clinical stages of Oral cancer (stage I – 4, stage II – 4, stage III – 13, stage IV – 9) were included and 30 normal healthy individuals (14 males and 16 females) without any systemic illness were included in the Study group.

[Figure 1] Most recent staging system by the American Joint Committee on Cancer (AJCC) TNM (Tumour Node Metastasis) staging system for oral cancer (2002) was utilized.^[8]

Data Collection

A thorough history was taken and all patients were examined by using mouth mirror and probe under artificial light. All patients with PMDs were confirmed with histopathological report after taking biopsy. Datas were collected in a specially designed proforma. Blood was collected from the mid cubital vein. The area was made aseptic by using absolute alcohol and by using bi - ended needle with connector and 4 ml of blood was drawn directly into yellow capped sterile vacuumed tubes which contained gel plus clot activator. The blood was allowed to clot for 60 minutes and then centrifuged at 2000 rpm for 10 minutes. Serum was separated with pipette and stored in a separate container. The samples were stored in a deep freezer at -20⁰C until analyzed. Hemolysed samples were discarded and fresh samples were taken for assay. The estimation of serum immunoglobulins [IgG, IgM, IgA] was done by Nephelometric technique in automatic analyzer (Olympus AU 400).

Statistical Analysis

After collection of data and results of Immunoglobulin values of all patients, data were tabulated for statistical analysis. SPSS (Statistical Package for Social Sciences) 15.0 for Windows (SPSS, Inc., Chicago, IL) was used for the statistical analysis and mean, standard deviation calculated and t- test applied. For all tests, P value < 0.05 (5%) was considered statistically significant.

Results

We observed significant elevated levels of Immunoglobulins (IgG,IgM,IgA) in all the oral cancer patients when compared to control groups. Mean value of Serum IgG of oral cancer group was 16.218 ± 2.505 g/l and for control group 11.712 ± 1.766 g/l, Mean value of Serum IgM of oral cancer group was 1.658 ± 0.677 g/l and for control group 0.702 ± 0.309 g/l Mean value of Serum IgA of oral cancer group was 3.280 ± 1.209 g/l and for control group 2.101 ± 0.646 g/l and it was statistically significant ($p < 0.05$).

[Table 1]

Table 1
Comparison of mean IgG, IgM, IgA between Oral cancer and Control groups

		Mean	Std. Deviation	Minimum	Maximum	P value
IgG	Cancer	16.218	2.505	11.027	19.839	< 0.05
	Control	11.712	1.766	9.008	14.934	
IgM	Cancer	1.658	0.677	0.549	3.235	< 0.05
	Control	0.702	0.309	0.230	1.600	
IgA	Cancer	3.28	1.209	1.563	5.658	< 0.05
	Control	2.101	0.646	1.260	3.809	

It was seen that increased levels correlated well with clinical stages of malignancies. In oral cancer, Mean value of Serum IgG in stage 1 was 12.986 g/l, stage 2 had 15.812 g/l, stage 3 16.144 g/l and stage 4 had 17.941 g/l. [Figure 2] For Serum IgM, Mean value of stage 1 was 1.007 g/l, stage 2 was 1.555 g/l, stage 3 was 1.652 g/l and stage 4 had mean value of 2.000 g/l. [Figure 3] For Serum IgA, stage 1 had mean value of 2.572 g/l, stage 2 had 3.040 g/l, stage 3 had 3.259 g/l and stage 4 had mean value of 3.732 g/l.

[Figure 4]

Discussion

Chewing areca nut with betel quid is practiced since two millennia. Four centuries back tobacco introduced and currently all areca nut chewers use it in combination. With the advent of 'pan masala' areca nut chewing has received a boost in last couple of decades.^[5] Due to easy availability, marketing, stress relieving effects, status trends and peer pressure, habit of smoking or smokeless tobacco and chewing betel nut in form of Panmasala or gutkha increased in youngsters which lead to premalignant and malignant diseases of oral cavity.^[9, 10]

Prevention and early detection is the best way to reduce incidence and mortality of oral cancer. There are many methods of early detection of oral cancer and immunological method is one of them.

Response to cancer is very complex process as it mainly depends on immune status of person, extent and severity of disease and response to treatment and resistance development plus effects of oncogenes or tumour suppressor genes and chromosomal changes.^[6] Defect in host's immune system with increase

in age might be reason behind occurrence of cancer, hence research in early cancer diagnosis has led to discovery of many immunological markers that contribute considerably to supplement the established method of diagnosis.

The tumour marker concept has evolved and new tumour markers are frequently introduced into clinical practice over the years but so far none of them meet all the criteria of an ideal tumour marker.

Biomarkers are unique molecular signature of each cell, which are identifiable by its levels or activities like the abilities of genes or proteins to perform their functions. So, for objective measure of evaluation of normal biological or pathogenic processes, or pharmacological responses to a therapeutic intervention, biomarkers can be used and it includes all diagnostic tests, imaging, and any objective measure of health status of an individual. Biomarkers are subject to dynamic modulation, and are expected to enhance our understanding of drug metabolism, drug action, efficacy and safety. These can also facilitate molecular definition of diseases, provide information about the course of disease and predict response to therapies.^[11]

Malignant cells display a broad spectrum of genetic alterations leading to disturbances in molecular pathways regulating cell growth, survival and metastasis. With a specific type of tumour, manifestation of such changes seen in majority of patients, these can be used as biomarkers for detection and developing targeted therapies, besides predicting responses to various treatments.^[11]

Immunoglobulins constitute 20–25 percent of the total serum proteins and are synthesized by plasma cells and also by lymphocytes and also found in other body fluids or tissues such as urine, spinal fluid, milk, saliva, tears, lymph nodes and spleen. Immunoglobulin serves as a specific link between epitopes on tumour cell antigens and the host-effectors cells. Many studies done for specific immunoglobulin response in oral cancer.^[12]

In serum the predominant immunoglobulin belong to IgG class. It is the only maternal immunoglobulin that is normally transported across the placenta and provides natural immunity in the newborn.^[13] In our study IgG was significantly elevated among the oral cancer compared to controls. The values of Serum IgG were increased from stage I to stage IV. Studies by Balan et al, Kohli et al, De-en et al and Parveen S et al have shown significant elevation of IgG in oral cancer stages. The results of these studies with respect to IgG and gradual increase in their levels with stages of malignancy were consistent with our study.^[4,14–16] In contrast, Neuchrist et al found no significant difference between tumour and controls.^[17]

The elevation of IgG levels in oral cancer patients may be due to dependencies of IgG on the intensity of the antigenic stimulation and functional capacity of the antibody producing mechanism. Consequently, the increase in IgG in this group of patients could be due to intensive antigenic stimulation that is possibly due to neoplastic process.^[16]

Elevated levels of IgG demonstrate an immune response which is helpful in antibody dependent cytotoxic–cell killing of tumor cells, however, tumor cell can release inhibitory substances and produce a

blocking factor (probably IgG2) to protect them from attacks by cytotoxic antibodies, and can also form complexes which act as blocking factors and further weaken cellular immunity.^[15] In contrast, Tsavarius found that the group of patients having increased IgG and perhaps IgM levels showed a longer time for progression of disease and prolonged overall survival time.^[18]

Banerjee S et al in their study of humoral response in malignancies with special reference to the effect of radiotherapy found the mid therapy fall of IgG and regain during post therapy. Possible explanation for midtherapy fall might be due to temporary exhaustion of regional lymphatic system by radiotherapy, regain probably due to stimulation of antibody response secondary to products of necrosis after application of radiotherapy.^[19]

Serum immunoglobulin (IgM) constitutes approximately 10% of normal serum immunoglobulins. It is the most common immunoglobulin expressed on the surfaces of B cells. In humoral immunity the B cell system of the marrow stem cells first give rise to IgM producing cells. So the first antibodies to appear in the serum after primary exposure to an antigen are of IgM class. IgM producing cells serve as the progenitors of other IgM producing cells and give rise to IgG progenitors.^[13]

The serum IgM levels are often increased in patients with oral cancer. Our study also shows higher values of IgM in the patients with oral cancer when compared to controls, which is in agreement with studies done by Balan et al, Kohli et al, De-en et al, Parveen S et al, Shah N et al and Veltri et al.^[4, 14–16, 20, 21] Khanna NN et al observed increased levels of IgM in cancer patients correlated with the clinical stages.^[22] In contrast, Banerjee S et al found decreased serum IgM than that of the controls level.^[19] This is probably due to the fact that the selective clones of cells producing different types of immunoglobulins are responding to the malignancies in different ways.

Serum immunoglobulin (IgA) is approximately 1/6th that of IgG. It is efficiently secreted in milk, colostrums, saliva, tears and secretions of GIT.^[13] In our study, increased levels of mean IgA values were found among oral cancer patients when compared to control groups which was consistent with studies by Parveen S et al, Veltri RW et al, Katz AE et al, Scully C et al and Vijay Kumar T et al.^[16,20,23–25] Schantz SP et al demonstrated significant difference in only IgA titers between cancer patients and controls.^[26]

Kohli GS et al found higher level of all three immunoglobulins than the control group in which levels of IgA being more than that of IgG and IgM.^[14] Khanna et al reported a significant increase in IgA as well as IgM levels,^[22] whereas Increased IgA and IgG levels were observed in oral cancer by Brown et al and Gupta et al.^[27, 28]

Higher levels were associated with advanced disease rather than early primary disease. We observed increased IgA levels with progression of oral cancer which was consistent with findings of Balan N et al and Schantz SP et al who observed highest values of IgA in patients with advanced disease.^[4, 26] Serum concentrations of IgA were increased in oral cancer patients as IgA is the predominant immunoglobulin secreted into mucosal surface of the nose, mouth, stomach, intestine, lungs, tears and colestrum.

Assuming a local immune response of the mucosal immune system to malignancies there could be an increased level of antigenic stimulation and cancer of such organs resulting in increased synthesis of IgA which is reflected in serum level.^[16]

Previous study conducted by Susal C et al in head and neck squamous cell carcinoma patients for IgA-anti-F_{ab} autoantibody activity and it was higher in carcinoma patients than the normal healthy controls and stage IV patients had significantly higher IgA-anti-F_{ab} than the stage I patients.^[29] Similarly, Lorenz et al found that higher IgA anti-Fab activity in stage IV compared to stage I and II demonstrated highest values in patients who died within 6 months after final diagnosis suggesting an association between autoimmunity and final disintegration of physiologic body functions.^[30]

Schantz SP et al found that elevated IgA blood levels reflect the autoimmune nature of cancer, an immunologic state defined by its tumor-promoting capacity, poor prognosis and higher probability of recurrent disease than those with normal IgA values.^[26] Brown AM et al reported two fold increase of serum and salivary IgA in primary oral and laryngeal carcinoma compared to controls.^[27] A follow up after radiotherapy confirmed a drop in salivary IgA with cure, and a spike with recurrence. Persistent elevation in advanced diseases and elevated levels after therapy may be due to persistence of tumour antigen / residual disease and appears to be directly related to tumour burden. De Souza et al evaluated serum and salivary IgA by two different methods in patients of cancer of mouth and oropharynx, result showed no difference between patients and normal subjects which was not matched with our result.^[31]

Increased level of immunoglobulins may reflect local infiltration of plasma cells adjacent to the growth and superadded infection in oral cancer may further increase immunoglobulin levels. Increased levels of immunoglobulins with advanced stage of oral cancer are indicative of an adverse prognosis.^[14, 16] Higher levels of these parameters may indicates poor prognosis and decrease survival rate. Therefore it is suggested that serial monitoring of Immunoglobulin could be used to know the prognosis, tumour burden or survival rate of patients.

Conclusion

Clinical oncology is poised to enter a new era in which oral cancer detection, diagnosis, and treatment will be guided increasingly by the molecular attributes of the individual patient, acquired from several different sources like diseased tissue, host cells/tissues that influence tumour behaviour and body fluids. The biomarkers will not only help the detection and diagnosis, but also answer fundamental questions about biologic behaviour of tumours, resistance to therapy and sensitivity to therapy. Higher levels of immunoglobulins in oral cancer are indicative of tumour burden or transformation of malignancy in higher stage and might be employed as prognostic indicators.

Declarations

- Ethics approval and consent to participate

Ethical approval taken from Ethical committee of Karnavati School of Dentistry, Gandhinagar, Gujarat, India where this study was conducted.

- Consent for publication

Consent taken from each participants of the study before taking any data, still no any individual data in published in this research paper.

- Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to it was college/university policy but are available from Karnavati School of Dentistry, Gandhinagar, Gujarat, India or from the corresponding author on reasonable request.

- Competing interests

The authors declare that they have no competing interests

- Funding

Not Applicable.

- Authors' contributions

VT has done data collection, study design, statistical analyses and write up.

NR did study design and proof reading.

DM did corrections in manuscript and proof reading.

MA did data collection and interpretation.

VA did acquisition and draft of manuscript

KB did proof reading and supervision.

- Acknowledgements

Not Applicable.

- Source(s) of support

NIL

- Conflicting Interest

NIL

- Prior Publication

NIL

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Figures

Figure 1

Total number of Male and Female patients in Oral cancer and Control group.

Figure 2

Mean values of IgG increase with clinical stages of oral cancer.

Figure 3

Mean values of IgM increase with clinical stages of oral cancer.

Figure 4

Mean values of IgA increase with clinical stages of oral cancer.