EVALUATION OF SERUM IMMUNOGLOBULIN (IGG, IGM, IGA) AS DIAGNOSTIC MARKERS IN ORAL PREMALIGNANT DISORDERS AND ORAL CANCER PATIENTS: A CASE CONTROL STUDY

Dissertation Submitted To

BABU BANARASI DAS UNIVERSITY, LUCKNOW,

UTTAR PRADESH

In the partial fulfillment of the requirement for the degree of

Master of Dental Surgery

in

Oral Medicine And Radiology

by

Dr. SARAH AFAQUE

Under the guidance of

Dr. NEETA MISRA

Professor

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,

BBDU LUCKNOW (U.P)

BATCH 2020-2023

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Evaluation Of Serum Immunoglobulin (IgG, IgM, IgA) As Diagnostic Markers In Oral Premalignant Disorders And Oral Cancer Patients: A Case Control Study"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Neeta Misra**, Professor and **Dr. Saurabh Srivastava**, Reader, Dept of Oral Medicine and Radiology, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Sarah Afaque

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Place: Lucknow

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CERTIFICATE BY THE GUIDE / CO-GUIDE

This is to certify that the Dissertation entitled **"Evaluation Of Serum Immunoglobulin (IgG, IgM, IgA) As Diagnostic Markers In Oral Premalignant Disorders And Oral Cancer Patients: A Case Control Study"** is a bonafide work done by **Dr. Sarah Afaque**, under our direct supervision and guidance as requirement of the partial fulfilment for the degree of Master of Dental Surgery in Oral Medicine and Radiology.

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ENDORSEMENT BY THE HEAD OF DEPARTMENT

This is to certify that this Dissertation entitled **"Evaluation Of Serum Immunoglobulin (IgG, IgM, IgA) As Diagnostic Markers In Oral Premalignant Disorders And Oral Cancer Patients: A Case Control Study" "** is a bonafide work done by **Dr. Sarah Afaque**, under the direct supervision and guidance of **Dr. DEEPAK U.**, Professor and Head, Dept of Oral Medicine and Radiology, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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Sarah Afaque

Date: 06/02/2023

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Place: Lucknow

Dr. Sarah Afaque

ACKNOWLEDGEMENT

“AT TIMES OUR OWN LIGHT GOES OUT AND IS REKINDLED BY A SPARK FROM ANOTHER PERSON. EACH OF US HAS CAUSE TO THINK WITH DEEP GRATITUDE OF THOSE WHO HAVE LIGHTED THE FLAME WITHIN US.”

— ALBERT SCHWEITZER

Words cannot express when the heart is full of praise and gratitude. The accomplishment of this study could not have been possible without the constant guidance, support, and exhortation of my mentor and guide, **Dr. Neeta Misra**, **Professor, Department of Oral Medicine and Radiology, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow.** Her guidance has helped me at all times in completing this dissertation; with her profuse knowledge of the subject and intense involvement, the dissertation's success was achievable.

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Dr. Sarah Afaque

Department of Oral Medicine and Radiology

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LIST OF ABBREVIATIONS

ABBREVIATION	
BBDCODS	Babu Banarasi Das College of Dental Sciences
BBDU	Babu Banarasi Das University
OMR	Oral Medicine and Radiology
H&N Mdt	Head And Neck Multidisciplinary Team
Opmd	Oral Premalignant Disorders
Oscc	Oral Squamous Cell Carcinoma
Ig	Immunoglobulin
Iga,	Immunoglobulin A
Igg	Immunoglobulin G
Igm	Immunoglobulin M
Tme	Tumor Microenvironment
Ecm	Extracellular Matrix
Tam	Tumor-Associated Macrophages
Treg	Regulatory T-Cells
Caf	Cancer-Associated Fibroblasts

Dc	Macrophages, Dendritic Cells
Mdsc	Myeloid Derived Suppressor Cells
Nkc	Natural Killer Cells
Hpv	Human Papilloma Virus
Pmd	Potentially Malignant Disorders
Who	World Health Organisation
Pvl	Proliferative Verrucous Leukoplakia
Osmf	Oral Submucous Fibrosis
Nsaid	Nonsteroidal Anti-Inflammatory Drug
Ace	Angiotensin-Converting Enzyme
Dc	Dyskeratosis Congenita
Nbi	Narrow Band Imaging
Shm	Somatic Hypermutation
Adcc	Antibody-Dependent Cellular Cytotoxicity
Cdc	Complement-Dependent Cytotoxicity
Adcp	Antibody-Dependent Cellular Phagocytosis
Mgcus	Monoclonal Gammopathy Of Undetermined Significance
Itam	Immunoreceptor Tyrosine-Based Activation Motif

Ra	Rheumatoid Arthritis
Sle	Systemic Lupus Erythematosus
Sis	Systemic Inflammatory Response
Nlr	Neutrophil-To-Lymphocyte Ratio
Lmr	Lymphocyte-To-Monocyte Ratio
Plr	Platelet-To-Lymphocyte Ratio
Crp	C-Reactive Protein
Nf-Kb	Nuclear Factor Kappa-Beta
Il	Interluekin
Cox-2	Cyclooxygenase

ABSTRACT

INTRODUCTION

Immunoglobulins are proteins of animal source bestowed with established antibody activity and specific other proteins which are related to them by its chemical structure and antigenic specificity. Based on their physiochemical and antigenic differences, Five immunoglobulins have been recognized – IgG, IgA, IgM, IgD, and IgE. Oral Premalignant disorders (OPMDs) are a substantial group of mucosal disorders that may pave the way to the diagnosis of oral squamous cell carcinoma (OSCC). Prevention and initial detection of Oral Premalignant Disorders (OPMD) help avoid oral cancer and the associated mortality and morbidity. Therefore, there is a need to identify markers that can indicate the occurrence of OPMDs and Oral cancer.

AIM

This study aims to evaluate the Screening of Immunoglobulins (IgG, IgM, IgA) in patients with Oral Premalignant Disorders and Oral Cancer Patients and their comparison with Healthy Subjects.

OBJECTIVES

- To Compare the levels of Serum Immunoglobulin G levels in Oral Premalignant Disorders and Oral Cancer Patients in comparison with Control group.
- To Compare the levels of Serum Immunoglobulin M levels in Oral Premalignant Disorders and Oral Cancer Patients in comparison with Control group.
- To Compare the levels of Serum Immunoglobulin A levels in Oral Premalignant Disorders and Oral Cancer Patients in comparison with Control group.

ABSTRACT

MATERIAL AND METHODS

The study was conducted in the Department of Oral Medicine and Radiology of Babu Banarasi Das College Of Dental Sciences, Lucknow (U.P.). For the Study Purpose, a total of 60 Patients were examined and were divided into 2 groups. The Case Group consisted of 40 patients with Oral Precancer and Oral Cancer, whereas the Control Group consisted of 20 healthy subjects. Patients were selected on the basis of inclusion and exclusion criteria. The collected data was tabulated on spread sheets and subjected to statistical analysis.

RESULTS

Estimation of the levels of immunoglobulins (IgG, IgM, IgA) in the OPMD and OSCC group when compared with the control group showed statistically significant results. Statistically, a significant difference was observed in the levels of IgG, IgM, IgA [**p<0.0001***]

CONCLUSION

The present study concluded that the level of immunoglobulins (IgG, IgM, IgA) was significantly higher in OPMD and OSCC compared to the Control. These factors may suggest a poor prognosis and a lower survival chance if they are elevated. It may help in the detection and diagnosis of OPMD and OSCC. Higher levels of immunoglobulins in oral cancer may indicate tumor burden or conversion of malignancy in higher stages and might be used as prognostic indicators.

KEYWORDS

Immunoglobulins, Oral Premalignant disorders, Oral squamous cell carcinoma, Biomarkers

1. INTRODUCTION

Patients with oral cancer provide the treating physician with a distinctive combination of difficult, intricate, and multidisciplinary therapeutic difficulties, the answers to which have a significant influence on both their survival and quality of life. A Multidisciplinary Head & Neck Oncology Team often treats all oral cavity tumors. [1] The Head and Neck Multidisciplinary Team (H&N MDT) includes numerous clinicians, including (but not limited to) throat surgeons and oral and maxillofacial, ear, nose, and plastic & reconstructive surgeons, radiologists, anatomical pathologists, radiation oncologists, medical oncologists, dieticians, anaesthetists, speech and language therapists, head and neck nurses, physiotherapists, oral medicine specialists, prosthodontist, and social workers. [2]

Oral Premalignant Disorders (OPMDs) are a significant group of mucosal disorders that can occur before diagnosing oral squamous cell carcinoma (OSCC). [3] Oral cancer accounts for about 30–40% of all cancers in India, compared to about 2–4% in other western countries. In males it is the most prevalent cancer and in females, it is the third most prevalent cancer. In regions of India where betel nut chewing and reverse smoking are prevalent, the prevalence of oral cancer is alarmingly high. [4,5]

Indicating the threat, incidence, significance, or future comportment of potentially malignant diseases or oral cancer, the notion of a tumor marker is utilized. [6] Consequently, it is necessary to identify indicators that can detect the presence of OPMDs and Oral cancer.

Due to the functional and aesthetic ramifications of treating tumors in this location, the treatment of oral cavity cancers is complicated. There are only a few essential head and neck activities that the tumor or its treatment may temporarily or permanently compromise, these include breathing, speaking, deglutition, visualisation, odour, taste, mastication, and jaw function. In addition, facial and oral aesthetics have a significant role in how others see us; Cancer and/or its treatment can significantly impact self-esteem and self-confidence. From the detection of premalignant lesions to the prompt detection of oral cancer, management of the oral cancer patient's dentition both before and after definitive treatment, shadowing recurrent or new primary tumors in unification with the treating specialist, and reintegration of missing teeth in collaboration with the treating maxillofacial surgeon and prosthodontist, Dentists tend play a vital role in the management of oral cancer. [7,8]

Immunoglobulins are proteins of animal source bestowed with established antibody activity and specific other proteins which are related to them by its chemical structure and antigenic specificity. There are five identified immunoglobulins: IgG, IgA, IgM, IgD, and IgE, based on physiochemical and antigenic distinctions. Numerous writers have investigated the particular immunoglobulin response generated by cancer. [9] In clinical practice, serum immunoglobulin levels are frequently measured because they offer vital evidence about the humoral immune state. Some humoral immunodeficiencies are characterized by low immunoglobulin (Ig) levels. Polyclonal gammopathy is witnessed in liver diseases, chronic inflammatory diseases, haematological disorders, infections, and malignancies.

Immunoglobulin levels also promote to diagnosing certain diseases, particularly liver ailments. [10,11]

It is essential to determine the distribution of immunoglobulin levels in broad populations, to evaluate reference values. The general guidelines for identifying and deciding reference intervals when there are substantial disparities between subgroups defined by age, sex, and common exposures such as smoking or alcohol intake, in the clinical laboratory state that segregating should be considered. [12] However, meagre studies have examined the potential impacts of these factors on blood immunoglobulin levels. According to reports, females have higher IgM levels than males. Additionally, immunoglobulin serum concentrations have a tendency to rise with age. It is well acknowledged that heavy drinkers with advanced liver disease frequently have elevated IgA levels. However, researches on the effects of smoking and alcohol consumption (from light to heavy) on serum IgA, IgG, and IgM has been yet to be examined [13] Collectively, these findings highlight the necessity for multivariate analysis to discover the perplexing interactions among all these factors allied to immunoglobulin levels and each other.

History of Oral Cancer and OPMDs

The notion of 'precancer' arose in 1805, given by a European panel of physicians, an implication that there are benign diseases that will incessantly develop into invasive malignancies if found for a long time. [14] In 1875, the term 'precancer' was first devised by Victor Babes, a Romanian physician. This concept was later expanded to include numerous diseases in the numerous organ systems of the body.

Some of the lesions convicted to be precancerous are Junctional nevi, Xeroderma pigmentosa of the skin, and leukoplakia, papillomas of the urinary bladder and larynx, polyps of the colon, and solitary thyroid adenomas. [15] With increase in research the preliminary depiction made in 1942 by C. H. Waddington: "the causal interactions between genes and their products bring the phenotype into being." has extended. The definition principally denoted to embryonic development— "the study of heritable changes in the gene expression that occurs independent of changes in the primary DNA sequence,"—a description that is further pertinent to the whole organism, including the stages of development and disease states. [16] Despite the same genetic material and similar cellular signaling patterns, epigenetic alterations facilitate the probability of discrepancy between the cells.

Oral precancers have had an intense and enthralling literature encompassing as far back as the 1870s, when one of England's most renowned surgeons, Sir James Paget, anticipated that 'leukokeratosis' or 'smoker's patch' of the hard palate (nicotine palatinus) or the tongue in innervate pipe smokers passed an amplified risk of ultimate cancer transformation. [17] Later on, various terminologies appeared in the literature concerning the 'precancer' concept like 'premalignant,' 'preneoplastic,' 'carcinoma prone,' 'intra-epithelial neoplasia,' etc. However, in the international literature, the information concerning the evolution of these terminologies is unavailable. The continuing progression of these lesions are standing to be a greater challenge.

For that reason, the ongoing challenge and uncertainty encircling the oral cancer concept, the WHO has periodically summoned International Workshops to redefine

the term ‘precancer’ and several precancerous lesions. A much previously working group of WHO, in 1978, used the term ‘precancer,’ which was further than classified into ‘lesions’ and ‘conditions. [18] The most recent workshop in London in 2005 commended using the term OPMDs and the abolition of the term ‘precancer.’ [19] Nevertheless, the latest WHO monograph on Head and Neck Tumors (2005) utilizes the term ‘epithelial precursor lesions [20]. Recently, the term potentially malignant disorders were coined by *Manne RK* for individuals with no recognized predisposing disorders or any clinically evident lesion, where the oral mucosa may be susceptible to cancer. [21]

2. AIM & OBJECTIVES OF THE STUDY

AIM

This study aims to evaluate the Screening of Immunoglobulins (IgG, IgM, IgA) in patients with Oral Premalignant Disorders and Oral Cancer Patients and their comparison with Healthy Subjects.

OBJECTIVES

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3. REVIEW OF LITERATURE

3.1 ORAL CANCER:

Cancers of the lips, tongue, floor of the mouth, palate, gingiva, alveolar mucosa, tonsils, uvula, and salivary glands are included in the definition of oral cancer. Oral cancer (OSCC) is largely self-inflicted because it is a multifaceted disease; identifying the variables will provide the knowledge necessary for preventing the disease and planning therapy for a better prognosis. In the post antibiotic era, oral cancer remains the disease with the highest mortality rate in countries where tobacco use in the form of chewing and/or smoking, with or without alcohol consumption, carries the highest risk of developing oral cancer; it is the sixth most prevalent cancer in this region worldwide. Recent research indicates that OSCC is more prevalent among younger persons. **[22]**

Oral SCC in young people accounts for about 0.4–2.6% of the total incidence and has a slight predominance in men. The most common location for this tumor is the tongue and occurrence of symptoms is rare unless the lesion reaches a wide size. Concerning the etiological factors for oral SCC in young adults who do not smoke and drink alcohol frequently, genetic abnormalities seem to have a preponderant role in development of the tumor. Additionally, human papilloma virus infection, specifically by HPV-16 and HPV-18, are more frequently detected in this group, but more studies are needed to confirm its influence in prognosis and clinical outcome of oral SCC in the younger. [23]

Changes in the amino acids and proteins generated by cells are caused by mutations at the level of genetic DNA. Multiple mutations are required for the malignant transformation that causes a rise in cell proliferation in potentially malignant illnesses, and when the cell escapes growth regulation, it becomes autonomous and malignant. The defining hallmark of a basement break is a malignant tumor with distal lymphatic and blood lymph node metastases. The affected organ demonstrates malfunction and death. [24]

Oral cancer is a disease with a high mortality rate. It is the ninth most prevalent form of male cancer worldwide. Currently, the incidence of oral cancer is prevalent in emerging nations, particularly in the southern region of Central Asia. Oral squamous cell carcinoma (OSCC) is the third most prevalent cancer in India, with a yearly incidence rate of 52,000. Despite the availability of diagnostic and screening facilities, the incidence in affluent nations such as the United States is 13% annually, with 30,000 new cases. The majority of cases are detected at an advanced stage, resulting in a low 5-year survival rate. [25]

Oral squamous cell carcinoma (OSCC) is distinguished by significant immune cell infiltration and is considered a highly immunogenic tumor. The tumor microenvironment (TME) consists of an extracellular matrix (ECM), stromal cells, and immune cells that synchronize and interact with tumor cells. Tumor-associated macrophages (TAMs), regulatory T-cells (Tregs), cancer-associated fibroblasts (CAFs), and endothelial cells are examples of such cells. The innate immune cells consist of macrophages, dendritic cells (DCs), neutrophils, myeloid derived suppressor cells (MDSCs), natural killer cells (NKs), and innate lymphoid cells, while

the adaptive immune cells are T cells and B cells. Crosstalk between cells of the tumor microenvironment (TME), extracellular matrix (ECM), and tumor cells greatly contributes to the growth of tumors. In addition, cancer-related inflammation, more specifically chronic inflammation, involves inflammatory cytokines, chemokines, and growth factors that can cause DNA damage, tumor angiogenesis, and genomic instability, as well as the struggle between immunosuppression and promotion that results in tumorigenesis.

However, research suggests that not all cells convert into malignant cells; this is attributable to a process known as "immunosurveillance" or, more broadly, "cancer immunoediting." Cancer immunoediting refers to the immune system's ability to both inhibit and stimulate tumor development. Immunoediting for cancer generally consists of three phases: elimination (immunosurveillance), equilibrium, and escape. Cancer immunoediting is a generally acknowledged concept, and tumor immune evasion is regarded an emerging cancer hallmark. [26] The immune system can also promote tumor progression through chronic inflammation, immunoselection of poorly immunogenic variants, and suppressing anti-tumor immunity. Sometimes, tumor cell variants may not be eliminated but instead enter into an equilibrium phase in which the immune system controls net tumor cell outgrowth; in this phase, adaptive immunity constrains the growth of clinically undetectable occult tumor cells and edits tumor cell immunogenicity.

3.2 Etiology:

3.2.1. Tobacco

Tobacco's carcinogenic compounds, such as nitrosamines, benzopyrenes, and aromatic amines, make it the most significant risk factor and cause of oral cancer. Cigarette smokers are three times as likely to acquire mouth cancer than non-smokers. Secondary passive smoking situations also pose a threat to individuals. Studies have revealed a synergistic association between alcohol, tobacco use and cancer risk, resulting in a higher incidence of malignancy. In numerous countries, tobacco is chewed or held in the mouth rather than smoked. The desired effect is produced via nicotine absorption through the mucosal membranes. Due to direct contact with afflicted tissues, it is most strongly connected with oral cavity cancers, but it is also linked to oropharyngeal cancers. Betel quid, also known as 'pan' or 'paan,' is a mixture of betel leaf, areca nut, slaked lime, and tobacco that is chewed. Due to the prolonged exposure of oral cells to carcinogens, it is a popular habit in Asia and is associated with an even greater risk of cancer than smoking tobacco alone.

Snuff/Snus: A wet kind of smokeless tobacco that is typically inserted beneath the upper lip for extended durations. This approach is especially prevalent in North America and Scandinavia. Further, snus appears to be a viable alternative to smoking tobacco. The World Health Organization (WHO) declared snus a carcinogen in 1985 and it was banned in most of the European Union countries except Sweden. Recently, a tobacco product, Chaini Khaini, identified as snus appeared in India, marketed as a safe alternative to other tobacco products contains very high levels of carcinogenic

nitrosamines and biologically available nicotine. Interventions are urgently needed to educate current and potential consumers of this product. [27]

3.2.2. Alcohol

Alcohol consumption raises the risk of oral cancer, especially when combined with smoking. Alcohol has been shown to increase the permeability of the oral mucosa, making it more susceptible to damage by other carcinogens despite the absence of any carcinogenic properties in ethanol itself.

3.2.3. HPV

The Human Papillomavirus (HPV), particularly strains 16 and 18, has been linked to cancer. Although it is most strongly connected with cervical cancer and oropharyngeal cancer (especially tonsillar and base of tongue tumors), there is some evidence that it is also linked to oral malignancies. In the oral cavity, persons with squamous cell carcinomas are four times more likely to be infected with HPV than those with healthy mucous membranes. Most infections are transmitted through oral sexual contact. [28]

3.2.4. Stem Cell Transplants

Patients receiving hemopoietic stem cell transplantation are four to seven times more likely than the general population to develop mouth cancer. Frequently, oral cavity evidence of graft-versus-host disease precedes this. Mucositis, xerostomia, and lichenoid alterations are symptoms. 5 to 9 years following transplant, the most prevalent cancer sites are the tongue and salivary gland.

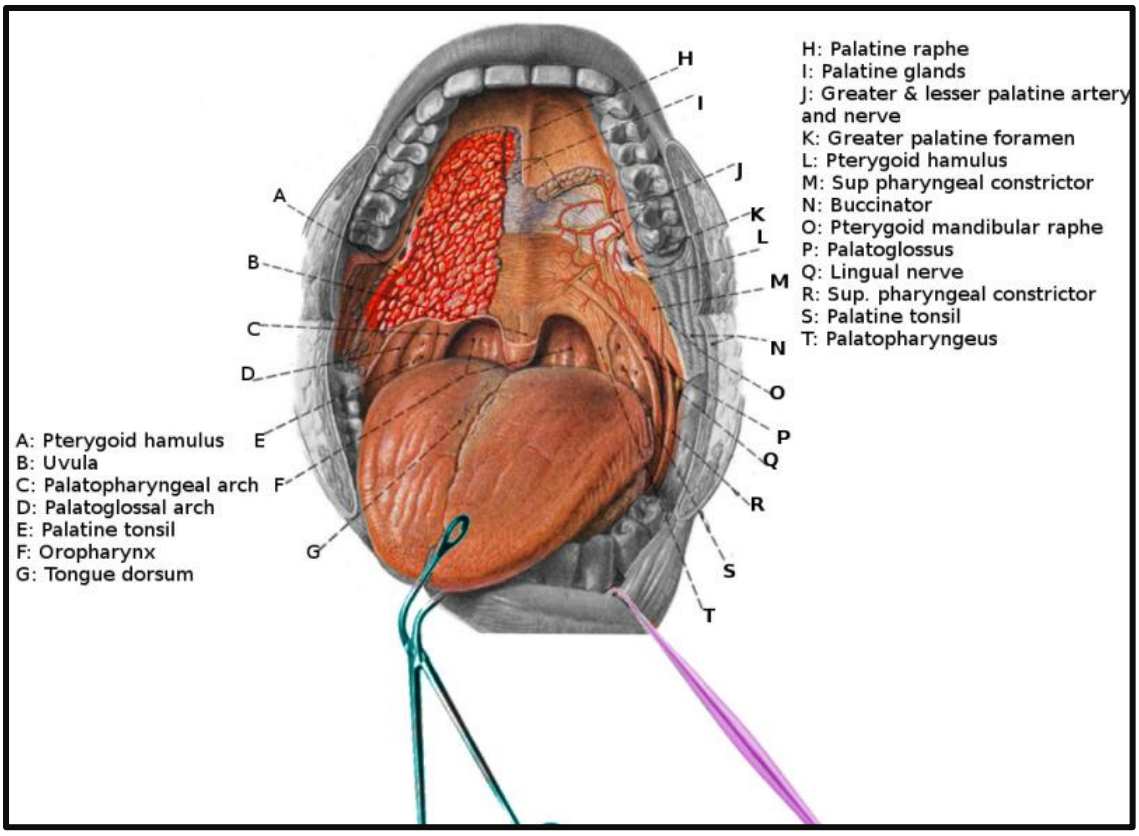


Figure 1 [29]***:*** *Oral cavity*

3.3. ORAL PREMALIGNANT DISORDER

3.3.1. Overview

A premalignant lesion of the oral cavity frequently manifests as an atypical area on the mucosal lining of the oral cavity, which can cause great concern in patients. Premalignant lesions can be discovered in any area of the oral cavity, and oral cavity anatomical landmarks include the lips, the mucosal lining of the face, the floor of the mouth, the anterior two-thirds of the tongue, the upper and lower gingiva (gums), and the hard palate. The specific etiology of premalignant lesions of the oral cavity is frequently diverse and may be linked to betel nut chewing, cigarette smoking, and alcohol drinking. Oral cavity precancerous lesions are observed in conjunction with and before to oral squamous cell carcinoma. According to the World Health Organization, potentially malignant illnesses are precancerous lesions of the oral cavity that may develop malignant transformation (PMD). Leukoplakia, Erythroplakia, lichen planus, oral submucous fibrosis, and actinic cheilitis are five lesions of the oral cavity classified as potentially malignant conditions. Preventing oral cavity squamous cell carcinoma requires early detection and timely treatment of premalignant lesions of the oral cavity. As a disordered mixing of dysplastic and non-dysplastic cells, oral cavity premalignant lesions are frequently difficult to diagnose based just on clinical signs.

The premalignant oral disease is an umbrella term for a multitude of disorders that can develop in the mouth cavity. Early detection and timely treatment are crucial for achieving good outcomes. However, there is still a substantial knowledge gap in this

field among medical professionals. A recent comprehensive analysis indicated that fewer than half of medical practitioners were aware of common risk factors for premalignant oral lesions or oral carcinoma. Consequently, there is substantial need to understand and recognize the appearance, etiology, and therapy of these disorders. [30]

The oral cavity comprises the lips, gums, buccal mucosa, gingiva/alveolar ridge, hard palate, the floor of the mouth, and the oral tongue (anterior 2/3). The vestibule consists of the buccal and labial sides of the teeth, as well as the mucosa of the alveolus and the wet margin of the lip. The bulk of the oral cavity is lined with stratified squamous epithelium, and abnormalities in this epithelium frequently give rise to premalignant oral lesions. Leukoplakia, Erythroplakia, lichen planus, and submucous fibrosis are the most often reported premalignant oral lesions. The following were designated as Potentially Malignant Disorders by the Oral Cancer Working Group of the World Health Organization:

• *Leukoplakia*

• *Erythroplakia*

• *Palatal lesion of reverse cigar smoking*

• *Oral lichen planus*

• *Oral submucous fibrosis (SMF)*

• *Discoid lupus erythematosus*

• *Hereditary disorders such as dyskeratosis congenita and epidermolysis bullosa.*

3.3.1.1. LEUKOPLAKIA

The word "leukoplakia" was coined in 1877 by Schwimmer of Budapest to describe whitish changes on the tongue that precede the development of lingual cancer in tertiary syphilis. It is the most frequent precancerous lesion and the PMD with the most research. The WHO working group defines leukoplakia as "a keratotic white patch or plaque that cannot be scraped off and cannot be clinically or pathologically distinguished from another disease." Consequently, the condition is diagnosed through a process of exclusion. [31]

Etiopathogenesis

The cause of leukoplakia is yet unknown. Numerous physical agents, including cigarettes, alcohol, prolonged friction, electro galvanic reaction between dissimilar restorative metals, and ultraviolet radiation, have been proposed. Tobacco smoking is the most widely established risk factor. Smokers are six times more likely to develop leukoplakia than nonsmokers. Clinically, leukoplakia can be differentiated into homogeneous **(Figure 2)** and nonhomogeneous types. The nonhomogeneous type is a white lesion that can be irregularly flat, speckled or nodular. Verrucous leukoplakia is an additional kind. Although verrucous leukoplakia typically has a white, uniform appearance, its verrucous texture distinguishes it from homogeneous type. Clinical characteristics of verrucous leukoplakia and verrucous cancer are identical. Proliferative verrucous leukoplakia (PVL) is a subtype of verrucous leukoplakia, distinguished by its multifocal presentation, resistance to treatment, and high rate of malignant transformation, seems more prevalent in elderly women.

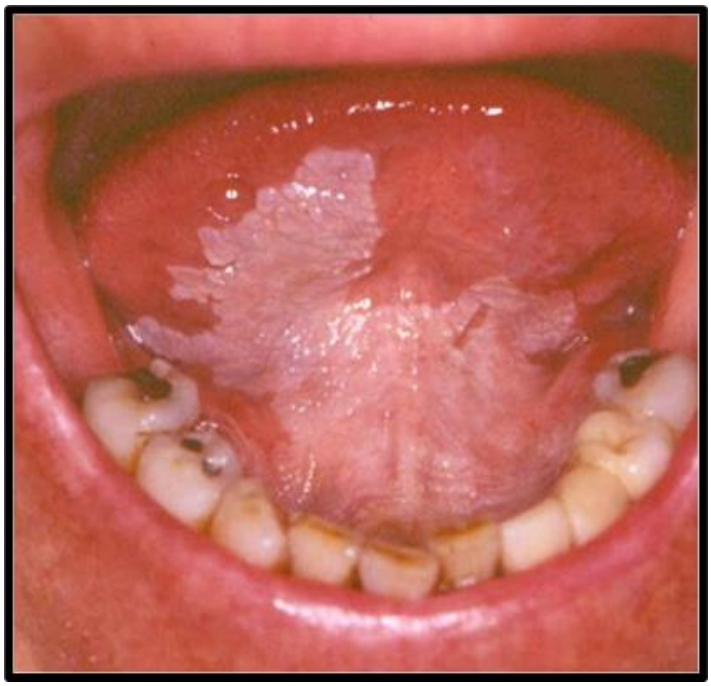


Figure 2 [30]***:*** *Leukoplakia of the ventral tongue and floor of mouth*

Table I: [31] **Risk factors associated with malignant transformation of the oral leukoplakia**

RISK FACTORS
Female sex
Long standing leukoplakia
Leukoplakia in non-smokers
Leukoplakia found on the tongue and/or floor of the mouth
Size of the leukoplakia >200 mm^2
Non-homogenous type of leukoplakia
Leukoplakia with presence of epithelial dysplasia

Various phases of leukoplakia have been described: [32]

Phase 1: Thin gray-white translucent plaques which are soft and flat.

Phase 2: Homogenous thick smooth or fissured leukoplakias.

Phase 3: Nodular or granular surface or verruciform leukoplakia

Phase 4: Erythroplakia, speckled leukoplakia, non-homogenous leukoplakia.

The possibility of malignant transformation of leukoplakias depends on multiple factors along with other risk factors. **(Table I)**

• Female gender

• Long duration of leukoplakia

• Leukoplakia in nonsmokers (idiopathic leukoplakia)

• Location on the tongue and/or floor of the mouth

• Size > 200 mm2

• Nonhomogeneous type

• Presence of *Candida albicans*

• Presence of epithelial dysplasia. [32]

3.3.1.2. ERYTHROPLAKIA

Erythroplakia is defined as "a flaming red patch or bright red velvety plaques that cannot be clinically or pathologically identified as any other disease."

Shear [33] suggested a classification of OE in 1972.

Classification [33]

(A) CLINICAL VARIATIONS

(1) Homogeneous Erythroplakia

(2) Erythroplakia interspersed with patches of leukoplakia

(3) Granular or speckled Erythroplakia (embracing the lesion described as speckled leukoplakia)

(B) MICROSCOPIC VARIATIONS

(1) Neoplastic

(a) Squamous carcinoma

(b) Carcinoma in situ (intra-epithelial carcinoma) and less severe forms of epithelial atypia

(2) Inflammatory

(a) Candida albicans infections (including denture stomatitis)

(b) Tuberculosis

(c) Histoplasmosis

(d) Miscellaneous specific, non-specific, and non-diagnosable lesions

Erythroplakia is less prevalent than leukoplakia, with a reported prevalence ranging from 0.02% to 0.83 %. It primarily affects those between the ages of 40 and 60. There

is no preference for either gender. This lesion has been dubbed "the dangerous oral mucosa" due to the fact that it generally appears as carcinoma in situ, severe epithelial dysplasia, or superficially invasive cancer under the microscope. In situations with a very high risk, such as lesions on the floor of the mouth in heavy smokers and alcoholics, eighty percent of these red patches may include microinvasive cancer foci at the time of the initial biopsy. Any region of the oral and oropharyngeal cavity may become affected, typically solitary. This solitary presentation is frequently useful for clinically separating Erythroplakia from erosive lichen planus, lupus erythematosus, and erythematous candidiasis, as these lesions nearly invariably occur bilaterally, symmetrically.

3.3.1.3. ORAL SUBMUCOUS FIBROSIS

Oral submucous fibrosis (OSMF) is a chronic condition characterized by fibrosis of the mucosal lining of the oral cavity, oropharynx, and frequently the upper third of the esophagus. With the exception of the earliest stages of the disease, the clinical appearance is characterized by fibrosis of the lamina propria and submucosa and a progressive loss of tissue mobility. **(Figure 3)**

Definition

An insidious, chronic potentially malignant fibrotic disorder affecting the entire oral cavity and sometimes the pharynx and esophagus. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial

inflammatory reaction followed by a fibroelastic change of the lamina propria with epithelial atrophy leading to stiffness of the oral mucosa, progressive decrement in mouth opening and inability to eat. [34]

OSMF is strongly related with areca nut chewing, which is the primary component of betel quid. Consequently, it is the most prevalent precancerous lesion that is exclusive to Southeast Asia and India. OSMF is caused by a variety of reasons, including areca nut consumption, chili pepper consumption, genetic and immunologic mechanisms, nutritional inadequacies, and others. It has been discovered that DR3 and DR7 are more prevalent in patients with OSMF.

Clinical manifestations

They are burning sensation, blanching and rigidity of the oral mucosa and oropharynx, and trismus. The patient may also experience pain and ulcerations in the oral cavity.

The most distinguishing trait is the creation of a prominent vertical fibrous ridge within the cheeks, as well as the board-like rigidity of the buccal mucosa. Fibrosis in the soft tissues causes trismus, feeding difficulties, and even dysphagia. Pathological characteristics include chronic inflammation, excessive collagen deposition in the connective tissues below the oral mucosal epithelium, local inflammation in the lamina propria or deep connective tissues, and degenerative muscle changes. OSF patients experience a severe burning sensation in the mouth after ingesting spicy foods. Other symptoms of OSF include dry mouth, pain, taste disorders, restricted tongue mobility, trismus, dysphagia, and altered tone. In advanced stages, fibrous bands form vertically in the cheeks, faucial pillars, and around the lips.

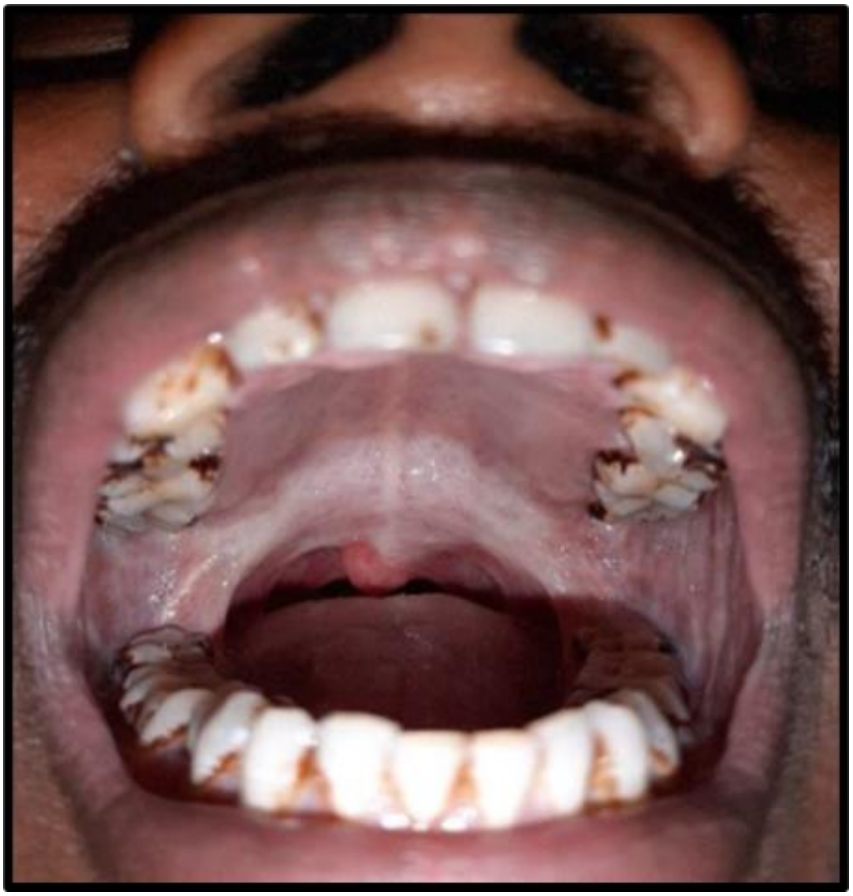


Figure 3 *[30]: Patient with oral submucous fibrosis showing whitish bands in the oral cavity*

The clinical presentation can be summarized into early and forms:

• Early forms are characterized by burning sensation exacerbated by spicy foods, vesiculation, blanching of mucosa, and leathery mucosa.

• Late forms are characterized by fibrous bands within the mucosa, limitation of mouth opening, narrowing of the oropharyngeal orifice with distortion of uvula and woody changes of the mucosa and tongue.

In India, 0–2% to 1.2% of the urban population accessing dental clinics are affected. There is a correlation between leukoplakia and oral cancer incidence and OSMF use. The reported frequency of malignant alteration ranges from 3% to 6%. The potential precancerous character of OSMF was initially recognized by Paymaster, who detected squamous cell carcinoma in one-third of his OSMF patients. [34]

Histopathologic features

Lamina propria fibrosis and hyalinization result in the atrophy of the underlying epithelium, which is caused by an unidentified process. In the presence of carcinogens, atrophic epithelium appears predisposed to squamous cell cancer growth. Masticatory muscle involvement and replacement with fibrous tissue were noted, pathologic changes in the blood vessels conspire to reduce perfusion in the affected muscles, which results in ischemia and necrosis of the muscles. This reduced perfusion of the muscles and their continued muscular activity can cause the further depletion of glycogen, which results in fibrosis. Due to the finding that tissue biopsies result in the creation of further fibrous scars and a worsening of symptoms, tissue biopsies are rarely performed.

3.3.1.4. ORAL LICHEN PLANUS

Lichen planus is an autoimmune condition of the skin and/or mouth membranes that typically affects middle-aged individuals but can occur at any age, with a significant tendency for females (M: F 1:2). Rarely with oral lesions also have skin lesions. It is an autoimmune disease caused by T cells in which autocytotoxic CD8+ T lymphocytes induce apoptosis of oral epithelial cells. Oral mucosal lichenoid lesions may develop following the treatment of systemic medicines, such as nonsteroidal anti-inflammatory drugs (NSAIDs), sulfonylureas, antimalarials, beta-blockers, and some angiotensin-converting enzyme (ACE) inhibitors. Varying intervals exist between the initiation of medication therapy and the clinical manifestation of oral lichen planus-like disease. The buccal mucosa, tongue, and gingiva are the most common sites, although palatal lesions are unusual; any oral area can be affected, with white streaks producing a reticular pattern like a spider web. The background membrane may be red, and the white lines may cause blisters and ulcers in certain individuals. Ulcers may have a metallic flavor and be somewhat painful. The bilateral and symmetrical nature of the lesions distinguishes them from erythroleukoplakia. Andreasen distinguished six kinds of oral lichen planus: reticular, papular, plaque-like, erosive, atrophic, and bullous. The reticular, papular, and plaque-like types are often painless and manifest as white keratotic lesions on the skin. In many instances, the erosive, atrophic, and bullous types are linked with a burning sensation and can cause considerable discomfort.The stated annual rate of malignant transformation is less than 1%. There are insufficient data to determine which kind of OLP is most likely to progress to squamous carcinoma, however some studies indicate a higher incidence in atrophic and erosive ulcerative varieties. The current absence of clinicopathologic

connection in the diagnosis further contributes to the muddle. Another entity, lichenoid dysplasia, contributes to the confusion. As there is no specific treatment to avoid malignant transformation, several experts advise close monitoring of patients.

3.3.1.5. NICOTINE STOMATITIS

Nicotine stomatitis is a hyperkeratotic thickening of the palate mucosa most commonly associated with pipe smoking or reverse smoking. The palatal mucosa gets thicker and hyperkeratotic, and its surface may develop fissures. Surface elevations with red centers, representing the inflamed apertures of the small salivary gland ducts, appear often. The term nicotine stomatitis is a misnomer because it is not nicotine that causes the alterations: rather, the extreme heat generated by smoking is responsible. Although nicotine stomatitis is a tobacco-related condition, it is not considered precancerous and is easily reversible with cessation of tobacco use.

3.3.1.6. PALATAL LESIONS IN REVERSE CIGAR SMOKERS

In several Southeast Asian and South American countries, the lit end, or the burning end of the cigarette or cigar is placed inside the mouth, a practice known as reverse smoking.

This behavior results in a more severe heat-related modification of the palatal mucosa known as reverse smoker's palate, which has been linked to an elevated risk of malignant transformation. [35]

3.3.1.7. ACTINIC KERATOSIS

Actinic keratosis is regarded as a potentially cancerous disorder that can manifest in numerous locations, including the lips. It is frequently related with sun exposure. The annual progression rate of actinic keratosis to invasive carcinoma ranges between 0.025 and 16%.

Actinic cheilitis is the clinical term for an ulcerative, crust-forming lesion of the mucosa of a portion or the complete vermilion border of the lower lip. The histology might range from hyperkeratosis with or without epithelial dysplasia to squamous cell carcinoma in its earliest stages.

3.3.1.8. TOBACCO POUCH KERATOSIS

The use of smokeless tobacco, such as snuff or chewing tobacco, is associated with a distinct modification of the oral mucosa. Typically, these lesions manifest in the buccal or labial vestibule where the tobacco is kept, although they can also affect the neighboring gingiva and buccal mucosa.

It is expected that 15 percent of chewing tobacco users and 60 percent of snuff users will have clinical lesions. Hyperkeratosis and acanthosis of the mucosal epithelium are visible under the microscope in smokeless tobacco keratoses. True epithelial dysplasia is uncommon; when identified, the dysplasia is typically minor.

Within two to six weeks following cessation of tobacco use, the majority of tobacco pouch keratoses are reversible.

HEREDITARY DISORDERS WITH INCREASED RISK OF MALIGNANT TRANSFORMATION

The disorders Dyskeratosis Congenital (DC) and Epidermolysis Bullosa are associated with an increased risk of oral cancer. They are rare inherited disorders. The majority of X-linked DC cases affect males. Patients with DC frequently acquire white plaques on the dorsal tongue that may be confused with leukoplakia; however, the absence of habits and their youth may indicate that this illness is inherited. There is a report of malignant alteration inside the white patch regions. In Xeroderma pigmentosum and Fanconi's anemia, malignancies, including mouth cancer, are more prevalent. [36]

3.3.2. DIAGNOSIS

Based on history, clinical symptoms, clinical examination and histological analysis, oral cavity premalignant lesions are typically identified. Brush cytology/biopsy is a minimally invasive procedure in which a brush is used to obtain a complete trans-epithelial specimen as opposed to only exfoliated surface cells. As this is a trans-epithelial approach that requires sampling the basal, intermediate, and superficial layers of the lesions, it cannot be used to discriminate carcinoma in situ from invasive carcinoma. The sensitivity of this approach ranges from 73% to 100%, and its specificity from 32% to 94%. 36 On the basis of the histological findings, leukoplakia can be classified as dysplastic or non-dysplastic.

The diagnosis is confirmed by a biopsy that also measures the severity of dysplasia. The histopathology of Erythroplakia reveals moderate or severe dysplasia. In actinic

cheilitis, histological examination reveals hyperplasia, acanthosis, or atrophy of the epithelium, keratin layer thickness, and/or mild to moderate dysplasia. In addition to these epithelial alterations, connective tissues exhibit a basophilic degeneration of collagen fibres known as sun elastosis. In OSMF, chronic inflammatory cells including eosinophilic components infiltrate the sub-epithelial tissues, as determined by histopathology. Long-standing lesions are characterised by diminished vascularity, fewer inflammatory cells, and the deposition of dense sheets of collagen immediately under the epithelium. Frequently, the diffuse hyalinization of the subepithelial stroma spreads into the submucosal tissues, which replace the fatty and fibrovascular tissues. Narrow band imaging (NBI) detects premalignant and malignant lesions with a high degree of precision. NBI aids in the identification of premalignant and early malignant illnesses of the oral cavity, as well as the evaluation of tumor invasion. [37]

3.3.3. TREATMENT

Diagnosis and treatment of premalignant lesions of the oral cavity can prevent the development of squamous cell carcinoma of the oral cavity.**[38]** If leukoplakia is treated in its earliest stages, the risk of developing oral cancer can be decreased. Although there are several therapeutic options for leukoplakia, none are suitable for limiting the malignant development of the condition. The choice of treatment options relies on the leukoplakia's clinical stage. The technique of treatment is determined by the presence or absence of dysplasia as determined by histological analysis. In the early stages of leukoplakia, the treatment technique tries to eliminate potential etiological factors, such as cigarette and alcohol cessation, as well as the elimination of mechanical and chemical damage. Two to four weeks are required for the

surveillance of leukoplakia. If the lesion does not improve during the observation period following discontinuation of the etiological causes, a histological diagnosis must be performed. The choice of treatment is determined by the presence or absence of dysplasia in oral cavity leukoplakia. In the absence of improvement or in the presence of dysplasia, surgical therapy is required. [38]

3.3.4. MANAGEMENT

The majority of PMD are asymptomatic, and the primary goal of treatment is to prevent or diagnose malignancy early on. There are three therapy options for PMD:

- Close observation,
- Surgical excision/ablation, and
- Medicinal treatment

Observation: There are patients with early, tiny, clinically benign-appearing lesions at advantageous places can be noted. The patient must be informed of the need for frequent follow-ups and the likelihood of malignant change.

The standard therapy for leukoplakia continues to be a surgical resection with negative margins. Surgical resection is undertaken to eliminate areas with a high likelihood of developing an early carcinoma or undergoing an early malignant transformation.

The attempts to eliminate all clinically apparent areas of leukoplakia or histologically identified areas of dysplasia are unfeasible in many cases. They can often result in more morbidity than benefit to the patient due to scarring and contracture. This difficulty to properly eliminate all precancerous areas with surgery is a result of the

vast "field effect" prevalent in the oral cavity, It is due to this effect, which exposure to carcinogens induces premalignant alterations over large areas of mucosa. In addition, excision i.e., surgically removing of the entire patch irrespective of its size can be associated with high rates of recurrence. In certain trials, however, excision of leukoplakia did not reduce the rate of malignant transformation.

Ablation using a laser has also been promoted for the elimination of Oral Premalignant Disorders. This method has the potential advantage of being non-invasive technique, quicker healing process, less scarring, but the absence of a resected specimen for histopathologic and genetic investigations is a major disadvantage. However larger scale studies are still essential to conclude an extensive efficacy of laser therapy and other effects of it.

Photodynamic therapy is another ablative treatment being researched for PMD. Intravenously or topically, a photosensitizing drug, such as hematoporphyrin derivatives or 5-ALA, which targets neoplastic cells preferentially is delivered. The targeted tissue is then subjected to a certain wavelength of light, which activates the photosensitizer and causes it to transfer energy to molecular oxygen, resulting in the formation of reactive oxygen species and consequent tissue damage. Several modest clinical studies have demonstrated good outcomes for the treatment of premalignant epithelial lesions. [39]

3.3.5. PROGNOSIS

'Early detection and treatment are among the leading solutions for preventing disease progression, improving patient quality of life and survival, and reducing morbidity. Oral potentially malignant disorders (OPMDs) comprise a group of oral mucosal diseases with different morphological characteristics that can progress to oral squamous cell carcinoma (OSCC). Given OSCC's poor prognosis and high mortality, early diagnosis is a priority step in OSCC. Development of potentially malignant disorders and oral SCC are multistep processes involving genetic changes due to exogenous or indigenous factors. There is currently no molecular or histopathological pathognomonic hallmark that can predict the malignant transformation of potentially malignant disorders. The analysis of the clinical aspects of these lesions remains the best way to control and prevent the development of oral SCC. Thus, accurate diagnosis and timely treatment may help prevent the transformation of potentially malignant disorders into OSCC.

Early treatment of oral cancer will increase patient survival, improve quality of life, and result in less morbidity and a better prognosis. To reach this goal, early detection of malignancies using technologies that can be used in remote and low-resource areas is desirable. There is a link between the degrees of dysplasia and malignant cell transformation. In epidemiological studies assessing the risk of OPMDs in India, it has been found that 80% of oral cancers were preceded by OPMDs. OPMDs management focuses on preventing risk factors and surgical excision of lesions with moderate and severe epithelial dysplasia. Non-surgical approaches involve the use of systemic and topical medications.

Early lesions screening is essential to ensure timely diagnosis or follow-up in case of confusing lesions to minimize the malignant transformation. Prognosis and patient survival is directly related to the stage and grade of cancer at initial diagnosis. The natural history of OPMDs has no consistent pattern, and it is difficult to predict who may develop cancer following the detection of an OPMD.

Most cancers tend to arise during the first two years after the detection of an OPMD, but follow-up studies indicate that the risk may continue to exist for 10–15 years. The high-risk OPMDs include Erythroplakia, erythroleukoplakia, proliferative verrucous leukoplakia, and oral submucous fibrosis, and risks are lower in homogeneous leukoplakia and in oral lichen planus. Though most OPMDs are asymptomatic at the time of presentation, careful observational studies during periods of follow-up indicate that around the time of malignant transformation, some patients may experience symptoms such as increased redness, ulceration, or tingling sensation and pain indicative of suspected malignancy.

The management protocols should therefore provide immediate access for a patient with an OPMD to consult a senior clinician in the follow-up team in such situations. A re-biopsy is indicated to detect any malignancy in an early stage or to plan its exclusion. An early diagnosis and management can, at the most, decrease the chances of turning to malignancy and may also cease it, ultimately not posing any threat to future malignancies. Patients diagnosed with an OPMD should have sufficient access to healthcare facilities for regular follow-up.

3.4. IMMUNE PROFILING OF ORAL CANCER

In the 8th edition of The American Joint Committee on Cancer (AJCC) Staging Manual's "Head and Neck", significant improvements have been made to the staging of oral cancer. Despite this, tumors of the same stage are observed to be heterogeneous in responsiveness to therapy and aggressiveness. In terms of predicting progression and overall survival, the incorporation of immunological contexture can provide information that is comparable or even superior to TNM staging. The genomic and molecular signatures of oral cancer and of the infiltrated immune system are focused on identifying key biological molecules that may be linked to cancer development, risk assessment, screening, predicting recurrence, prognosis, and invasion/metastasis, and are significant determinants of immunotherapeutic response. The significance of immune profiling is attributable to the fact that patients belonging to distinct molecular subgroups may respond optimally to different treatments. In addition, technological advancements have changed immune profiling, which has shifted from analyzing a single marker using immunohistochemistry (IHC) to sequencing a vast number of immune-related genes. This portion of the article highlights the significance of profiling TME and tumor cells, their biomarkers, identifying immune cell-associated genes and proteins, and the techniques applied for immunological profiling in oral cancer. [40]

3.5. IMMUNOGLOBULINS

Immunoglobulin (Ig) molecules consist of two immunoglobulin heavy (IgH) chains and two immunoglobulin light (IgL) chains connected by disulfide bridges to produce a twofold symmetric structure. IgH chains are separated into five isotypes: Igμ, Igγ,

Igα, Igδ and Igϵ, as well as four subclasses of Igγ (Igγ1, Igγ2, Igγ3 and Igγ4) and two subclasses of Igα (Igα1 and Igα2). IgL chains contain two distinct isotypes: Igκ and Igλ. Variable (V) regions at the N-terminus of the IgH and IgL chains are very variable in their sequences and are responsible for antigen recognition. In contrast, the C-terminal sections of the IgH and IgL chains are sequence-constant and are hence referred to as constant (C) regions. The creation of V region diversity is crucial for functional Ig expression and the capacity of Ig molecules to recognize a variety of antigens. This process involves multiple molecular processes, including V(D)J recombination, somatic hypermutation (SHM), and class-switch recombination (CSR). Ig molecules are essential components of humoral immune responses. By neutralizing antigens and mediating antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP), these molecules serve as antibodies in immunological defence (ADCP).

B cells are believed to be entirely responsible for Ig production of the five known classes (IgA, IgM, IgG, IgD, and IgE). Igs of the classes IgG, IgA, and IgM, as well as their respective mRNAs, were detectable in the cytoplasm and supernatant of malignant non-B cells.

Due to their neoantigenic properties, tumor-specific V(D)J patterns can be used not only as biomarkers but also as immuno-therapy targets, thanks to the distinctiveness of the expressed Ig repertoire. In addition to Igs directly derived from solid tumors, a disproportionately high number of malignant plasma cells may be the source of unusual Igs. Plasma cell neoplasms are characterized by an abundance of benign or

malignant plasma cells. Monoclonal gammopathy of unknown significance (MGUS) and multiple myeloma are typical neoplasms. B cells generated from MGUS release copious amounts of monoclonal Igs. Multiple myeloma is known to originate from MGUS and expresses 1% of specific Ig types. These malignant B cells produce predominantly IgG, followed by IgA, single free light chains (Bence Jones myeloma), and IgD, all of which are detected in serum or urine testing. In addition to proteinuria, renal insufficiency and amyloidosis are caused by an abnormal number of Igs. Patients' immunodeficiency is caused by the relative decrease of polyclonal antibodies. [42]

3.5.1. Serum immunoglobin; IgA

IgA is the most prevalent antibody on mucosal surfaces and the second most prevalent antibody in serum (~15%; 2–3 mg mL^{-1}), behind IgG (80%; ~10–20 mg mL^{-1}). More IgA is produced daily than all other antibody isotypes combined (66 $mgkg^{-1}day^{-1}$), however serum IgA is rapidly degraded, resulting in a relatively short half-life (4–6 days). IgA is distinguished from other antibody isotypes by its N-linked glycan and disulfide bridge configurations. **(Figure 4)** The fragment antigen-binding region (Fab) is required for antigen binding, neutralization, and opsonization, whereas the Fc region is crucial for beginning innate immune effector actions. IgA is composed of two heavy chains and two light chains, each of which is folded into several globular domains, including four heavy-chain domains (VH, Cα1, Cα2 and Cα3) and two light-chain domains.

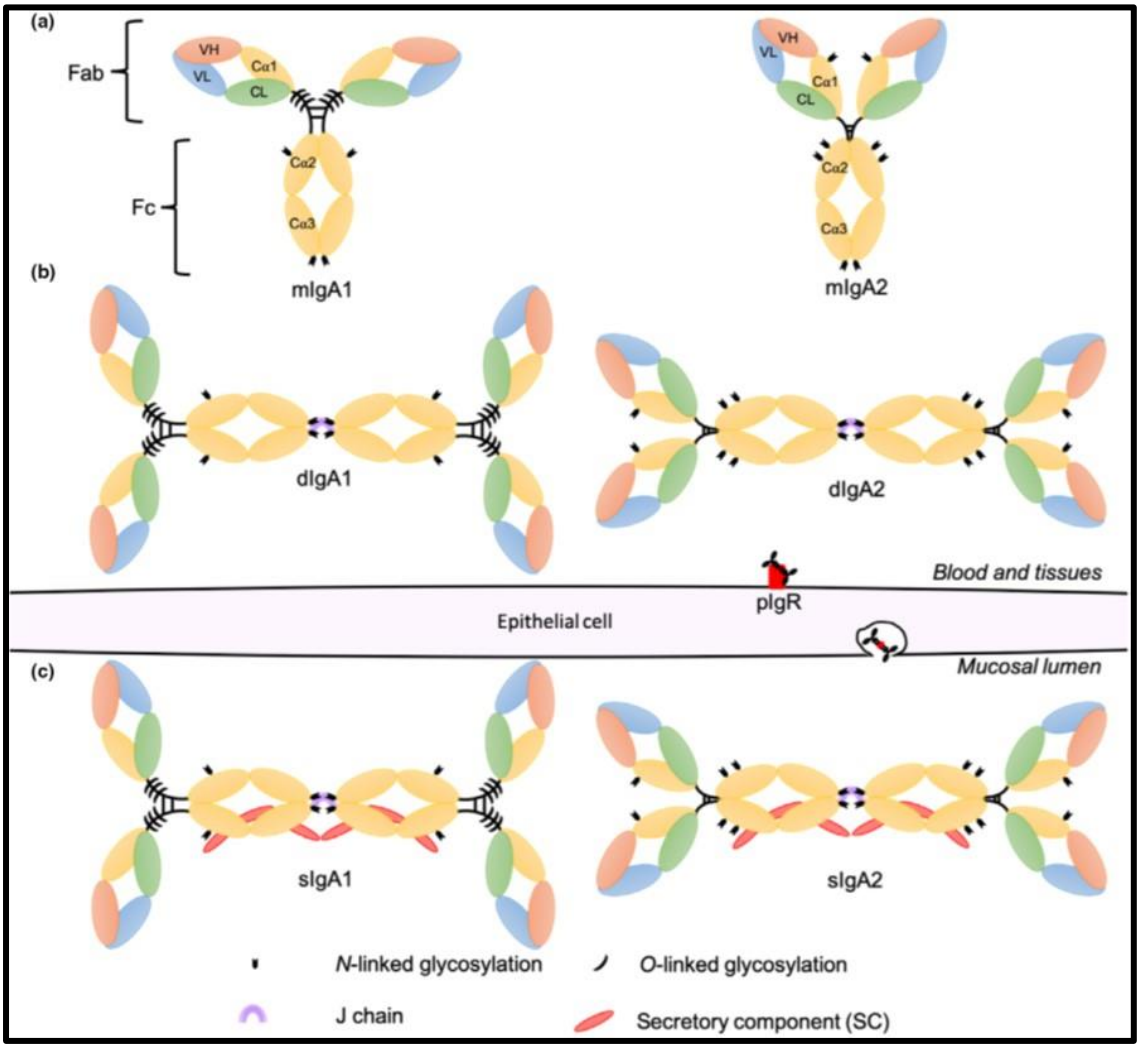


Figure 4 [43]*: Schematic diagram of immunoglobulin A (IgA) subclasses IgA1 and IgA2, glycosylation patterns and their respective heterogenous molecular forms.*

Humans possess heterogeneous IgA molecule forms including monomeric (mIgA), dimeric (dIgA), polymeric (pIgA), and sIgA. In addition to IgA subclasses, these molecular forms are variably dispersed throughout body compartments. IgA is predominantly mIgA1 (90%) in serum, which is produced in the bone marrow and transferred to the blood. In contrast, the protease-resistant hinge region results in a proportionate rise of IgA2 in the majority of mucosal secretions. In addition, mucosal IgA is generated locally as dIgA in organised gut-associated lymphoid tissues, where IgA2 plasmablasts home to specific sites. dIgA undergoes transcytosis across epithelial cells and into the mucosal lumen via polymeric immunoglobulin receptor. [41]

Historically, IgA was regarded as a noninflammatory antibody due to the role of sIgA in the downregulation of proinflammatory responses to pathogens and food antigens by preventing binding to other Fc receptors, rather than by activating anti-inflammatory pathways such as those described in a later section. In sIgA-deficient patients, where an increased risk of autoimmunity and allergy is found, the importance of sIgA as a noninflammatory antibody is underlined. Extensive research on mucosal secretions supports the function of sIgA in the passive and maybe active immune protection of neonates in colostrum and breast milk IgA. In addition, adult sIgA maintains microbiota diversity and growth equilibrium and contributes to passive defence against invading pathogens. Comparatively, the function of serum IgA (mIgA, dIgA, and pIgA) is comparatively unexplored.

Serum IgA and FcαRI: Human FcαRI (CD89) is constitutively expressed on monocytes, eosinophils, certain macrophages, intestinal dendritic cells, Kupffer cells, and neutrophils, which are the most prevalent blood cells expressing FcRI. FcαRI has a ligand-binding chain that maps to chromosome 19 alongside the genes for natural killer cell receptors (KIR) and leukocyte immunoglobulin-like receptors, in contrast to IgG (FcγR) and IgE (FcεR), which map to chromosome 1.

The FcαRI α chain contains two extracellular domains resembling immunoglobulins, a transmembrane region, and a short cytoplasmic tail devoid of recognized signaling patterns. FcαRI must connect with the immunoreceptor tyrosine-based activation motif (ITAM), which can be phosphorylated to activate signal transduction, for signaling to occur. 1 IgA:2 FcαRI are required for the binding of monomeric serum IgA Cα1 and Cα2 Fc domains to the membrane distal domain of FcαRI. In addition, two FcαRI single-nucleotide polymorphisms have been discovered in humans: Ser248/Gly248 and Asp92/Syn92. Gly248 FcαRI has been linked to an increase in the proinflammatory potential of serum IgA, whilst Asn92 FcαRI has been linked to an increased risk of myocardial infarction. [42] **(Figure 5)** There are no recorded cases of low or no FcαRI expression on myeloid cells in humans, in contrast to FcαRI abnormalities that correlate with susceptibility to autoimmunity, chronic inflammation, and infection, underlining the potential role of FcαRI in homeostasis and inflammation in humans. It is essential to note, however, that IgA deficiency in humans has been linked to increased vulnerability to viral illness and autoimmune.

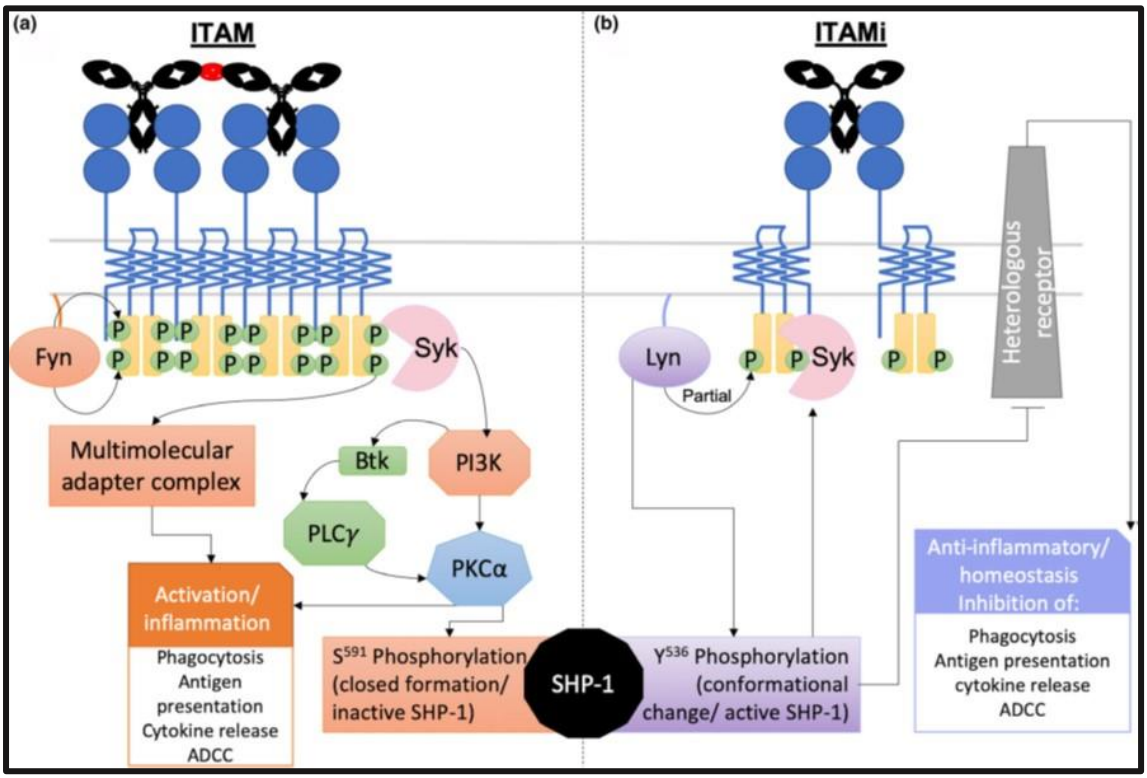


Figure 5 [43]*: Initiation of immunoglobulin A (IgA)/Fc alpha receptor I (FcaRI) immunoreceptor tyrosine-based activation motif (ITAM) and ITAM inhibitory (ITAMi) signal cascades and resulting Fc effector functions*

IgA appears to be potent in the recruitment and activation of neutrophils via the FcRI to kill tumors, making it an attractive target for mAb antitumor therapy. Most research has focused on IgG in mAb therapy due to its potent antitumor mechanisms, such as complement activation and natural killer cell mediated ADCC. Modifications to IgA mAbs can enhance their half-life as well as stability. Combinations of IgG and IgA mAbs can increase tumor killing, and "cross-type antibodies" such as IgGA and tandem antibodies combine the best anticancer effects of IgG (complement binding) and IgA (cytotoxicity/phagocytosis). [43]

3.5.2. Serum immunoglobin; IgG

Immunoglobulin G (IgG) is generated as a result of daily allergen exposure. Peanut, milk, and other natural allergens induce antibody responses, notably IgG, upon inadvertent intake, and every individual has a unique and varied repertoire of antibodies to their food and environment.

Immunoglobulin G class or subclass antibodies are correlated with allergies and Th2 cytokine responses to allergens that sensitize the immune system. Several autoimmune inflammatory disorders, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), are characterized by elevated immunoglobulin G antibodies, in which the pathology recapitulates the immunological damage. [44] In a few instances, the expression of complete IgG, or at least the expression of entire Ig chains, was also observed in noncancerous, nonlymphoid tissues, such as normal human lung and colon tissues. **(Figure 6)**

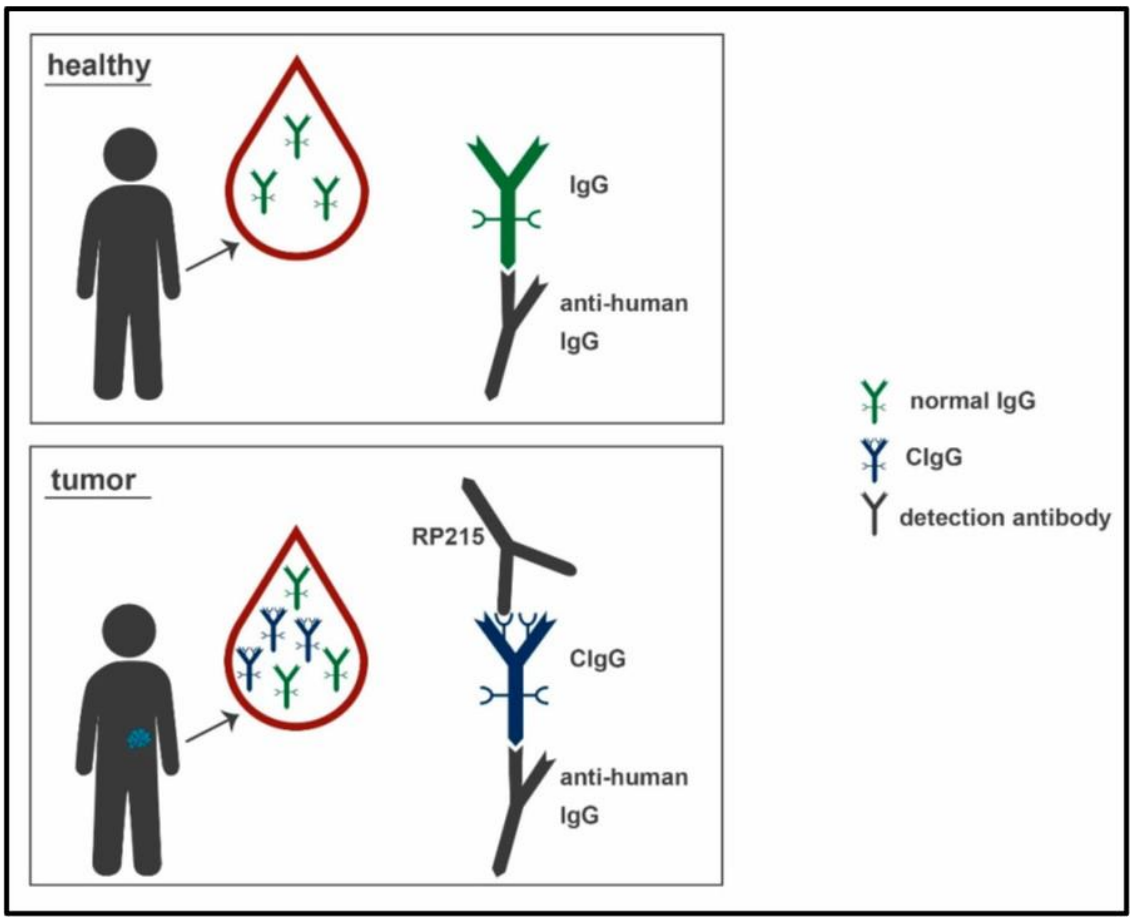


Figure 6 [44]*: Human IgG (green) circulates in the bloodstream of healthy individuals and tumor patients, while CIgG (blue) is only present in the blood of tumor patients. Anti-human IgG antibodies (grey).*

These non-lymphoid and non-cancerous Igs have unknown roles. Similarly, the precise function of cancer-derived Igs (CIg) remains largely unknown; however, there is evidence of a cancer-promoting effect. Inhibiting the generation of cancer-derived IgG (CIgG) with anti-human IgG antibodies and siRNA results in an increase in tumor cell death, as well as a reduction in colony formation and invasion. The significant difference between Igs produced by B cells and CIgs produced by cancer cells appeared as restricted patterns of V(D)J recombination in CIgGs and Igs of normal tissue controls relative to IgGs produced by B cells. RP215, an antibody that detects CIgG selectively and has a potent anticancer effect in vivo, makes it possible to differentiate B-cell-derived IgG from CIgG.

Multiple research groups discovered IgG expression in tumor tissues originating from various organs. Regarding the peritumoral and possibly systemic effects of CIgG, very little is understood. Some evidence points to a mechanism of action for CIgG on T cells involving sialic acid-binding immunoglobulin-type lectins (siglecs). It is believed that they are more essential in the immediate tumor microenvironment than in the circulation. CIgG has also been shown to have direct effects on platelets. **(Figure 7)** This suggests not only direct impacts, but also systemic ones. Several groups have studied the impact of platelets on cancers. In this instance, platelets envelop circulating tumor cells, protecting them from NK-cell-mediated cytolysis. For stable adherence, tumor cells can release platelet-activating substrates such as ADP and proteins such as thromboxane A2 or high-mobility group. By binding toll-like receptor 4, these substances trigger activation. Platelets can also control tumor development, angiogenesis, and metastasis. They impact tumor cells because of their

high number of surface receptors and secreted chemicals, which include thromboxane, platelet-derived growth factor, and vascular endothelial growth factor. Upon activation, P-selectin (CD62P) migrates from an internal site to the cell surface. Blocking CD62P decreased metastatic colon cancer foci in the lungs of mice and decreased platelet binding to tumor cells, suggesting that CD62P is directly implicated in the protumoral characteristics of platelets. [45]

3.5.3. Serum immunoglobin; IgM

IgM, which has μ heavy chains, is the initial class of antibody generated by and appearing on the surface of a developing B cell, despite the fact that many B cells subsequently switch to other classes. It is also the principal type of antibodies released in the blood during the initial phases of a primary antibody response to an antigen. IgM is an organism's initial line of defence. IgM is a pentamer composed of 5 4-chain units in its secretory form, giving it a total of 10 Ag-binding sites and a higher valency than the structures of other immunoglobulins (Igs), allowing it to bind Ags with high avidity. Each pentamer comprises one copy of a J (joining) chain, which is another polypeptide chain. IgM controls B cell formation, promotes the removal of apoptotic cells, affects inflammatory reactions and autoimmune disorders, and mediates the elimination of cancer cells. [46]

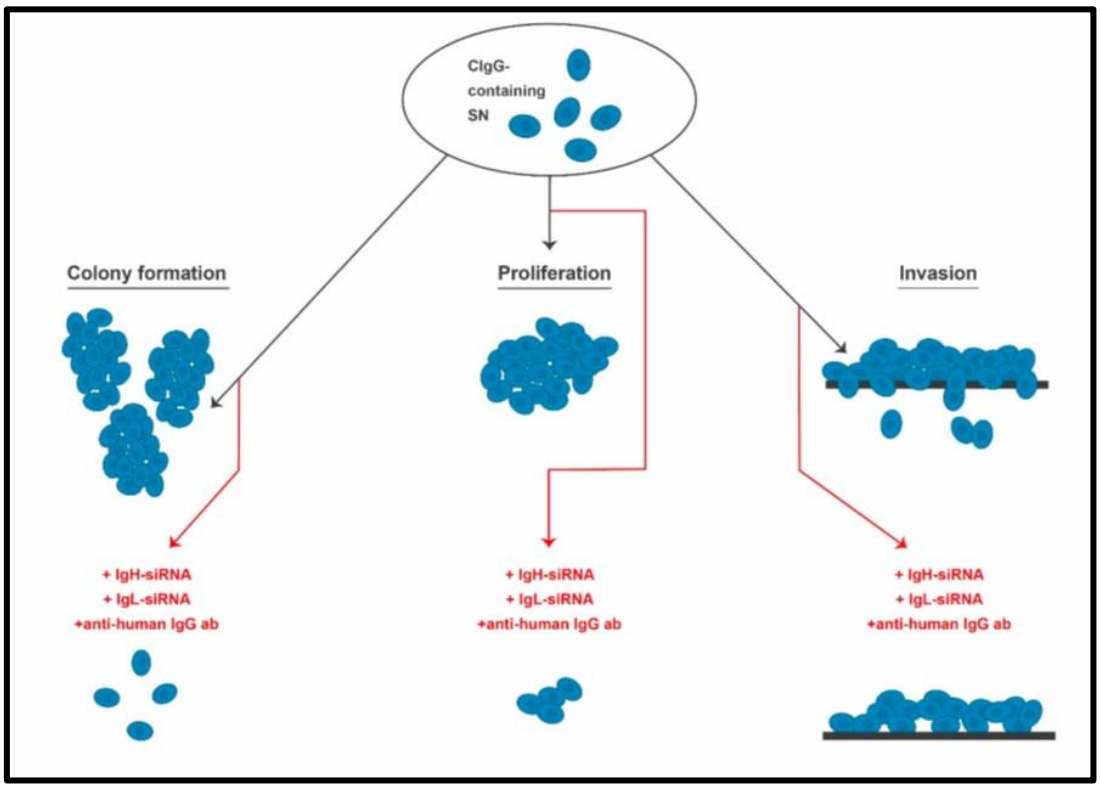


Figure 7 [45]***:*** *CIgG expression increases the cancer cells' abilities in terms of colony formation, proliferation, and invasion.*

The complement system is initiated by the binding of Ag to a single, pentameric, secretory IgM molecule. This activation designates pathogens and altered cells for phagocytosis or kills them directly when the Ag lies on the surface of an invading pathogen, senescent cells, cell debris, or precancerous or cancer cells.

Natural IgM antibodies

Natural Abs are primarily IgM, with smaller amounts of IgA and IgG, are polyreactive, and have a low affinity. Without exogenous antigenic stimulation or Ag-driven selection, IgM circulates naturally in healthy persons. Serum IgM concentrations of infants and animals raised in sterile circumstances on an Ag-free diet are identical to those of normal animals. IgMs are also present in humans. IgM has a crucial role in the major defensive systems. Reacting with cell surface receptors and identifying and eliminating apoptotic and senescent cells, cell debris, and self-Ags, they participate in the early recognition and clearance of bacterial and viral invaders and changed self-material from an organism. Natural IgM Auto-Abs assist in inhibiting pathogenic IgG auto-Ab responses.

IgM is linked to the identification and elimination of precancerous and cancerous cells. Identifying the conserved structures of carbohydrate epitopes, natural IgM binds preferentially to tumor-specific, post-transcriptionally changed cell surface Ags, recognizing their carbohydrate epitopes. Carbohydrate epitopes identified by natural IgM are persistently produced in numerous tumor precursor stages.

In contrast to epitope-based single-peptide chains, glycoepitopes share structural homologies that extend beyond the protein families; hence, they can cross-react and are the preferred targets for natural IgM Abs. Produced by a limited subset of B1 cells (CD5+) and B cells in the marginal zone (MZ), natural IgM does not require affinity maturation to give early protection. Germline-encoded IgM antibodies are not affinity-matured.

Adaptive IgM antibodies

Adaptive IgM is the first antibody to arise in response to an immunological challenge, although its production typically declines as IgG develops. As a result, IgM is not widely thought to have a large role in long-term immunity, despite its effectiveness in host defence. Typically, durable humoral immunity is coupled with the formation of high-affinity Ab and isotype switching. Immunization-induced IgM varies from naturally occurring IgM in terms of its structure in the Ag-binding centres, affinity, specificity repertoire, and spectrum of functions. These IgM Abs represent a small proportion of the circulating molecules; they are monoreactive, have a high affinity (10-7 to 10-11 mol-1), and their variable regions contain point mutations. 35 hours is the half-life of monoreactive IgM. [47,48] **(Figure 8)**

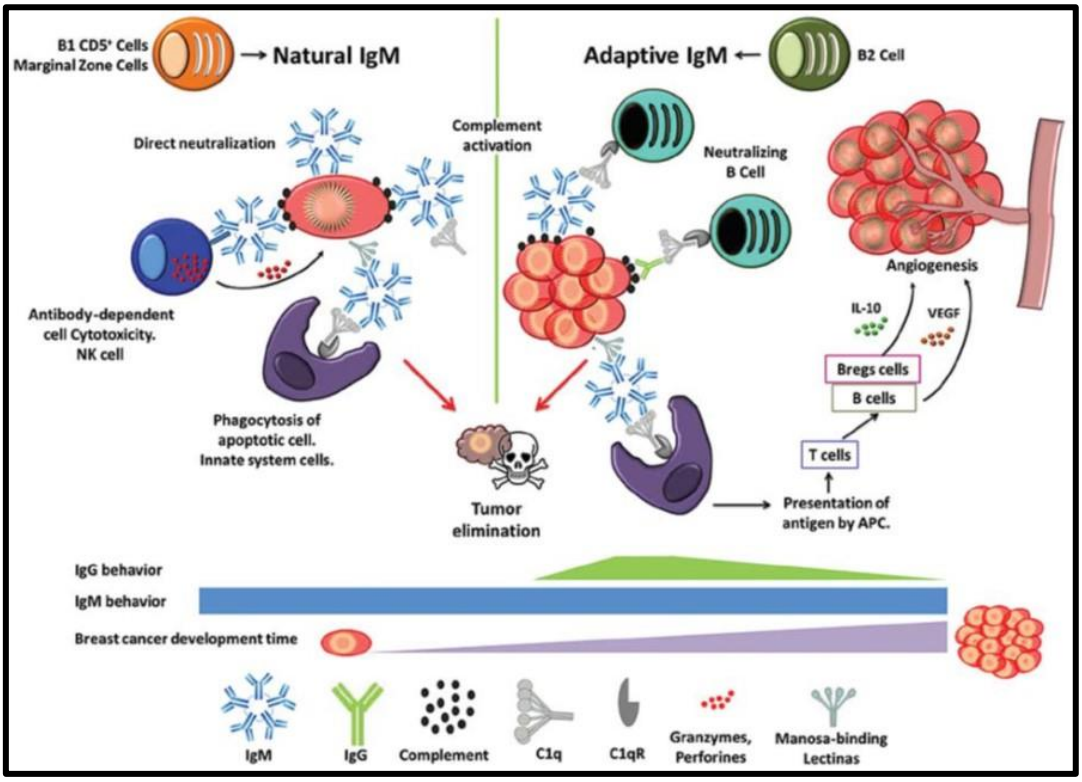


Figure 8 [47]***:*** *Natural IgM is produced by B1 cells and marginal zone cells, and adaptive IgM is synthesized by B2 cells.*

3.6. KEY BIOMARKERS

3.6.1. Immune and Non-Immune Cell Markers

OSCC is associated with significant differences in the amount of T cells, MDSCs, TAMs, and numerous other immune and non-immune cells. This profile of cells with biomarkers can not only aid in the diagnosis and prognosis of oral cancer patients, but it can also identify parameters for the optimal immunotherapeutic strategy. CD11c, CD80, and HLADR are some of the hallmark immune cell biomarkers utilized in OSCC investigations to detect M1 TAMs, while CD163, CD11b, CD206, and MRC1 are used to detect M2 TAMs. CD68 is a pan macrophage marker, despite not being specific. Several markers, including S100, CD1a, CD83, CD207, CD208, CD80, CD11c, CD86, and HLA-DR, are used to detect DCs, but the most frequently accepted markers are CD1a for immature DCs and CD83, which has been proven to be expressed on activated and mature DCs.

The T-helper cell markers CD4 and Tregs, a subset of CD4+ cells, are utilized to identify OSCC, as are several other markers. CD25, CD127, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), lymphocyte activation gene-3 (LAG3), and fork/winged-helix transcription factor box P3 are involved in this process (FOXP3). Nevertheless, the most well acknowledged Treg markers are CD4+ CD25+ FOXP3+ and CD4+ CD25+ CD127 low. Pan B cell markers include CD19 and CD20, and CD19+ B cells have been identified as a predictive factor for oral tongue squamous cell cancer (OTSCC). Other cells include MDSCs, which lack consistent markers but have been identified as expressing CD33 and CD11b; CAF, which express SMA; and endothelial cells that express CD31 and CD34. [49] **(Figure 9)** Inflammatory

Biomarkers There is evidence that chronic inflammation plays a major role in the development of OSCC. The creation of inflammatory mediators serves to activate the immune system against the tumor, but inflammatory mediators can also encourage tumor growth and could be used as potential biomarkers. In malignancies, the systemic inflammatory response (SIS) has been the subject of extensive research. Neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and C-reactive protein (CRP) are the most often used biomarkers for SIS evaluation. According to a study conducted by NLR, disease-specific survival in young OSCC patients is also substantially associated with NLR. CRP is an early indicator of inflammation, and it was found that the preoperative serum CRP level effect was more pronounced in oral cancer and served as a prognostic indicator in OSCC. The nuclear factor kappa-beta (NF-B) plays an important function in the inflammatory process and has been associated to carcinogenesis. By modulating NF-kB, the receptor for activated C kinase 1 enhances the polarization of M2 macrophages and accelerates the progression of OSCC. IL-1, IL-6, IL-8, TNF-α, and TGF-β are examples of proinflammatory cytokines, while IL-2, IL-12, IL-4, IL-10, and IFN-γ are examples of anti-inflammatory cytokines. Cytokines play a key role in modulating the immune response. Cyclooxygenases are enzymes that convert arachidonic acid to prostaglandins; chronic inflammation is strongly associated with the production of cyclooxygenase (COX- 2) in the onset of carcinogenesis. Cell migration and the control of cytokine levels at the site of inflammation are mediated by MMP, and their potential as biomarkers have been investigated by a large number of OSCC researchers. [50]

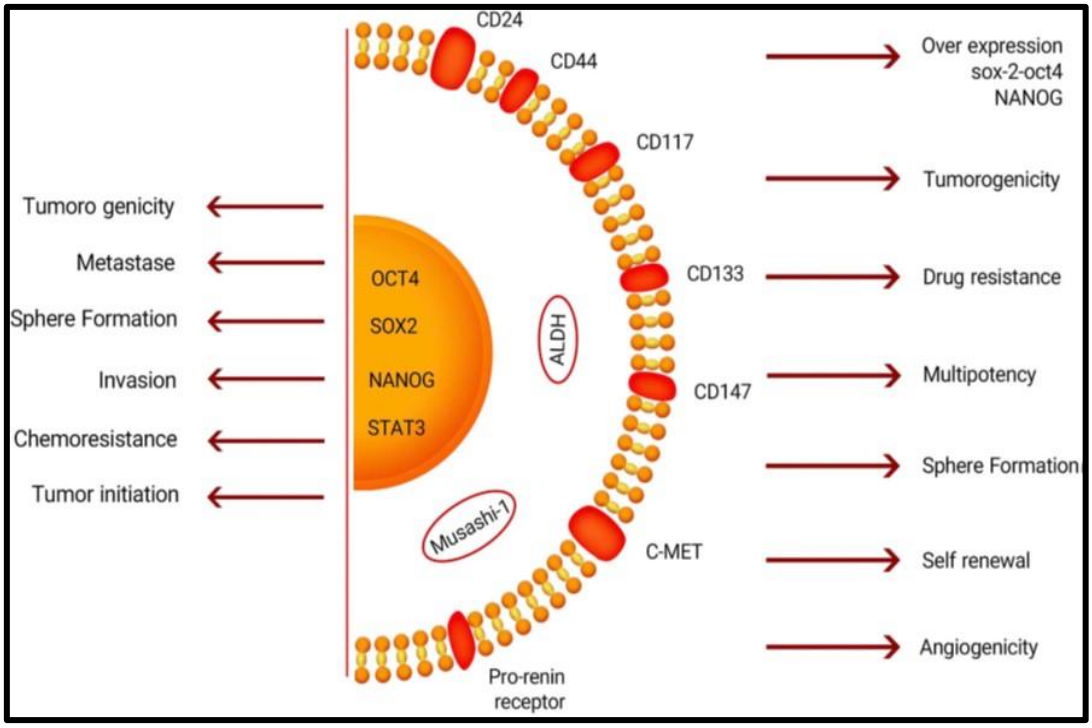


Figure 9 [48]***:*** *Molecular markers used for isolation and identification of oral cancer stem cells*

3.6.2. Serum biomarkers

Biomarkers **(Table II)** in serum are molecules that undergo quantitative changes during tumor development. Typically, malignant cells produce a marker and release it into the bloodstream or express it abundantly on the cell surface. These indicators can be utilized to predict tumor recurrence or metastasis due to the fact that the development of the malignant tumor alters their concentrations.

Tumor markers are often termed as measurable biochemicals that are associated with malignancy. These markers are either produced by tumor cells (tumor-derived) or the body in response to tumor cells (tumor-associated). Although tumor markers are usually imperfect as screening tests for detecting occult (hidden) cancers, once a particular tumor has been found using a marker, the marker may be a way of monitoring the success (or failure) of treatment.

The tumor marker or molecule is both tumor-specific and tumor-associated. Tumor-specific substances are considered to be a direct outcome of oncogenesis, whereas tumor-associated markers are numerous proteins, enzymes, hormones, and immunoglobulins that are found in the blood and are mediated by the tumor itself or by the tumor's influence on the implicated organs.

Repeated serum biomarker testing enables for the monitoring of tumor development and metastasis, as well as the monitoring of therapy response and treatment efficacy. However, there are currently no standardized criteria for determining whether biomarker is useful for oral cancer. [49]

Table II [50]: Biomarkers Identified In Studies

1. Adiponectine	Adiponectin is an adipokine produced predominantly by Adipocytes. It functions as an anti-diabetic, anti-atherogenic, anti-inflammatory and anti-**a**ngiogenic hormone	Associated biomarker
2. Annexin A1 mRNA	Annexin A1, an anti-inflammatory and calcium-dependent protein of the superfamily of annexins, may have important regulatory roles in tumor development and progression	Associated biomarker
3. CRP	C-Reactive Protein (CRP)is a functional analogue to immunoglobulin G, which synthesis by pro-inflammatory cytokines	Associated biomarker
4. Cycling D1	Cyclin D1, the product of the CCND1 gene located on chromosome 11q13	Associated biomarker
5. DCR3	Decoy receptor 3. DcR3 functions as a death decoy inhibiting apoptosis mediated by the tumor necrosis factor receptor family	Specific biomarker
6. GDF 15	Growth-differentiation factor 15 (GDF 15) is involved in tumor pathogenesis. Its expression is increased in many types of cancers. (Associated biomarker)	Specific biomarker

7. Hb	Hemoglobine level mediates tumor response to radiation through the delivery of oxygen to the tumor	Associated biomarker
8. TNFa	Tumor necrosis factor-alpha	Specific biomarker
9. IL6	Interleukin 6. Proinflammatory cytokines	Associated biomarker
10. MiCB	Major histocompatibility complex class I-related chain A/B (MICA/B), a ligand of natural killer group 2D (NKG2D) immunoreceptors	
11. MMP-3	Matrix metalloproteinase-3 is a member of MMP family which is capable to degrade a broad range of substrates. MMP-3 reveals pathological expression in many tumors	Associated biomarker
12. MMP-9	Matrix metalloproteinase-9. Potent factors involved in angiogenesis. Under physiological conditions MMP are capable of degrading extracellular matrix and basement membrane components	Associated biomarker
13. Nitric Oxide	Nitric Oxide concentration plays an essential role in the process of lipid peroxidation	Associated biomarker
14. PDEs	Phosphodiesterases have a fundamental role	Associated

	in thetransduction of the intracellular signals and tumor growth by influencing angiogenesis	biomarker
15. PlGF	Placenta growth factor is a member of the vascular endothelial growth factor (VEGF) family. PlGF stimulates proliferation, differentiation, and survival of endothelial cells	Associated biomarker
16. SCCAg	Squamous cell carcinoma antigen. A tumor-associated protein, an adjunct in the diagnosis of the disease (associatedbiomarker)	Specific biomarker
17. Serum fucose	L-fucose, is a monosaccharides that compounds serum glycoproteins	Associated biomarker
18. Serum Leptin	Leptin is a protein of cytokine family, related to body weight, metabolism and reproductive function	Associated biomarker
19. Sialic acid levels	Siacilic acids are acetylated derivatives of neuramic acid. They are attached to the non-reduced residue of carbohydrate chains of glycoproteins and glycolipids	Associated biomarker
20. Th17 cells	TH17 cells are the third subset of CD4+ T helper cells (Tlymphocytes that belong to the CD4+ subset). Important role in inflammation	Associated biomarker

21. TPA	Tissue Polypeptide Antigen One of the most frequently used cytokine evaluated as a serum marker	Associated biomarker
22. VEGF	Vascular endothelial growth factor VEGF is a multifunctional cytokine that plays a pivotal role in angiogenesis. (induction of angiogenesis in tumor growth)	Associated biomarker
23. Visfatin/pre-b cell colony enhancing factor	Nicotiamide phosphoribosyl transferase or pre-B cell colony enhancing factor, is a pro-inflammatory cytokine. Itregulates growth, apoptosis, and angiogenesis.	Associated biomarker

The following studies act as background to the objective:

1. **Gutiérrez-Corrales A et al. 2017** [51] studied the effect that the acute inflammatory process occurring post extraction could have on these parameters has not been studied. Certain salivary biomarkers may be influenced by the initial inflammatory process following the extraction of a retained lower third molar, despite the fact that these biomarkers are not explicitly inflammatory. This study evaluated three biomarkers: total protein, immunoglobulin A (IgA), and alpha-amylase. 15 patients were recruited in all. Comparing the average values of each marker across the various stages of the study. Only alpha-amylase, among the three salivary biomarkers, was statistically related with an inflammatory response to surgery ($P<0.05$). These data imply that salivary alpha-amylase levels may be altered by the acute inflammation that occurs after extraction; consequently, this marker would not be suitable for use in further studies unless this interference is controlled for.

2. **Kaczor-Urbanowicz KE et al. 2017** [52] provided an update on the current and future applications of saliva for diagnostic purposes. There are numerous benefits of utilising saliva as a biofluid. Its collection is quick, painless, economical, and noninvasive. As a "mirror of the body," saliva can also reveal the physiological and pathological state of the body. The name "Salivaomics" was coined in 2008 to emphasise the rapid expansion of information regarding multiple "omics" components of saliva, such as the proteome, transcriptome, micro-RNA, metabolome, and microbiome. In recent years, scientists have developed new

technologies and verified a vast array of salivary biomarkers, paving the way for the clinical application of saliva. Nevertheless, there is a significant demand for noninvasive diagnostic tools that are both convenient and accurate and may be used at the point of treatment. In addition, there is a pressing need to comprehend the scientific reasoning and mechanisms underlying the transmission of systemic disorders to saliva. Liquid biopsy, an additional promising method, enables the identification of circulating tumor cells (CTCs) and tumor DNA fragments in saliva, allowing for the non-invasive early detection of many cancers. The newly developed technology, electric field-induced release and measurement (EFIRM), enables near-perfect detection of actionable mutations in patients with lung cancer. Recent improvements have expanded the salivary diagnostic method from the oral cavity to the entire physiological system, indicating a bright future for salivary diagnostics in personalized medical applications, such as clinical decisions as well as post-treatment outcome predictions.

3. **Zhang S et al. 2017** [53] aimed to determine changes in the concentration of secretory immunoglobulin A (SIgA) and interleukin 6 (IL-6) in the saliva of patients with oral cancer, to evaluate the abnormal expression of cluster of differentiation (CD) 1a, CD83, CD80 and CD86 on dendritic cells (DCs) of oral cancer tissues and to discuss the interaction between SIgA, IL-6 and DCs in oral cancer. The content of SIgA in the saliva of patients with oral cancer dropped, but the level of IL 6 increased considerably compared to the control group ($P<0.05$). In addition, there was a negative association between the decrease in SIgA level and the increase in IL 6 level ($r=0.543$, $P<0.05$). According to the IRS score, the

expression levels of CD1a, CD83, CD80, and CD86 were lower in cancer tissue than in the control group (P<0.05). In addition, there was no link between CD80 and CD86 expression and histological grade or pathological type (P>0.05), however there was a negative correlation between CD80 and CD86 expression and clinical stage and lymph node metastases (P<0.05). The concentration of SIgA and IL 6 in saliva can be used as a diagnostic supplementary sign for oral cancer. The identification of CD80 and CD86 on DCs within oral cancer tissue may be relevant for tumor diagnosis and prognosis evaluation. In the present investigation, it was anticipated that SIgA vaccines or IL 6 inhibitors may be effective for restoring the immunological deficit caused by DCs in oral cancer.

4. **Alshagroud R et al. 2017** [54] characterized the various in vivo ANA patterns detected in the oral mucosa by direct immunofluorescence to describe the associated hematoxylin and eosin findings, and determine whether patients with these findings had a coexisting systemic connective tissue disease. 72 of the 2019 patients studied displayed ANA staining in vivo. Immunoglobulin G was the predominant immunoreactant (71 of 72 cases), and speckled nuclear staining was the predominant in vivo ANA pattern (52 of 72). Hematoxylin and eosin staining of biopsy specimens revealed mucositis in the majority of cases (24 of 34). All ten patients for whom detailed clinical information was available had an autoimmune illness. The incidence of ANA staining with direct immunofluorescence in oral epithelial biopsy specimens was comparable to that reported for skin. The presence of ANA in the oral epithelium in vivo may be indicative of an immune-mediated illness. Patients whose oral mucosal biopsies reveal ANA deposits

should be evaluated for systemic connective tissue disease and persistent ulcerative stomatitis.

5. **Mozaffari HR et al. 2018** [55] in their meta – analysis aimed to assess the serum and salivary levels of Igs as more important immunoglobulins in patients affected by oral lichen planus (OLP) compared to the healthy controls. To determine the publication bias of the studies, the CMA 2.0 software was utilized. The meta-analysis included and examined eight of the seventy studies reported in the databases. 282 OLP patients and 221 healthy controls were included in the meta-analysis. The pooled MDs of serum IgA, IgG, and IgM levels were 0.13 g/L [95% CI: 0.24, 0.02; P = 0.02], 1.01 g/L [95% CI: 0.91, 2.93; P = 0.30], and 0.06 g/L [95% CI: 0.25, 0.14; P = 0.56]; while, the salivary IgA and I gG levels were 71.54 mg/L [95% CI: 12.01, 131.07; P = 0.02] and 0.59 mg/L [95% CI: −0.20, 1.38; P = 0.14], respectively. Considering the small number of research conducted on saliva, the results indicated that salivary levels, particularly IgA levels, were higher than serum levels. Consequently, salivary immunoglobulins may play an important role in the aetiology of OLP.

6. **Felix CE et al. 2017** [56] examined the clinical utility of CIC and different classes of immunoglobulin, they evaluated and correlated the serum levels at different disease stages and treatment groups in women with malignant and pre-malignant disease conditions of the breast. The descriptive comparison of CIC and serum

immunoglobulins (IgG, IgM, and IgA) across illness stages and therapy groups revealed no significant (P>0.05) changes. There were inconsistent associations between CIC and immunoglobulins across illness groups and treatment phases. Results indicate that serum concentrations of CIC and immunoglobulins have limited usefulness for breast cancer surveillance in our setting. Other immunological variables with potential clinical usefulness are required.

7. **Felix CE et al. 2018** [57] compared the major serum immunoglobulin levels (IgA, IgG, and IgM) in patients with malignant and pre-malignant breast conditions of the breast across disease stages and treatment groups. In all stages of breast cancer and therapy groups, the mean serum IgG, IgM, and IgA levels were not substantially elevated (P > 0.05), according to the results. In addition, the majority of patients (59%) presented at an advanced stage of disease, according to the findings. Low educational attainment and low income were prevalent risk variables. The majority of cases (63%) had a body mass index indicative of obesity (>30 kg/m2) Results indicate that serum immunoglobulin (IgG, IgM, and IgA) levels are of little utility in the detection and monitoring of breast cancer in our environment. On the basis of their data, it is also possible to assume that low levels of education and income are risk factors. Prioritize advocacy and evidence-based policies focused at the disease's early detection and prevention.

8. **Khowal S et al. 2018** [58] assessed cellular and serum proteome from tongue squamous cell carcinoma patient lacking addictive proclivities for tobacco, betel nut, and alcohol. Intriguing molecular processes associated with oral

carcinogenesis remain obscure. Oral squamous cell carcinoma (OSCC) is the most prevalent form of oral cancer, accounting for more than 90 percent of all cases identified worldwide. The ten-year increase in the OSCC incidence rate has had a worrying impact on human healthcare. OSCC is characterized by a delayed diagnosis, a high metastatic rate, and a dismal five-year survival rate. The present research is predicated on a reverse genetic technique and entails the discovery of genes displaying expressional variability in an OSCC patient devoid of cigarette, betel nut, and/or alcohol addiction. In 16 patients with oral pre-cancerous and cancerous histopathologies, the expression modulations of the discovered genes were examined. SCCA1 and KRT1 were discovered to be downregulated, while DNAJC13, GIPC2, MRPL17, IG-Vreg, SSFA2, and UPF0415 were found to be elevated in oral pre-cancerous and cancerous diseases, indicating that these genes are critical in oral carcinogenesis.

9. **Hammody RH et al. 2018** [59] estimated the serum immunoglobulins (IgA, IgM, and IgG) levels, and complements (C3 and C4) level in Iraqi women with breast cancer pre-treatment and post-treatment. Patients' pre-treatment values of IgA, IgG, and C3 were substantially greater (p0.01) than their post-treatment values. Highly significant differences were found in the concentrations of IgA, IgM, and IgG in patients with breast cancer (390.37±13.19 mg/dl, 292.86±14.35 mg/dl, and 1416.66±49.73 mg/dl, respectively). The present investigation observed no statistically significant difference in IgM levels between the pre-treatment and post-treatment groups; this may be due to the fact that IgM levels are still within the normal range. Pre-treatment serum concentrations were substantially greater

than post-treatment concentrations. The present investigation shown that a rise in serum IgA, IgG, and C3 levels may serve as biomarkers for breast cancer diagnosis before to and during treatment.

10. **Jesinda Pauline K et al. 2018** [60] aimed to identify potential molecular biomarkers associated to OSCC using a combination of genomics and proteomics technologies. In comparison to other malignancies, LRG, A1BG, PRO2044, ACTBM, HBB, CRNS1, HBA, F8WAH6, and SCND3 were discovered to be specifically expressed in OSCC. These proteins may have the potential to serve as OSCC-specific biomarkers. SYNE1 (Nesprin-1) was the sole biomarker identified using both genomic and proteomic techniques. In addition, functional enrichment and pathway analysis were done on these 77 putative biomarkers using ConsensusPathDB, DAVID v6.8, and STRING v10.1 to identify the biological function and pathways related with OSCC. Based on our findings, the most important biological role of these indicators in OSCC was their association with exosomes. In contrast, the platelet activation, signaling, and aggregation pathway was determined to be the most significant. The found biomarkers play a crucial role in cancer metastasis, according to the results of both the biological function and pathway analyses. In conclusion, the study found a combination of thirteen unique potential biomarkers and enhanced our understanding of the molecular activities and pathways linked with OSCC. However, additional research is required to validate these biomarkers in a larger population and to completely comprehend their significance in OSCC.

11. **Tyagi KK et al. 2018** [61] aimed at the estimation of immunoglobulins in smokers in a study population. The purpose was to estimate the immunoglobulin levels of smokers in the study population. In group I, the mean level of IgG was 14.24, while in group II it was 7.45. The change was statistically significant (P<0.05). In group I, the mean level of IgA was 3.12 while in group II it was 1. The change was statistically significant (P<0.05). The elevated levels of IgG and IgA in smokers relative to the control group improve the likelihood of early detection of lesions. Serum concentrations of immunoglobulins, C3, C4, and IL-8 in smokers and non-smokers showed that nicotine stimulates dendritic cells and enhances their ability to drive T cell proliferation and cytokine release. Therefore, people with a smoking habit should have their IgG and IgA levels periodically evaluated.

12. **Mozaffari HR et al. 2019** [62] investigated interleukin 4 (IL-4) concentration in patients affected by oral lichen planus (OLP). Only 10 of the 108 studies collected from the databases were included in the quantitative synthesis. The pooled mean difference (MD) of serum and salivary IL-4 levels in OLP patients relative to controls was 6.36 picograms/milliliter (pg/mL; 95% confidence interval [CI]: 1.47 to 11.24; P =.01) and 2.67 pg/mL (95% CI: 2.66 to 2.68; P<.00001), respectively. In addition, the pooled MD of serum and salivary IL-4 levels were 1.30 pg/mL (95% CI: –0.35, 2.95; P =.12) and 1.83 pg/mL (95% CI: 0.26, 3.40; P =.02) in patients with erosive, erythematous, bullous, and ulcerative types of OLP, respectively, compared with patients with reticular OLP. This meta-analysis revealed that OLP patients have higher blood and salivary IL-4 levels, suggesting that IL-4 may serve as a salivary biomarker for the condition. In contrast,

practitioners must be mindful that other factors (such as secondary infection) may also affect its concentration.

13. **Weed DT et al. 2019** [63] reported for the first time interim results of their ongoing phase I clinical trial (NCT02544880) in patients with recurrent HNSCC that evaluated the safety of and immunological effects of combining Tadalafil with the antitumor vaccine composed of Mucin1 (MUC1) and polyICLC. However, this analysis also reveals that CD163-negative cells within the tumor upregulate PDL1 in response to treatment, indicating the establishment of new immune evasion mechanisms. In conclusion, their findings corroborate the safety and immunologic potential of PDE5 inhibition in HNSCC, while pointing to PDL1 as an additional tumor evasion mechanism. This reinforces the logic for combining PDE5 and checkpoint inhibitors in the treatment of human cancers.

14. **Kouketsu A et al. 2019** [64] aimed to investigate the role of the PD-L1/PD-1 pathway in oral squamous cell carcinoma (OSCC) and oral epithelial precursor lesions (OEPL). Expressions of PD-L1 and PD-1 were significantly positively correlated in OEPL and OSCC samples ($P<0.001$). Immunoreactivity for PD-L1 and PD-1 was substantially correlated with tumor size ($P<0.05$). PD-L1 and PD-1 immunoreactivity was substantially higher in instances with advanced TNM staging than in cases with modest staging ($P<0.01$). There were substantial connections between PD-L1 and PD-1 expression in OSCC specimens and pathological factors such as stromal lymphocytic response ($P<0.05$) and invasion depth ($P<0.01$); however, there was no link between PD-L1 and PD-1 expression

and survival. In oral epithelial lesions, the immunohistochemistry status of PD-L1 and PD-1 may be associated with carcinogenesis, tumor progression, and prognosis. Agents that target PD-1 and PD-L1 may be useful for treating OSCC.

15. **de Vicente JC et al. 2019** [65] evaluated NANOG expression by immunohistochemistry in 55 patients with oral epithelial dysplasia, and 125 OSCC patients. Assessing correlations with clinical and follow-up data. Oral dysplasias exhibited nuclear (3.6%) and cytoplasmic (16.4%) NANOG expression, respectively. NANOG expression increased as dysplasia severity increased. Expression of cytoplasmic NANOG and dysplasia grade were substantially linked with oral cancer risk, while dysplasia grade was the only independent predictor of oral cancer development in multivariate analyses. NANOG expression was also found in the cytoplasm of 39 (31%) OSCC samples. Positive NANOG expression was substantially related with cigarette and alcohol intake and was more prevalent in early I-II stage pN0 malignancies. These findings demonstrate the clinical importance of NANOG in the early stages of OSCC carcinogenesis, as opposed to in advanced malignancy. Expression of NANOG appears as an early indicator of oral cancer risk in OPMD patients.

16. **Chen XJ et al. 2019** [66] investigated the expression level of PD-L1 in OSCC and OPMDs and examine its relationship with CD8 expression and different clinicopathological features. The PD-L1 high expression OSCC group had a trend toward higher overall survival (OS) and disease-free survival (DFS) compared to the low expression group, but the differences were not statistically significant.

PD-L1 expression in OSCC was strongly connected with pathological grade (P<0.0001), but was independent of age, gender, smoking, drinking, tumor size, lymph node status, and recurrence (P > 0.05). In addition, PD-L1 expression was significantly upregulated in the OLK group compared to the control group (P<0.0001) PD-L1 positive in OLK patients was correlated with gender and smoking (P<0.05), but not with age, alcohol use, or dysplasia (P > 0.05). In oral premalignant and malignant lesions, the overexpression of PD-L1 may be related with disease progression and CD8+ tumor-infiltrating cells.

17. **Tarsariya VM et al., 2022** [67] estimated the serum immunoglobulins level (IgG, IgM, IgA) in leukoplakia, OSMF and oral lichen planus (OLP) patients and its comparison with levels among control groups and Weather these values can be used to predict severity of disease or not. In comparison to the control group, they detected significantly raised levels of all immunoglobulins in leukoplakia, OSMF, and oral lichen planus, and these levels increased with the clinical stages of OSMF (p<0.05). All of these immunoglobulins can be used as prognostic indicators for the aforementioned conditions, as they are correlated with tumor burden or malignant transformation at advanced stages.

18. **Shukla AK et al., 2020** [68] estimated the level of IgG and IgA in SLT patients and establish a correlation between them. A total of 60 participants, including 34 khaini users (32 males and 2 females) and 26 gutkha users (22 males and 4 females) with a mean age of 36.9 years were chosen for the study. Serum IgA and IgG levels in SLT recipients exhibited significant variations. Serum IgG levels

were positively correlated with the form of SLT, whereas serum IgA levels were negatively correlated and statistically insignificant. In their study, males dominated the 30-to-40-year-old age group. This study may act as an early diagnostic tool and aid in educating the Indian populace about the dangers of using SLT as a deplorable alternative to smoking tobacco. In addition, it plays a crucial role in SLT-mediated effects on immunoglobulin levels.

19. **Sollie S et al., 2020** [69] evaluated associations between pre-diagnostic serum markers of the overall humoral immune system [immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM)], and the risk of pancreatic cancer in the Swedish Apolipoprotein-related MORtality RISk (AMORIS) study. Compared to the reference level of 6.10–14.99 g/L, individuals with IgG levels <6.10 g/L had an increased risk of pancreatic cancer [HR: 1.69 (95% CI: 0.99–2.87)], but an inverse correlation was detected among those with IgG levels ≥15.00 g/L [0.82 (95% CI: 0.64–1.05); Ptrend = 0.027]. The connection appeared to be greater for women than for males [HR: 0.64 (95% CI: 0.43–0.97) and 0.95 (95% CI: 0.69–1.29)]. There were no connections found with IgA or IgM. There was an inverse relationship between pre-diagnostic serum IgG levels and pancreatic cancer risk. Their findings underscore the necessity for additional research into the involvement of immune response in the etiology of pancreatic cancer.

20. **Peppas I et al., 2020** [70] aimed to evaluate the association of serum immunoglobulin classes with solid cancer and test their hypothesis that the

immune escape of tumors is accompanied by dysregulated systemic immunoglobulin class-switching. 11 relevant studies comparing serum IgA levels in 1,351 patients and 560 controls found a statistically significant SMD (1.50; 95% confidence interval [CI]: 0.96–2.04). SMDs were nonsignificant for the 14 selected studies analyzing serum IgG [SMD, 0.02 (95% CI, 0.22 to 0.18)] and the 10-research reporting serum IgM [SMD, 0.11 (95% CI, 0.30 to 0.32)]. Despite sensitivity analyses by immunoglobulin measurement method, control matching, cancer kind, illness stage, and sequential study exclusion, substantial variability was detected between studies. Serum immunoglobulin levels in patients with solid tumors may be skewed toward class-switching to IgA, which may be indicative of Th2-polarized immunity.

21. **Madki P et al., 2020** [71] aimed to evaluate the role of immunoglobulins (IgA, IgG, and IgM) and circulating immune complexes (CIC) as tumor marker in oral cancer and precancer patients. In their investigation, the mean serum IgA levels were 161.00 (±118.02) mg/dL for oral precancer, 270.67 (±171.44) mg/dL for oral malignancies, and 133.73 (±101.31) mg/dL for healthy controls. Mean IgG serum levels in oral precancer were 1,430.87 (±316) mg/dL, while they were 1,234.27 (±365.42) mg/dL in oral tumors and 593.87 (±323.06) mg/dL in healthy controls. Serum IgG and IgA levels were consistently higher in both the precancer and cancer groups, but serum IgM levels were enhanced exclusively in the precancer group. In addition, a substantial rise in serum CIC levels was seen in the oral precancer and cancer groups compared to the control group.

22. **Monteiro de Oliveira Novaes JA et al., 2021** [72] performed this study to further characterize the immune landscape of oral premalignant lesions (OPL) and determine the impact of targeting of the PD-1, CTLA-4, CD40, or OX40 pathways on the development of OPLs and oral carcinomas in the 4-nitroquinoline 1-oxide model. Targeting the immune pathways with mAbs or, in the case of the PD-1/PD-L1 pathway, PD-L1–knockout (PD-L1ko) mice. Following the intervention, tongues and cervical lymph nodes were removed and examined for tumor development and immune milieu alteration, respectively. Targeting CD40 with an agonist mAb was the most effective treatment for preventing progression of OPLs to OSCC; PD-1 alone or in conjunction with CTLA-4 inhibition, or PD-L1ko were also effective, but to a lesser extent. CD40 agonists induced a sustained proliferation of experienced/memory cytotoxic T lymphocytes and M1 macrophages, whereas blockage of the PD-1/PD-L1 axis with or without CTLA-4 blocking led to the depletion of regulatory T cells, among other alterations. CD40 agonists merit consideration as potential immunopreventive medicines in this environment, based on these findings, which show that distinct techniques may be employed to target different stages in the development of OSCC and that CD40 agonists may be useful immunopreventive therapies in this setting.

23. **Shahi Y et al., 2021** [73] aimed to determine the influence of smoking or tobacco chewing and the association of Interleukin 6 (IL-6) polymorphism, where G is substituted by A at the position − 596 (IL-6 − 596 G/A) and substitution of G by cytosine (C) at position − 572 (IL-6 − 572 G/C) on the susceptibility of precancerous oral lesions and oral cancer. Subjects with precancerous oral lesions

and oral cancer possessed the genotypes GG and GA for the IL-6 596 G/A SNP, however no patient possessed the AA genotype. IL-6 596 G/A was closely linked to oral precancerous lesions, but not oral malignancy. The current study found that smokers with an IL-6 gene allele frequency more than 596 G/A had an increased incidence of oral precancerous lesions by a factor of three. Cigarette smokers with GC and CC for IL-6 572 G/C had an increased risk of developing oral precancerous lesions. The interaction of the variant A allele of IL-6 596 G/A and the variant C allele of IL-6 596 G/C polymorphism with smoking increases the incidence of oral precancerous lesions. Oral precancerous lesions and oral cancer were not associated with IL-6 596 G/A or IL-6 596 G/C.

24. **Gupta R et al., 2022** [74] evaluated IgG and IgM in serum of oral submucous fibrosis, thereby observing any possible association of these immunoglobulins in the pathogenesis of this disease. Patients in the study group ranged in age from 15 to 74 years, with a mean age of 37.47±16.24 years. Seventy percent of the participants in the study chewed solely gutka. In their study, all 30 participants (100%) reported a burning sensation, 27 (90%) had difficulties opening their mouths, and 15 (50%) had trouble swallowing. Three (10%) of thirty OSMF patients were in stage I, thirteen (43.33%) were in stage II, and fourteen (46.67%) were in stage III. The greatest number of cases, 14, or 46.67 percent, were in stage III of OSMF. All OSMF patients had higher serum IgG levels than the control group, and the differences were statistically significant ($P > 0.05$). Comparing IgM levels between the control group and the study group revealed no statistically

significant changes (P<0.457). The increase in immunoglobulin levels lends credence to the theory of autoimmune.

25. **Sarmadi MH et al., 2022** [75] aimed to determine the carcinoembryonic antigen (CEA) and IgG serum levels in different oral lichenoid lesions before and after treatment with local corticosteroids. Before the intervention, there was no significant difference between the control and case groups' CEA serum levels (P=0.19). In addition, CEA serum levels in the case group did not differ significantly before and after treatment (P=0.30). While IgG serum levels were significantly greater before the intervention (P=0.01), they reduced significantly after treatment (P=0.01) in the case group. In addition, the case group experienced a substantial reduction in pain severity (P=0.05). Statistically, 8.2% of the 21.7% of patients with positive staining results displayed dysplasia symptoms. However, neither CEA nor IgG serum levels differed between dysplasia-positive and -negative patients (P > 0.05). Consequently, this treatment can be deemed an efficient and low-complication technique for treating lichenoid lesions.

4. MATERIALS AND METHOD

The study was conducted in the Department of Oral Medicine and Radiology of Babu Banarasi Das College Of Dental Sciences, Lucknow (U.P.). Ethical clearance for the dissertation was obtained from the institutional ethical committee [(IEC code - 34), BBDCODS/04/2022] following the declaration of Helsinki for research involving the human subject.

For the Study Purpose, a total of 60 Patients were examined and were divided into 2 groups. The Case Group consisted of 40 patients with Oral Precancer and Oral Cancer, whereas the Control Group consisted of 20 healthy subjects.

The following comprised the eligibility Criteria:

INCLUSION CRITERIA

GROUP A - CASE GROUP

SUBGROUP 1

- Patients with different oral potentially malignant disorders have been clinically diagnosed. (WHO Collaborating Centre 2020)
- Patients of either sex, aged between 18 to 70 years

SUBGROUP 2

- Patients with different clinical stages of Proven Oral Cancer (AJCC TNM, January 2018)
- Patients of either sex, aged between 18 to 70 years

GROUP B – CONTROL GROUP

- Normal healthy individuals
- Subjects of either sex, aged between 18 to 70 years

EXCLUSION CRITERIA

- Patients having any major systemic illness like diabetes, hypertension, or under medication for any other systemic condition.
- Any patient who has previously undergone any treatment for oral cancer.
- Patients suffering from any immunologically associated disease.
- Pregnant and lactating women.
- The patient is not willing to participate.

A detailed case history was recorded in a case history Proforma. **(Annexure 1)** Following the establishment of the diagnosis, each patient was informed about the protocol and given appropriate instructions after obtaining written consent.

3.1 Methodology:

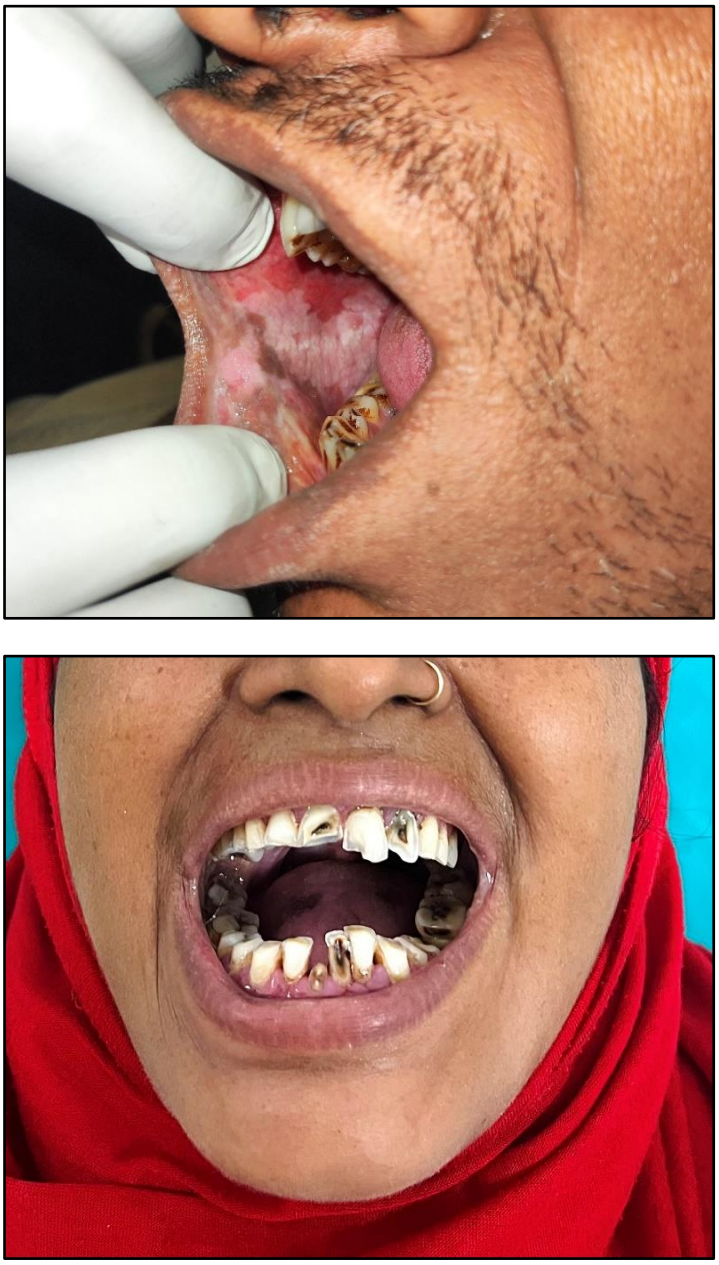
In the present study, all the subjects fulfilling the inclusion and exclusion criteria were enrolled for Evaluating the Serum Immunoglobulins (IgG, IgM, IgA) levels. One group with 40 subjects was selected for the Case Group , and One group with 20 subjects was selected for the Control Group. After written informed consent was taken from the participant, a thorough history was taken, and all patients were examined using a mouth mirror and a probe under artificial light, following universal precautions. The list of armamentarium used has been summarized in the following table. **(Table III) (Photograph 1)**

Table III: Armamentarium Used for Diagnosis

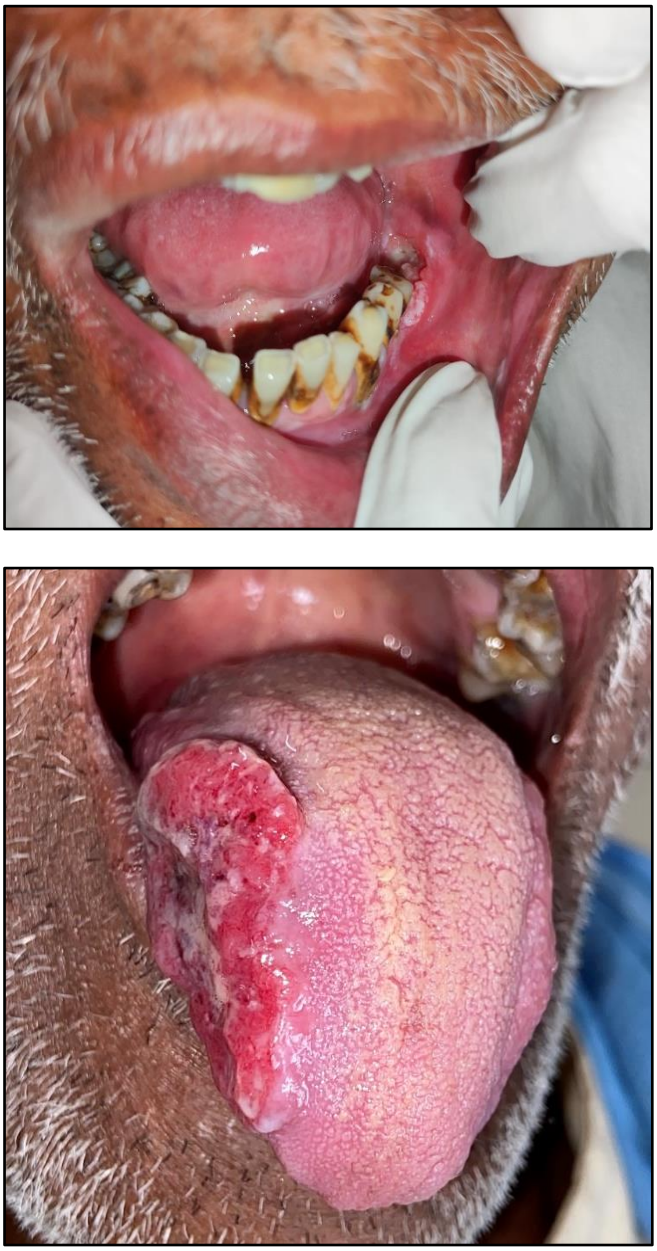
Mouth Mirror	
Straight Probe	
Double Ended Probe	
Tweezer	
Instrument Trays	
Kidney Trays	
Cotton Holder	
Sterile cotton	
Surgical gloves	
Mouth mask	
Digital Vernier Caliper	
Lidocaine Topical Aerosol Spray	
Cheek Retractor	
Gauze	

Photograph 1: Armamentarium Used for Diagnosis

A thorough diagnosis was carried out. The patients were further categorized into the following Groups, Case and Control group. The Case group comprised of the patients with Oral Premalignant Disorders (OPMD) and Oral Cancer. **(Photograph 2, Photograph 3)**



PHOTOGRAPH 2: OPMD Patients



PHOTOGRAPH 3: Oral Cancer Patients

Following which, 4.5 mL of blood samples were taken from all the patients to estimate their Serum immunoglobulin (IgG, IgM, IgA) levels. The armamentarium **(Table III) (Photograph 4)** for fresh blood samples consisted of a 5 ml syringe, an alcohol Swab, a plain vial, and a vial containing ethylenediaminetetraacetic acid (EDTA), a tourniquet, sterile cotton, and surgical gloves and antiseptic bandage.

Table III: Armamentarium Used for Phlebotomy

5 ml syringe	
Alcohol Swab	
Plain vial	
Vial containing ethylenediaminetetraacetic acid (EDTA)	
Tourniquet	
Sterile cotton	
Surgical gloves	
Antiseptic Bandage	

Photograph 4: Armamentarium Used for Phlebotomy

4.5 ml of venous blood was collected from all subjects using routine venipuncture and was stored in a vial containing EDTA. **(Photograph 5)** EDTA containing whole blood samples must be run within 1 hour at room temperature (68-77 ˚F) and may be stored refrigerated (36-46 ˚F) for up to 12 hours. Blood should return to room temperature before estimation. The sample taken from the patient was tested for total immunoglobulins (IgG, IgM, IgA). The quantitative estimation of immunoglobulin IgG, IgA, and IgM was done by Electro chemiluminescence immunoassay technique using the Roche Cobas 8000 modular analyzer, e602, Roche Diagnostics, USA, **(Photograph 6)** which is a mid-volume throughput immunoassay module. The anticoagulant used was ethylenediaminetetraacetic acid (EDTA) during sample collection. EDTA chelates the free Calcium ions (Ca2+) and inhibits coagulation. Cell preservation is optimum in Electrochemiluminescence immunoassay. Serum and plasma were separated by centrifuging the blood for 20 minutes and then transferred to analyze. Since mixing can cause bubble formation (which interferes with the Cobas sample detection system), care must be taken to remove these bubbles before analysis begins. This can be done by poking the bubbles with a wooden stick or by a short (1 minute) centrifugation at 1,500 x g. The plasma collected with anticoagulants was centrifuged for 15 minutes and then transferred to further analyze it. Samples were loaded into the machine, and tests were programmed by the user. A probe measured an aliquot part of the sample and placed it into a reaction vessel. Reagents were added from an onboard refrigerated supply.

Photograph 5: Phlebotomy

SPECIFICATIONS	
Manufacturer	Roche Diagnostics
Series	Cobas 8000
CLIA Complexity	Moderate
Sample Types	Serum, CSF, Plasma, Urine
Number of Assays	61
Maximum Throughput	170 tests/hour
Sample Cycle Time	(sec)
Sample Cycle Min	1.5 µL
Sample Cycle Max	3.5 µL
Direct sample (STAT)	Yes
Auto Sample Handling	Yes
Sample Input Type	Sample Cup
Sample input closed Tube	No
Patient Sample Capacity	300
Optical Source	Halogen Lamp
Test Method	Electro Chemiluminescence (ECLIA)
Reagent Type	Liquid
Reagent System	Closed
Reagent Capacity	25
On-board Refrigeration	Yes
Standby Mode	Yes
Configuration	Floor Model
Height	134 cm
Width	149 cm
Depth	116 cm
Weight	545 Kg
Power Supply	220V
Point of Care	No

Photograph 6: Roche Cobas 8000 modular analyzer, e602, Roche Diagnostics, USA fully automated, random-access, software- controlled system for immunoassay

According to the specific Immunoglobulin, specific reagents were used.

IgG - Anti-human IgG antibody (Goat); TRIS buffer: 20mmol/L, pH 8.0; NaCl: 150mmol/L; preservative

IgA - Anti-human IgA antibody (Goat); TRIS buffer: 20mmol/L, pH 8.0; NaCl: 150mmol/L; preservative

IgM - Anti-human IgM antibody (Goat); TRIS buffer: 20mmol/L, pH 8.0; NaCl: 150mmol/L; preservative

Incubation time was allowed, following which the unbound reagent and sample were washed away with a buffer. In the reaction, the chemiluminescent reaction was electrically estimated to produce light. The amount of light the reaction provides is indirectly proportional to the concentration of antigen/antibody present in the sample tested. The signal (light) can be amplified, measured and the concentration of the analyte was calculated. Results were displayed on screen or sent to a printer or computer. This method was fully automated.

During electrochemical reactions between analytes and antigen/antibody complexes in the reacting solutions. Intermediates that are produced undergo exothermic reaction to produce an excited state that illuminates upon relaxation to a lower-level state. This luminance of the emitted photon correlates to the energy gap between the excited and relaxed state of the molecules. Electrochemiluminescence or electrogenerated chemiluminescence is a kind of luminescence produced.

This estimation of Serum Immunoglobulins (IgG, IgM, IgA) was done by a lab with Certification from the National Accreditation Board for Testing and Calibration Laboratories (NABL), the results were examined thoroughly. **[Annexures 2, 3a, 3b]**

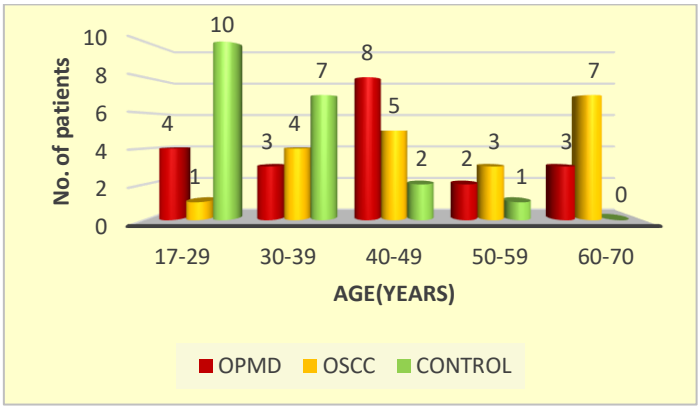
5. OBSERVATIONS AND RESULT

This case-control study was carried out at the Department of Oral Medicine and Radiology of Babu Banarasi Das College Of Dental Sciences, Lucknow (U.P.). After obtaining ethical clearance and informed consent, 60 patients with Oral Precancer and Oral Cancer were enrolled per inclusion-exclusion criteria. All the patients were divided into two groups, i.e., Case Group, which is further divided into two subgroups [(OPMD group; n=20; Patients with different oral potentially malignant disorders which have been clinically diagnosed); (OSCC group; n=20; Patients with different clinical stages of Proven Oral Cancer)] and Control Group (n=20; Normal healthy individuals).

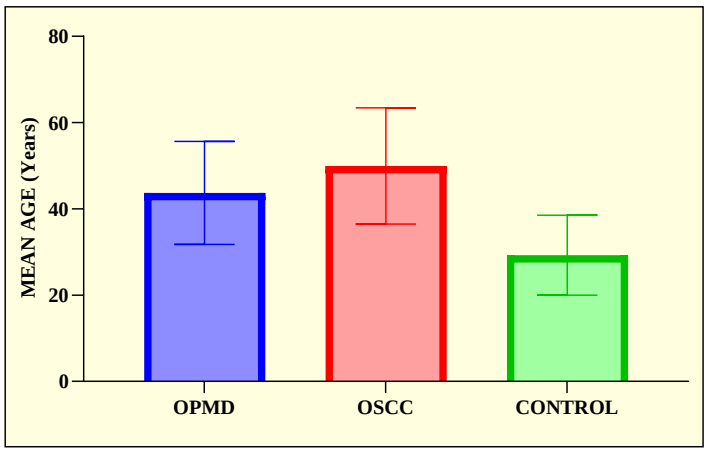
Table 1: Age-wise distribution of patients enrolled in case and control groups

AGE(YEARS)	**OPMD**		**OSCC**		**CONTROL**		**P-VALUE**
	N	**%**	**N**	**%**	**N**	**%**	
17-29	4	20.00%	1	5.00%	10	50.00%	
30-39	3	15.00%	4	20.00%	7	35.00%	
40-49	8	40.00%	5	25.00%	2	10.00%	X=22.26
50-59	2	10.00%	3	15.00%	1	5.00%	**p=0.0045***
60-70	3	15.00%	7	35.00%	0	0.00%	
Grand Total	**20**	**100.00%**	**20**	**100.00%**	**20**	**100.00%**	
MEAN±SD	43.70	11.95	49.95	13.50	29.25	9.25	F=16.47 **p<0.0001***

The majority of the patients in the OPMD group were 40-49 years [8(40.00%)], whereas, in group OSCC and control, most of the patients were 60-70 years [7(35.00%)] and 17-29 years [10(50.00%)], respectively. The mean age was higher in the OSCC group [49.95±13.50], followed by OPMD [43.70±11.95] and the control group [29.25±9.25]. Statistically, a significant difference was observed in the age-wise distribution among groups. **[Table 1; Graph 1a and Graph 1b]**



Graph 1a: Graphical representation of the age-wise distribution of patients enrolled in case and control groups

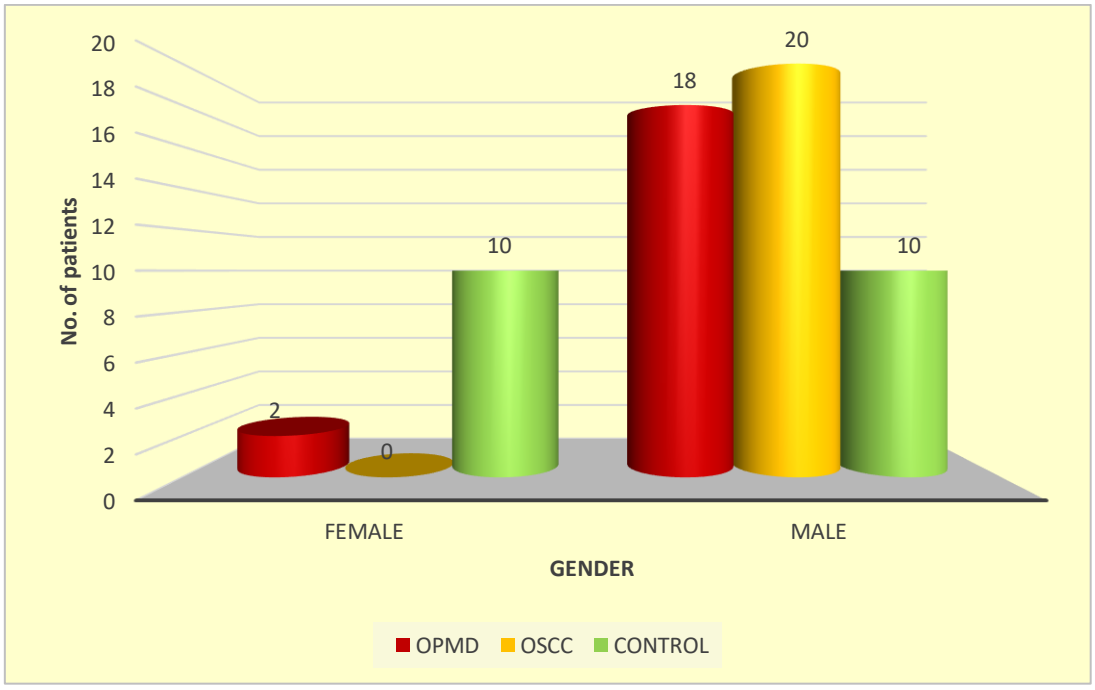


GRAPH 1b: Graphical representation of the mean age of patients enrolled in case and control groups

Table 2: Gender-wise distribution of patients enrolled in case and control groups

GENDER	OPMD		OSCC		CONTROL		P-VALUE
	N	%	N	%	N	%	
FEMALE	2	10.00%	0	0.00%	10	50.00%	X=17.50 **p=0.0002***
MALE	18	90.00%	20	100.00%	10	50.00%	
Grand Total	**20**	**100.00%**	**20**	**100.00%**	**20**	**100.00%**	

Overall male preponderance was observed in all three groups, where the OSCC group had no females, and in the control group, males [10(50.00%)] and females [10(50.00%)] were equal in numbers. Statistically, a significant difference was observed in gender among groups [**p=0.0002***]. **[Table 2; Graph 2]**

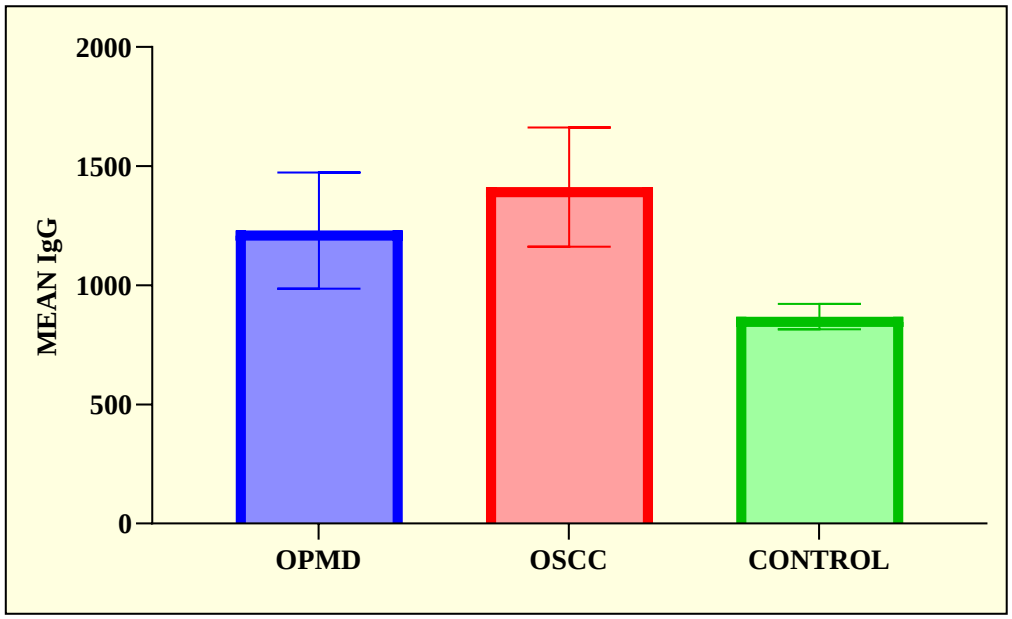


Graph 2: Graphical representation of Gender-wise distribution of patients enrolled in case and control groups

Table 3: Level of Immunoglobulin G in patients enrolled in case and control groups

IgG	OPMD		OSCC		CONTROL		P-VALUE
	MEAN	SD	MEAN	SD	MEAN	SD	
Mean±SD	1229.95	243.98	1411.90	250.21	868.15	53.60	F=36.77 **p<0.0001***

The mean IgG was higher in the OSCC group [1411.90±250.21], followed by OPMD [1229.95±243.98] and Control group [868.15±53.60]. Statistically, a significant difference was observed in the level of IgG [**p<0.0001***]. **[Table 3; Graph 3]**

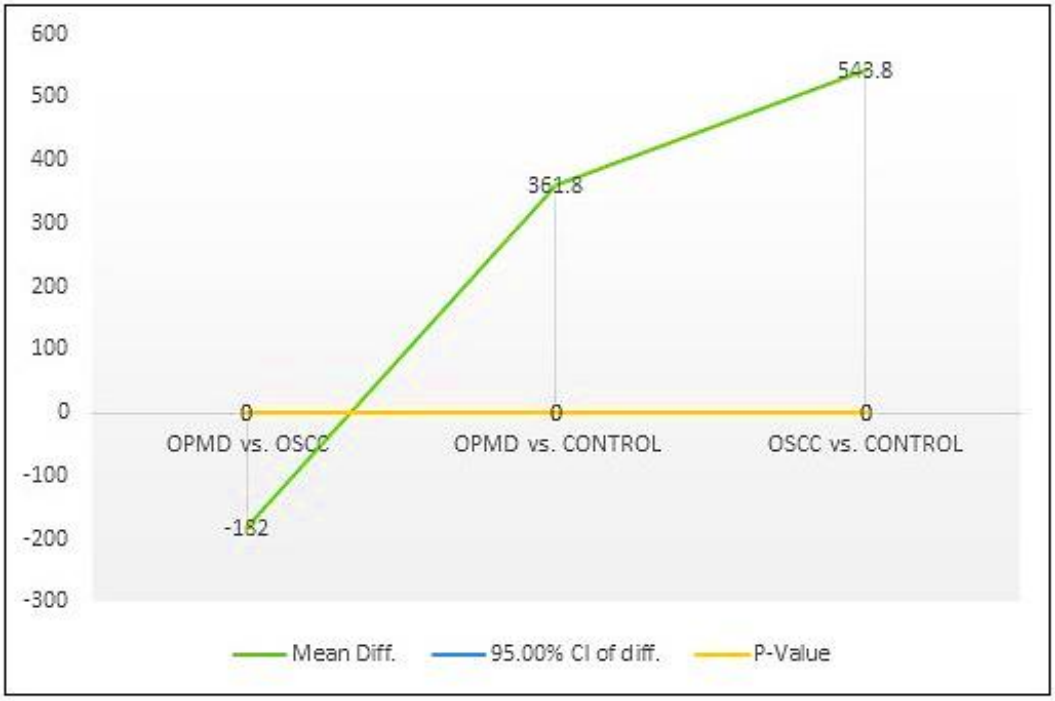


Graph 3: Graphical representation of the mean level of IgG in patients enrolled in case and control groups

Table 4: Tukey's multiple comparison test for the level of Immunoglobulin G in patients enrolled in case and control groups

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	P-Value
OPMD vs. OSCC	-182.0	-337.3 to -26.61	**0.0179***
OPMD vs. CONTROL	361.8	206.5 to 517.1	**<0.0001***
OSCC vs. CONTROL	543.8	388.4 to 699.1	**<0.0001***

Tukey's Multiple Comparisons Test for the level of IgG showed a significant value among all the groups, i.e., OPMD vs OSCC [**p=0.0179***], OPMD vs Control [**p<0.0001***] and OSCC vs Control [**p<0.0001***]. **[Table 4] [Graph 4]**

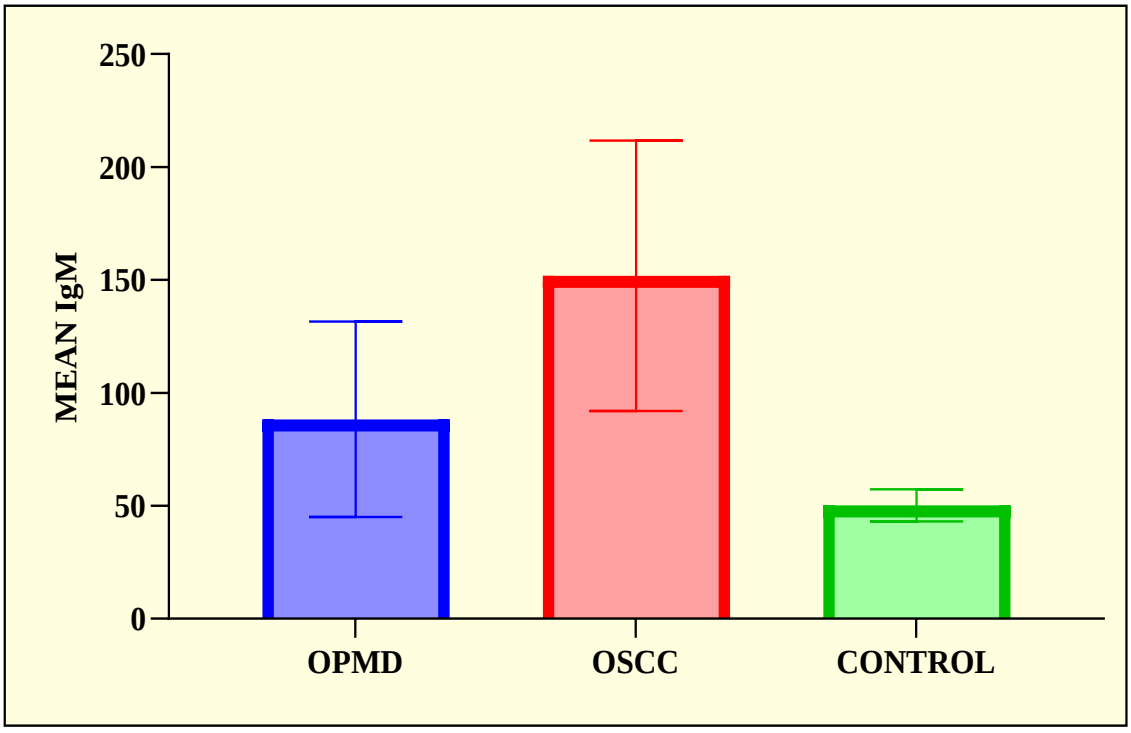


Graph 4: Graphical representation of Tukey's multiple comparison test for the level of Immunoglobulin G in patients enrolled in case and control groups

Table 5: Level of Immunoglobulin M in patients enrolled in case and control groups

IgM	OPMD		OSCC		CONTROL		P-VALUE
	MEAN	SD	MEAN	SD	MEAN	SD	
Mean±SD	88.28	43.29	151.84	59.93	50.13	7.09	F=28.72 **p<0.0001***

The mean IgM was higher in the OSCC group [151.84±59.93], followed by OPMD [88.28±43.29] and Control group [50.13±7.09]. Statistically, a significant difference was observed in the level of IgM [**p<0.0001***]. **[Table 5; Graph 5]**

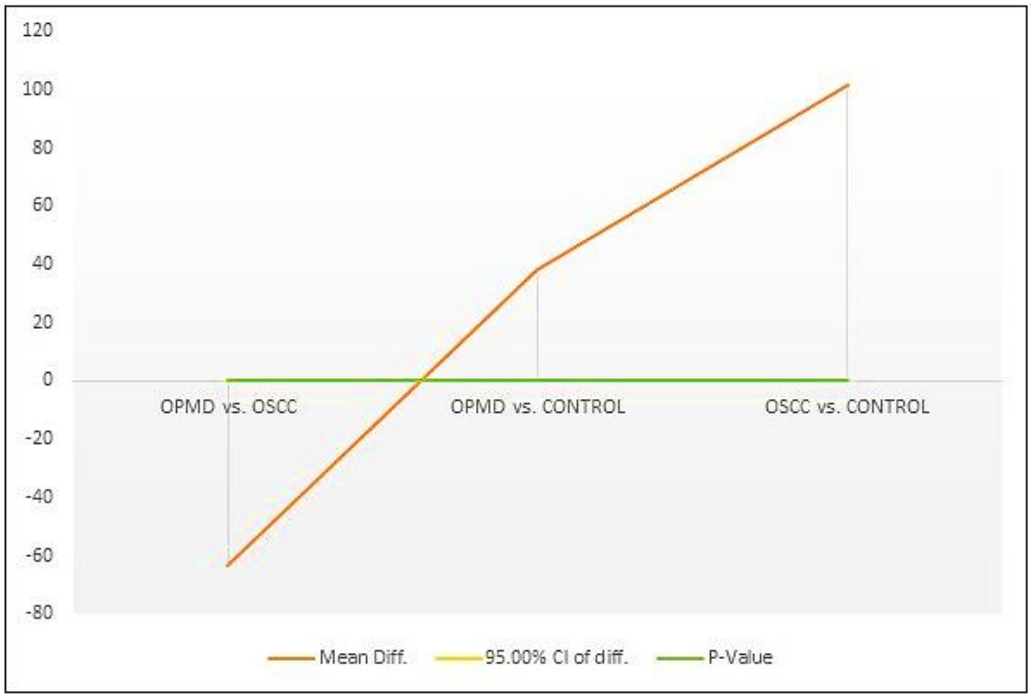


Graph 5: Graphical representation of the mean level of IgM in patients enrolled in case and control groups

Table 6: Tukey's multiple comparison test for the level of Immunoglobulin M in patients enrolled in case and control groups

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	P-Value
OPMD vs. OSCC	-63.56	-96.19 to -30.93	**<0.0001***
OPMD vs. CONTROL	38.15	5.520 to 70.78	**0.0182***
OSCC vs. CONTROL	101.7	69.08 to 134.3	**<0.0001***

Tukey's Multiple Comparisons Test for the level of IgM showed a significant value among all the groups, i.e., OPMD vs OSCC [**p<0.0001***], OPMD vs Control [**p=0.0182***] and OSCC vs Control [**p<0.0001***]. **[Table 6] [Graph 6]**

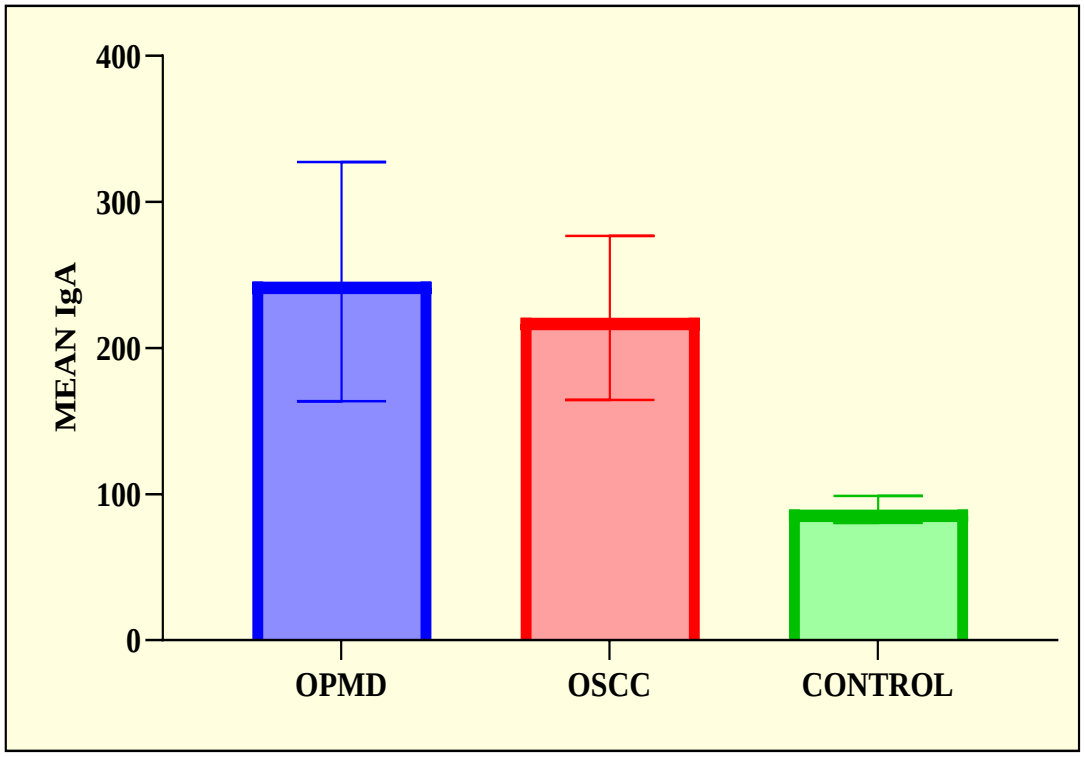


Graph 6: Graphical representation of Tukey's multiple comparison test for the level of Immunoglobulin M in patients enrolled in case and control groups

Table 7: Level of Immunoglobulin A in patients enrolled in case and control groups

IgA	OPMD		OSCC		CONTROL		P-VALUE
	MEAN	SD	MEAN	SD	MEAN	SD	
Mean±SD	245.51	81.83	220.69	56.10	89.44	9.21	F=42.51 **p<0.0001***

The mean IgA was higher in the OPMD group [245.51±81.83], followed by OSCC [220.69±56.10] and Control group [89.44±9.21]. Statistically, a significant difference was observed in the level of IgA [**p<0.0001***]. **[Table 7; Graph 7]**

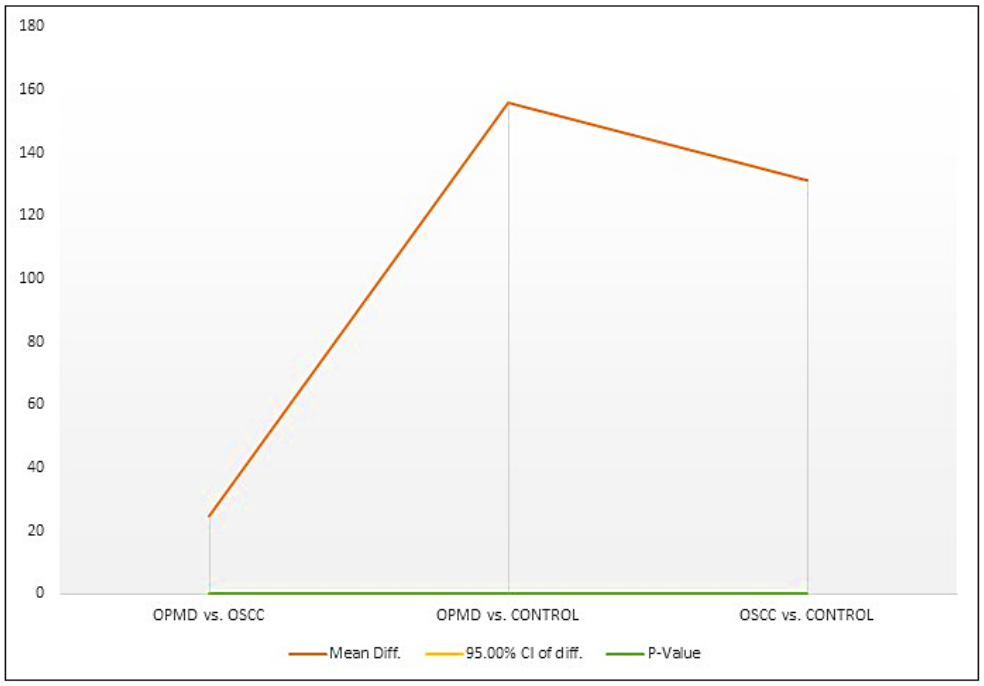


Graph 7: Graphical representation of the mean level of IgA in patients enrolled in case and control groups

Table 8: Tukey's multiple comparison test for the level of Immunoglobulin A in patients enrolled in case and control groups

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	P-Value
OPMD vs. OSCC	24.82	-18.96 to 68.60	0.3663
OPMD vs. CONTROL	156.1	112.3 to 199.8	**<0.0001***
OSCC vs. CONTROL	131.3	87.47 to 175.0	**<0.0001***

Tukey's Multiple Comparisons Test for the level of IgA showed a significant value among OPMD vs Control [**p<0.0001***] and OSCC vs Control [**p<0.0001***]. **[Table 8] [Graph 8]**



Graph 8: Graphical representation of Tukey's multiple comparison test for the level of Immunoglobulin A in patients enrolled in case and control group

6. DISCUSSION

Oral cancers are prevalent in Southern Asian nations such as India, Sri Lanka, and the Pacific Islands. They are the leading cause of cancer-related mortality among men in India and Sri Lanka. Oral cavity cancer is the most prevalent form in Northern India due to the natives' chewing and smoking habits. Leukoplakia, lichen planus, and submucosal fibrosis are common oral precancerous lesions that can lead to oral cavity cancer. Oral cancer accounts for 30–40% of all cancers in India, compared to 2–4% in other western countries, and it is the most common form of cancer seen in males and the third most common form of cancer in females.

Oral cancer is disturbingly widespread in areas of India where betel nut chewing and reverse smoking are common. [4,5] Tumor markers indicate the risk, presence, status, or future behavior of potentially malignant illnesses or oral cancer. Therefore, it is vital to find OPMD and oral cancer detection signs. To evaluate reference values, it is necessary to determine the distribution of immunoglobulin levels in large groups. The general guidelines for the design and determination of reference intervals in the clinical laboratory suggest that partitioning should be considered when significant differences between subgroups are defined by age, sex, and common exposures such as smoking or alcohol consumption. [12]

However, few research has investigated these factors' potential effects on blood immunoglobulin concentrations. Reports indicate that females have higher IgM levels than males. In addition, immunoglobulin serum levels tend to increase with age. It is generally known that heavy drinkers with the advanced liver disease typically have higher IgA levels. Still, fewer studies have evaluated the impact of smoking and light

to heavy alcohol intake on serum IgA, IgG, and IgM. [13] Collectively, these findings highlight the need for multivariate analysis to discover confounding or interactions among all these factors associated with immunoglobulin levels and each other. Hence a hypothesis was made for the present study, which aimed to evaluate the serum level of immunoglobulin (IgG, IgM, IgA) as diagnostic markers in oral premalignant disorders and oral cancer patients.

Comparative analysis of Sociodemographic parameters with the previously available literature: (Table 1, Graph 1a and 1b; Table 2, Graph 2)

In the present study, the majority comprised of 8 (40.00%) patients in the OPMD group were aged 40-49 years, whereas, in the OSCC group, the majority comprised 7 (35.00%) patients that were aged 60-70 years, and 10 (50.00%) patients in the control group. The mean age was higher in the OSCC group, which was found to be 49.95±13.50, followed by OPMD, which was found to be 43.70±11.95mm, and the control group was 29.25±9.25. In all three groups, males outnumbered females, except in the OSCC group, where there were no females, and in the control group, where males and females had equal representation. Most patients, i.e., 18 (90.00%) in the OPMD group, were males, followed by 2 (10.00%) females. In India, the prevalence of OSMF is estimated to be between 0.2% and 0.5%, with prevalence ranging by gender (0.2% to 2.3% in males and 1.2% to 4.57% in females), with smokeless tobacco consumption being more widespread among females than males. [74] Similarly, **Shahi Y et al. (2021)** [71] noted a higher mean age in the oral cancer group [45.3±11.7], followed by the oral precancerous group [44.2±11.9] and the control group [41.7±12.3]. In addition, a majority of men were found in all three groups. In addition, **Madki P et al. (2021)** [69]

also reported patients older than 20 years. Sociodemographic parameters between groups showed statistically significant differences. Likewise, **Nayyar A et al. (2019)** [74] reported that the majority of patients in the OSCC group were aged 50 to 59 [16(64%)], followed by 40 to 49 [6(24%)]. The majority of members in group OPMD were aged 40 to 49 [13(52%)], followed by those aged 30 to 39 [6(24%)]. Lastly, the majority of individuals in the control group were between the ages of 20 and 29 [16(64%)], followed by 30-39 [8(32%)]. The mean age was highest in the OSCC group [53.0±5.46], followed by the OPMD group [41.32±8.02] and the control group [28.36±5.79]. They also found a male preponderance in all groups.

Comparative analysis of levels of Immunoglobulins with the previously available literature:

Comparative analysis of levels of Immunoglobulins IgG (Table 3, Graph 3; Table 4, Graph 4)

In the present study, the OSCC group had the highest mean IgG level, 1411.90±250.21 mg/dl, followed by the OPMD group, 1229.95±243.98 mg/dl, and the Control group, 868.15±53.60. Statistically, a significant difference was observed in the level of IgG [**p<0.0001***]. Similarly, **Tarsariya V et al. (2020)** [65] found a significantly higher level of IgG in both OPMD [14.722±2.773] g/l and controls [11.713±1.766] g/l. **Taneja et al. (2015)** [77] found that IgG and IgM blood levels were elevated in cases of oral submucous fibrosis. Cancer cells can release inhibitory substances to defend themselves from cytotoxic antibodies and produce a blocking factor (likely IgG2). They can also create complexes that act as blocking factors and further reduce cellular defense. [75] Multiple comparisons have revealed a significant difference in the mean

IgG concentration between the OPMD, OSCC, and Control groups. In contrast, **Rajendran R et al. (1986)** [23] observed no variation in the serum IgG concentration of OPMD patients. The increase in IgG levels may result from IgG's dependence on antigenic stimulation and the antibody-producing mechanism's functional ability. Consequently, the increase in IgG in the OPMD group may result from a neoplastic process involving strong antigenic stimulation. [39] Some patients with normal immunoglobulin levels and no immunoglobulin increase may indicate a malfunction in the secretory immune system and immunologic dysfunction. [69,46] All these studies were per the present study.

Comparative analysis of levels of Immunoglobulins IgM (Table 5, Graph 5; Table 6, Graph 6)

The OSCC group had the highest mean IgM level of 151.84±59.93 mg/dl, followed by the OPMD group of 88.28±43.29 mg/dl and the Control group of 50.13±7.09 mg/dl. Statistically, a significant difference was observed in the level of IgM [**p<0.0001***]. Similarly, **Tarsariya V et al. (2020)** [65] found a significantly higher level of IgM in OPMD [1.0807±0.579] g/l than in controls [0.702±0.309] g/l. In contrast, **Chaturvedi et al. (1991)** [78] and **Neuchrist et al. (1994)** [79] observed no increase in IgM levels as the progression of oral cancer. **Griffith et al. (1974)** [80] observed IgM values within normal ranges in OLP patients and indicated that decreasing serum immunoglobulins are not always related to lowered mucosal defenses; hence there is no relation between host susceptibility to an infectious agent and serum immunoglobulin levels. Studies have also shown that 2.3% to 7.0% of OSMFs undergo malignant transformation. [74, 46] Statistically significant difference between the mean levels of IgM in the OPMD,

OSCC, and Control groups was found. In contrast, **Tsavarius et al. (1992)** [81] showed that the patients with elevated IgG and maybe IgM levels had delayed cancer progression and longer overall survival time.

Comparative analysis of levels of Immunoglobulins IgA (Table 7, Graph 7; Table 8, Graph 8)

In the present study, the mean IgA was higher in the OPMD group [245.51±81.83], followed by OSCC [220.69±56.10] and Control group [89.44±9.21]. Statistically, a significant difference was observed in the level of IgA [p<0.0001*]. Consequently, **Dawood** and **Hasan (2013)** [76] observed that the levels of IgG and IgA in the serum of patients with oral malignancies were significantly elevated compared to healthy controls. **Madki P et al. (2021)** [69] evaluated serum IgG and IgA levels in oral precancers, oral malignancies, and controls, showing statistically significant increases in IgG and IgA levels in precancer and oral cancer patients, highlighting the significance of active immunological phenomena and rapid body defense. Between 2.3% to 7.0% of OSMFs undergo malignant transformation. [74, 46] The OPMD group had the highest mean IgA level [245.51±81.83] mg/dl, followed by the OSCC group [220.69±56.10] mg/dl and the Control group [89.44±9.21] mg/dl. Similarly, **Tarsariya V et al. (2020)** [65] observed a greater mean IgA level in OPMD [2.514±1.274] g/l than in controls [2.1006±0.646] g/l. IgA serum level is approximately one-sixth that of IgG. It is secreted efficiently in milk, colostrum, saliva, tears, and GIT secretions. [82] Similarly, **Rajendran R et al. (1986)** [23] found a substantial increase in IgA in OPMD relative to the control group. [14] Statistically, a significant difference in IgA levels between OPMD and OSCC compared to the Control [**p<0.0001***] was noted. In their

research, **Griffith M et al. (1974)** [80] and others reported higher levels of serum IgA in Oral Lichen Planus. [83] Similarly, **Gupta K et al. (1994)** [84] found serum immunoglobulin in patients with oral lichen planus in which all immunoglobulins were elevated relative to normal controls, but only IgA reached statistical significance. Various research showed varied outcomes for these variables at elevated, decreased, and normal levels. [85] These alterations may be explained by the fact that cancer cell antigens typically induce the production of particular serum antibodies, resulting in increased immunoglobulins. Several pathways enable these antibodies to eradicate the tumor protectively. These markers may suggest a bad prognosis and a lower survival chance if they are elevated. Therefore, it is proposed that serial monitoring of immunoglobulin could be utilized to determine a patient's prognosis, tumor incidence, or survival rate.

Inter-Group Comparison of Present Study

In this present study, the level of IgG showed a significant value among all the groups, i.e., OPMD vs. OSCC **[p=0.0179*],** OPMD vs. Control **[p<0.0001*]** and OSCC vs. Control **[p<0.0001*].** The level of IgM showed a significant value among all the groups, i.e., OPMD vs. OSCC **[p<0.0001*],** OPMD vs. Control **[p=0.0182*]**, and OSCC vs. Control **[p<0.0001*],** and IgA showed a significant value among OPMD vs. Control **[p<0.0001*]** and OSCC vs. Control **[p<0.0001*]** as well. Immunoglobulin (IgG, IgM, IgA) and circulating immune complex (CIC) levels are frequently elevated in oral cancer patients compared to healthy controls. This has already been reported by authors such as **Balaram et al. (1987)** [85] and **Abraham et al. (1987)** [86]

The study by **Parveen S et al. (2010)** [39] was in complete concordance with prior research indicating that patients with oral cancer have greater levels of Ig and CIC than healthy controls. Further, **Khanna et al. (1982)** [87] reported a correlation between the elevated levels of IgM in oral cancer patients and their clinical stages. In contrast, **Rajendra et al. (1986)** [23] found no significant increase in IgM levels as the disease progressed. Another study estimated levels of circulating immune complexes, which they found to be appropriate, and concluded that 60% of patients with carcinoma of the buccal mucosa had a markedly higher amount of immune complexes. They also noted that the amount of immune complexes in the patient's sera showed no correlation with serum levels of IgG, IgA, and IgM. [88]

Various research showed varied outcomes for these variables at elevated, decreased, and normal levels. [89] These alterations may be explained by the fact that cancer cell antigens typically induce the production of particular serum antibodies, resulting in increased immunoglobulins. Several pathways enable these antibodies to eradicate the tumor protectively. These markers may suggest a bad prognosis and a lower survival chance if they are elevated. Therefore, serial monitoring of immunoglobulin could be utilized to determine a patient's prognosis, tumor incidence, or survival rate.

7. CONCLUSION AND SUMMARY

Immunoglobulins are proteins of animal origin endowed with known antibody activity and specific other proteins related to them by chemical structure and antigenic specificity. Based on physiochemical and antigenic differences, five immunoglobulins have been recognized – IgG, IgA, IgM, IgD, and IgE. Many authors have studied specific immunoglobulin response caused by malignancy. Prevention and early detection of Oral Premalignant Disorders helps avoid oral cancer and the associated mortality and morbidity. Oral Premalignant disorders are a significant group of mucosal disorders that may precede the diagnosis of oral squamous cell carcinoma. In India, cancer is the sixth leading cause of 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. The concept of a tumor marker is applied to indicate the risk, presence, status, or future behavior of potentially malignant disorders or oral cancer. Therefore, there is a need to identify markers that can indicate the occurrence of OPMDs and Oral cancer.

The present study titled, “Evaluation Of Serum Immunoglobulin (IgG, IgM, IgA) as Diagnostic Markers In Oral Premalignant Disorders And Oral Cancer Patients: A Case Control Study” aimed to estimate the Serum Immunoglobulins (IgG, IgM, IgA) level

in oral precancer, oral cancer patients and its comparison with the control group and whether these values can be used to predict severity of disease or not.

The study aimed to evaluate the Screening of Immunoglobulins (IgG, IgM, IgA) in patients with Oral Premalignant Disorders and Oral Cancer Patients and their comparison with Healthy Subjects.

Nevertheless, numerous studies have been in accordance of the observations, while a few have been dogmatic. In this analytical study, 60 patients were enrolled. We can conclude from the present study that the level of immunoglobulins (IgG, IgM, IgA) was significantly higher in OPMD and OSCC compared to the Control. These parameters may suggest a poor prognosis and a lower survival chance if they are elevated. It may help in the detection and diagnosis of OPMD and OSCC. Higher levels of immunoglobulins in oral cancer indicate tumor burden or transformation of malignancy in higher stages and might be used as prognostic indicators.

However, this study could have been conducted with a larger sample size and a multi-centric study. In addition, more clinical trials are required to study the closure technique and its benefits. Additionally, studies with more parameters are needed to approve the reliability of Immunoglobulins as diagnostic markers in oral premalignant disorders and oral cancer patients.

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9. ANNEXURES

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

Babu Banarasi Das College of Dental Sciences, Lucknow (U.P.)

1. CASE HISTORY PROFORMA

OPD No:

Name: Age: Sex:

Occupation:

CHIEF COMPLAINT

PAST MEDICAL HISTORY

COVID VACCINATION

PAST DENTAL HISTORY

PERSONAL HISTORY

Abusive Habit ☐ Non-Abusive Habit ☐

Abusive Habit	Duration	Frequency	Site	Duration in Oral Cavity
Smokeless Tobacco				
Smoking Tobacco				
Alcohol				

INTRAORAL EXAMINATION

Hard Tissue Examination:

Mouth Opening:

Lip Competency:

Soft Tissue Examination:

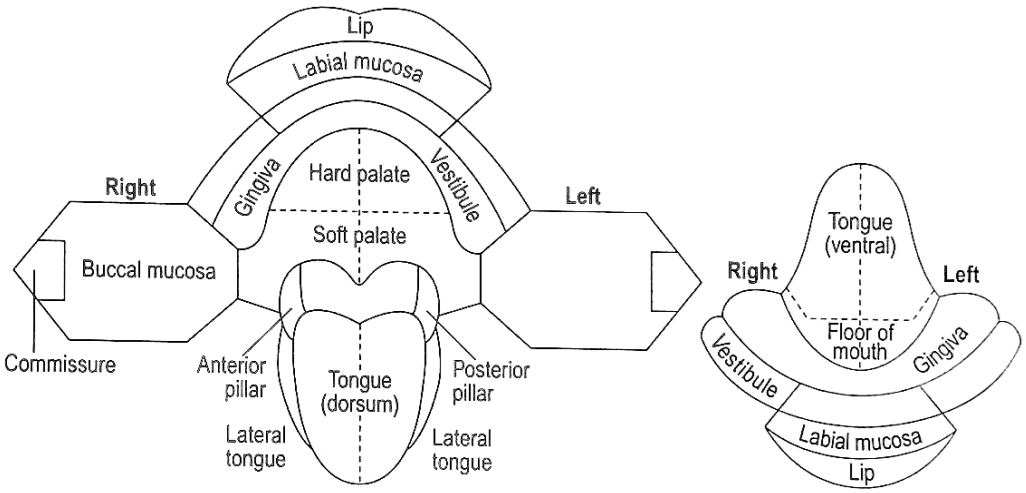
SITE	BLANCHING	STIFNESS AND FIBROUS BANDS	ANY WHITE OR RED LESION
Labial Mucosa			
Buccal Mucosa			
Vestibule			
Tongue			
Floor of the Mouth			
Hard and Soft Palate			

Uvula	DEVIATED	INFLAMED	BLANCHED	SHRUNKEN

CLINICAL EXAMINATION

Oral Lichen Planus	Yes	No
Leukoplakia	Yes	No
OSMF	Yes	No
Any Other Oral Premalignant Disorder	Yes	No
Oral Cancer	Yes	No

SITE OF ORAL MUCOSAL LESIONS



DATE

INVESTIGATION

Reports	Serum IgG	
	Serum IgM	
	Serum IgA	

Along with Other Blood Investigations advised

TREATMENT PLAN

SIGNATURE OF STUDENT SIGNATURE OF GUIDE

2. LAB REPORT OF CONTROL GROUP

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Accredited Lab

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info@ldpldiagnostics.com

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Laboratory Test Report

Name	**: Mrs. NEHA PAL**	**Patient UID.**	**: 230353**
Age/Gender	: 27 Yrs/Female	Visit No.	: 32262301270006
Referred Client	: LDPL3706-LDPL-DK	Collected on	: 27-Jan-2022 02:51PM
Referred By	: NA	Received on	: 28-Jan-2022 09:44AM
Doctor Name	: Dr. SELF	Reported on	: 28-Jan-2022 11:29AM
Sample Type	: Serum - 962346		

IMMUNOLOGY

Test Name	Results	Unit	Bio. Ref. Interval
	IMMUNOGLOBULIN IGG		
IMMUNOGLOBULIN IgG	1,479.00	mg/dL	767 - 1590

Methodology: ECLIA

CLINICAL NOTES

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells in response to bacteria, viruses, and other microorganisms as well as exposures to other substances that are recognized by the body as "non-self" harmful antigens.

Immunoglobulin G (IgG) – About 70-80% of the immunoglobulins in the blood are IgG. Specific IgG antibodies are produced during an initial infection or other antigen exposure, rising a few weeks after it begins, then decreasing and stabilizing. The body retains a catalog of IgG antibodies that can be rapidly reproduced whenever exposed to the same antigen. IgG antibodies form the basis of long-term protection against microorganisms. In those with a normal immune system, sufficient IgG is produced to prevent re-infection. Vaccinations use this process to prevent initial infections and add to the catalog of IgG antibodies, by exposing a person to a weakened, live microorganism or to an antigen that stimulates recognition of the microorganism. *IgG is the only immunoglobulin that can pass through the placenta. The mother's IgG antibodies provide protection to the fetus during pregnancy and to the baby during its first few months of life.* There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4.

SIGNIFICANCE

- Polyclonal IgG increase is seen in SLE, Chronic liver diseases, Infectious diseases and Cystic fibrosis.
-Monoclonal IgG increase is seen in IgG Myelomas.
-Decreased synthesis of IgG is found in Congenital and Acquired Immunodeficiency diseases and selective IgG subclass deficiency.
-Decreased IgG levels are seen in Protein losing enteropathies, Nephrotic syndrome and skin burns

	IMMUNOGLOBULIN IGM		
IMMUNOGLOBULIN IgM	182.90	mg/dL	37.0 - 224.0

Methodology: ECLIA

CLINICAL NOTES

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells in response to bacteria, viruses, and other microorganisms as well as exposures to other substances that are recognized by the body as "non-self" harmful antigens.

Immunoglobulin M (IgM) – IgM antibodies are produced as a body's first response to a new infection or to a new "non-self" antigen, providing short-term protection. They increase for several weeks and then decline as IgG production begins.IgM is the largest immunoglobulin molecule that makes 6% of the total immunoglobulins. It is the first specific antibody to appear in serum after infection which is capable of activating complement and killing bacteria. Post infection IgM returns rapidly to normal levels as compared to IgG. If IgM is prevalent, the infection is acute whereas if IgG predominates, the infection is chronic. Polyclonal IgM increase is seen in viral, bacterial and parasitic infections, Liver diseases, Rheumatoid arthritis, Scleroderma, Cystic fibrosis & heroin addiction. Monoclonal IgM increase is seen in Waldenstroms macroglobulinemia. Decreased synthesis of IgM is found in Congenital and Acquired Immunodeficiency diseases. Decreased IgM levels are seen in Protein losing enteropathies and skin burns

SIGNIFICANCE

-Polyclonal IgM increase is seen in viral, bacterial and parasitic infections, Liver diseases, Rheumatoid arthritis, Scleroderma, Cystic fibrosis & heroin addiction.
-Monoclonal IgM increase is seen in Waldenstroms macroglobulinemia.
-Decreased synthesis of IgM is found in Congenital and Acquired Immunodeficiency diseases- Decreased IgM levels are seen in Protein losing enteropathies and skin burns

	IMMUNOGLOBULIN A (IGA)		
IMMUNOGLOBULIN A (IgA)	183.50	mg/dL	61.0 - 356.0

DR. MD ARIF
MBBS, MD(PATHOLOGY)
LAB DIRECTOR
Reg. No. 34518

DR. EKTA TIWARI
MBBS, MD
CONSULTANT PATHOLOGIST
Reg. No. 78767

DR. SAUMYA GUPTA
MD DNB PATHOLOGY
CONSULTANT HISTOPATHOLOGIST
Reg.No. 96898

DR.PIYUSH DIXIT
Ph.D(MEDICAL BIOCHEMISTRY)
CONSULTANT BIOCHEMIST

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3a. LAB REPORT OF CASE GROUP (SUBGROUP 1)

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Laboratory Test Report

Name	**: Mr. SANJAY SINGH**	**Patient UID.**	**: 1396313**
Age/Gender	: 49 Yrs/Male	Visit No.	: 07702206040001
Referred Client	: LDPL522-RELIABLE PATH LAB	Collected on	: 03-Jun-2022 10:00AM
Referred By	: SELF	Received on	: 04-Jun-2022 09:12AM
Doctor Name	:	Reported on	: 04-Jun-2022 10:02AM
Sample Type	: Serum - 8967633		

IMMUNOLOGY

Test Name	Results	Unit	Bio. Ref. Interval
	IMMUNOGLOBULIN IGG		
IMMUNOGLOBULIN IgG	1,335.00	mg/dL	767 - 1590

Methodology: ECLIA

CLINICAL NOTES

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells in response to bacteria, viruses, and other microorganisms as well as exposures to other substances that are recognized by the body as "non-self" harmful antigens.

Immunoglobulin G (IgG) – About 70-80% of the immunoglobulins in the blood are IgG. Specific IgG antibodies are produced during an initial infection or other antigen exposure, rising a few weeks after it begins, then decreasing and stabilizing. The body retains a catalog of IgG antibodies that can be rapidly reproduced whenever exposed to the same antigen. IgG antibodies form the basis of long-term protection against microorganisms. In those with a normal immune system, sufficient IgG is produced to prevent re-infection. Vaccinations use this process to prevent initial infections and add to the catalog of IgG antibodies, by exposing a person to a weakened, live microorganism or to an antigen that stimulates recognition of the microorganism. *IgG is the only immunoglobulin that can pass through the placenta. The mother's IgG antibodies provide protection to the fetus during pregnancy and to the baby during its first few months of life.* There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4.

SIGNIFICANCE

- Polyclonal IgG increase is seen in SLE, Chronic liver diseases, Infectious diseases and Cystic fibrosis.
-Monoclonal IgG increase is seen in IgG Myelomas.
-Decreased synthesis of IgG is found in Congenital and Acquired Immunodeficiency diseases and selective IgG subclass deficiency.
-Decreased IgG levels are seen in Protein losing enteropathies, Nephrotic syndrome and skin burns

	IMMUNOGLOBULIN IGM		
IMMUNOGLOBULIN IgM	47.80	mg/dL	37.0 - 224.0

Methodology: ECLIA

CLINICAL NOTES

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells in response to bacteria, viruses, and other microorganisms as well as exposures to other substances that are recognized by the body as "non-self" harmful antigens.

Immunoglobulin M (IgM) – IgM antibodies are produced as a body's first response to a new infection or to a new "non-self" antigen, providing short-term protection. They increase for several weeks and then decline as IgG production begins.IgM is the largest immunoglobulin molecule that makes 6% of the total immunoglobulins. It is the first specific antibody to appear in serum after infection which is capable of activating complement and killing bacteria. Post infection IgM returns rapidly to normal levels as compared to IgG. If IgM is prevalent, the infection is acute whereas if IgG predominates, the infection is chronic. Polyclonal IgM increase is seen in viral, bacterial and parasitic infections, Liver diseases, Rheumatoid arthritis, Scleroderma, Cystic fibrosis & heroin addiction. Monoclonal IgM increase is seen in Waldenstroms macroglobulinemia. Decreased synthesis of IgM is found in Congenital and Acquired Immunodeficiency diseases. Decreased IgM levels are seen in Protein losing enteropathies and skin burns

SIGNIFICANCE

-Polyclonal IgM increase is seen in viral, bacterial and parasitic infections, Liver diseases, Rheumatoid arthritis, Scleroderma, Cystic fibrosis & heroin addiction.
-Monoclonal IgM increase is seen in Waldenstroms macroglobulinemia.
-Decreased synthesis of IgM is found in Congenital and Acquired Immunodeficiency diseases- Decreased IgM levels are seen in Protein losing enteropathies and skin burns

	IMMUNOGLOBULIN A (IGA)		
IMMUNOGLOBULIN A (IgA)	334.50	mg/dL	61.0 - 356.0

DR. MD ARIF
MBBS, MD(PATHOLOGY)
LAB DIRECTOR

DR. EKTA TIWARI
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CONSULTANT PATHOLOGIST

DR. NISHTHA MALIK
MBBS, MD
CONSULTANT MICROBIOLOGIST

Find us on

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Regional Lab :- Shop No. 410, Near Ladla Steel, Old Char Chaman, Karnal 132001 (Haryana)

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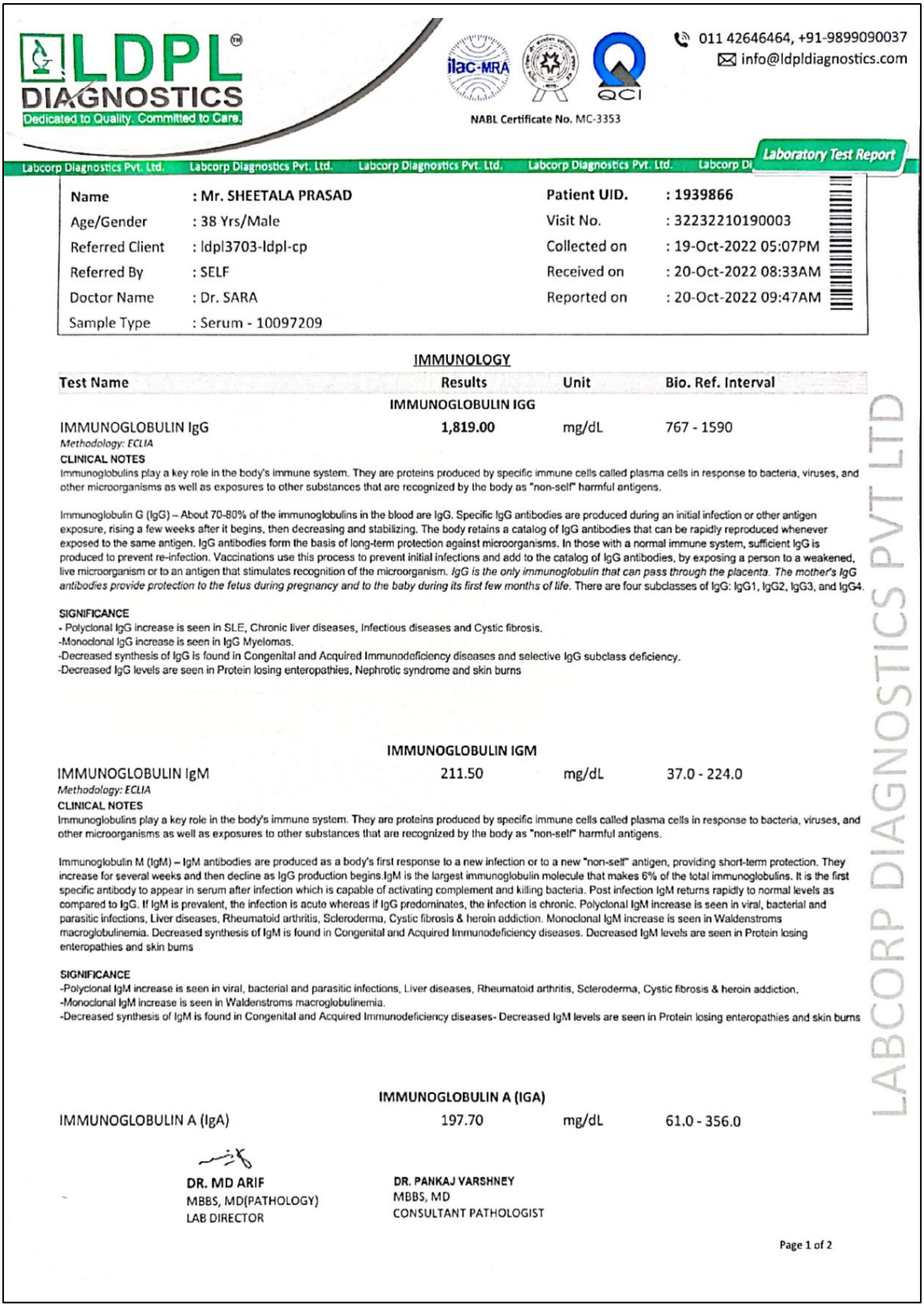
3b. LAB REPORT OF CASE GROUP (SUBGROUP 2)



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NABL Certificate No. MC-3353

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info@ldpldiagnostics.com

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Laboratory Test Report

Name	: Mr. SHEETALA PRASAD	Patient UID.	: 1939866
Age/Gender	: 38 Yrs/Male	Visit No.	: 32232210190003
Referred Client	: ldpl3703-ldpl-cp	Collected on	: 19-Oct-2022 05:07PM
Referred By	: SELF	Received on	: 20-Oct-2022 08:33AM
Doctor Name	: Dr. SARA	Reported on	: 20-Oct-2022 09:47AM
Sample Type	: Serum - 10097209		

IMMUNOLOGY

Test Name	Results	Unit	Bio. Ref. Interval
IMMUNOGLOBULIN IGG			
IMMUNOGLOBULIN IgG	**1,819.00**	mg/dL	767 - 1590

Methodology: ECLIA

CLINICAL NOTES

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells in response to bacteria, viruses, and other microorganisms as well as exposures to other substances that are recognized by the body as "non-self" harmful antigens.

Immunoglobulin G (IgG) – About 70-80% of the immunoglobulins in the blood are IgG. Specific IgG antibodies are produced during an initial infection or other antigen exposure, rising a few weeks after it begins, then decreasing and stabilizing. The body retains a catalog of IgG antibodies that can be rapidly reproduced whenever exposed to the same antigen. IgG antibodies form the basis of long-term protection against microorganisms. In those with a normal immune system, sufficient IgG is produced to prevent re-infection. Vaccinations use this process to prevent initial infections and add to the catalog of IgG antibodies, by exposing a person to a weakened, live microorganism or to an antigen that stimulates recognition of the microorganism. *IgG is the only immunoglobulin that can pass through the placenta. The mother's IgG antibodies provide protection to the fetus during pregnancy and to the baby during its first few months of life.* There are four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4.

SIGNIFICANCE

- Polyclonal IgG increase is seen in SLE, Chronic liver diseases, Infectious diseases and Cystic fibrosis.
-Monoclonal IgG increase is seen in IgG Myelomas.
-Decreased synthesis of IgG is found in Congenital and Acquired Immunodeficiency diseases and selective IgG subclass deficiency.
-Decreased IgG levels are seen in Protein losing enteropathies, Nephrotic syndrome and skin burns

IMMUNOGLOBULIN IGM			
IMMUNOGLOBULIN IgM	211.50	mg/dL	37.0 - 224.0

Methodology: ECLIA

CLINICAL NOTES

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells in response to bacteria, viruses, and other microorganisms as well as exposures to other substances that are recognized by the body as "non-self" harmful antigens.

Immunoglobulin M (IgM) – IgM antibodies are produced as a body's first response to a new infection or to a new "non-self" antigen, providing short-term protection. They increase for several weeks and then decline as IgG production begins.IgM is the largest immunoglobulin molecule that makes 6% of the total immunoglobulins. It is the first specific antibody to appear in serum after infection which is capable of activating complement and killing bacteria. Post infection IgM returns rapidly to normal levels as compared to IgG. If IgM is prevalent, the infection is acute whereas if IgG predominates, the infection is chronic. Polyclonal IgM increase is seen in viral, bacterial and parasitic infections, Liver diseases, Rheumatoid arthritis, Scleroderma, Cystic fibrosis & heroin addiction. Monoclonal IgM increase is seen in Waldenstroms macroglobulinemia. Decreased synthesis of IgM is found in Congenital and Acquired Immunodeficiency diseases. Decreased IgM levels are seen in Protein losing enteropathies and skin burns

SIGNIFICANCE

-Polyclonal IgM increase is seen in viral, bacterial and parasitic infections, Liver diseases, Rheumatoid arthritis, Scleroderma, Cystic fibrosis & heroin addiction.
-Monoclonal IgM increase is seen in Waldenstroms macroglobulinemia.
-Decreased synthesis of IgM is found in Congenital and Acquired Immunodeficiency diseases- Decreased IgM levels are seen in Protein losing enteropathies and skin burns

IMMUNOGLOBULIN A (IGA)			
IMMUNOGLOBULIN A (IgA)	197.70	mg/dL	61.0 - 356.0

DR. MD ARIF
MBBS, MD(PATHOLOGY)
LAB DIRECTOR

DR. PANKAJ VARSHNEY
MBBS, MD
CONSULTANT PATHOLOGIST

Page 1 of 2

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4a. CONSENT FORM (ENGLISH)

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)

BBD City, Faizabad Road, Lucknow – 227105

(INDIA)

Title of the Study

Study Number

Subject's Full Name

Date of Birth/Age

Address of the Subject

Phone no. and e-mail address

Qualification

Occupation: Student / Self Employed / Service /Housewife/ Other (Please tick as appropriate)

Annual income of the Subject

Name and of the nominees(s) and his relation to the subject _____
(For the purpose of compensation in case of trial related death)

1. I confirm that I have read and understood the Participant Information Document dated _____ for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor's

behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.

4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes [ ] No [ ] Not Applicable [ ]**
6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally acceptable Representative:

_____

Signatory's Name _____ Date _____

Signature of the Investigator _____ Date _____

Study Investigator's Name _____ Date _____

Signature of the witness _____ Date _____

Name of the witness _____

Received a signed copy of the PID and duly filled consent form

Signature/thumb impression of the subject or legally acceptable Representative

_____

Date _____

4b. CONSERT FORM (HINDI)

Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

सहमति पत्र

अध्ययन शीर्षक.....
अध्ययन संख्या.....
प्रतिभागी के पूर्ण नाम.....
जन्म तिथि / आयु.....
प्रतिभागी का पता .....
फोन नं. और ई-मेल पता .....
योग्यता .....
व्यवसाय: छात्र / स्व कार्यरत / सेवा / ग्रहिणी .....
अन्य (उचित रुप मे टिक करें) .....
प्रतिभागी की वार्षिक आय .....
प्रत्याशीयो के नाम और प्रतिभागी से संबंध...(परीक्षण से संबंधित मौत के मामले मे मुआवजे के प्रयोजन के लिए)

1. मेरी पुष्टि है कि मैने अध्ययन हेतु सुचना पत्र दिनांक ..... को पढ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।

2. मैंने यहाँ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है और किसी भी दबाव के बिना स्वतंत्र इच्छा के साथ दिया है किसी भी समय किसी भी कारण के बिना , मेरे इलाज या कानूनी अधिकारो को प्रभावित किए बिना , अध्ययन में भाग न लेने के लिए स्वतंत्र हुँ ।

3. मैंने यह समझ लिया है कि अध्ययन के प्रायोजक , प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नही है, चाहे मैने इस अध्ययन से नाम वापस ले लिया है। हॉलाकि मै यह समझता हुँ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नही दी जायेगी।

4. मै इससे सहमत हूँ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नही है।
5. भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक/रक्त) पर अध्ययन के लिए अपनी सहमति देता हुँ।
हॉ [ ] नही [ ] अनउपयुक्त [ ]

6. मै परीक्षण की अनुमति देता हुँ। मुझे इसके द्वारा यदि कोई परेशानी होती है, इसके बारे में जानकारी दे दी गई है। मैने रोगी जानकारी सूचना पत्र को पढ तथा समझ लिया है।

प्रतिभागी / कानूनी तौर पर स्वीकार्य प्रतिनिधि का हस्ताक्षर (या अंगूठे का निशान.....

हस्ताक्षरकर्ता का नाम..... दिनांक .....अन्वेषक के हस्ताक्षर ..... दिनांक .....

अध्ययन अन्वेषक का नाम .....

गवाह के हस्ताक्षर ..... दिनांक .....गवाह के नाम .....

मैनें पीआईडी और विधिवत भरे सहमति फार्म का एक हस्ताक्षर की नकल प्राप्त की.

प्रतिभागी कानूनी तौर पर प्रतिनिधि का हस्ताक्षर/ अंगूठे का निशान ..... दिनांक.....

5. INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Evaluation of Serum Immunoglobulin (IgG, IgM, IgA) as Diagnostic Markers in Oral Premalignant Disorders and Oral Cancer Patients: A Case Control Study."** submitted by **Dr Sarah Afaque** Post graduate student from the **Department of Oral Medicine & Radiology** as part of MDS Curriculum for the academic year 2020-2023 with the accompanying proforma was reviewed by the Institutional Research Committee present on **12th October 2021** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

6. INSTITUTIONAL ETHICAL CLEARANCE

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the IXth Institutional Ethics Sub-Committee

IEC Code: 34 **BBDCODS/04/2022**

Title of the Project: Evaluation of Serum Immunoglobulin (IgG, IgM, IgA) as diagnostic markers in oral premalignant disorders and oral cancer patients: A case control study.

Principal Investigator: Dr Sarah Afaque **Department:** Oral Medicine & Radiology

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Research

Dear Dr Sarah Afaque,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 07th April, 2022.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. Puneet Ahuja)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

7. STATISTICAL ANALYSIS

Arithmetic mean ($\bar{x}$)

Mean is one of the measures of central tendency. It finds the average value for the given data/observations. Arithmetic mean is defined as the sum of all the numbers in the data divided by the total count of numbers. The formula for finding the mean is given by,

$$\bar{x} = \frac{\sum x}{n}$$

Where $\sum x$ is summation of all observations

n = Total number of observations

Standard Deviation (σ)

Standard deviation measures the amount of variation/dispersion of a set of values. Dispersion tells how much data is spread out. A lower standard deviation indicates that data is close to the center. The higher value of standard deviation represents that data spread is more.

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Standard Error

The standard error is one of the mathematical tools used in statistics to estimate the variability. It is abbreviated as SE. The standard error of a statistic or an estimate of a parameter is the standard deviation of its sampling distribution. We can define it as an estimate of that standard deviation.

Standard Error Formula

The accuracy of a sample that describes a population is identified through the SE formula. The sample mean which deviates from the given population and that deviation is given as.

$$SE_x = \frac{S}{\sqrt{n}}$$

Where,

S is the standard deviation

n is the number of observation

P-Value

P-Value or probability value can be defined as the measure of the probability that a real-valued test statistic is at least as extreme as the value actually obtained.

The mentioned P in the text indicates the following:

P value	**Wording**	**Summary**
≥ 0.05	Not significant	ns
0.01 to 0.05	Significant	*
0.001 to 0.01	Very significant	**
0.0001 to 0.001	Extremely significant	***
< 0.0001	Very Highly significant	****

ANOVA

Analysis of Variance (**ANOVA**) is a statistical formula used to compare variances across the means (or average) of different groups. A range of scenarios use it to determine if there is any difference between the means of different groups.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F Value
Between Groups	$SSB = \Sigma n_j(\bar{X}_j - \bar{X})^2$	$df_1 = k - 1$	$MSB = SSB / (k - 1)$	$f = MSB / MSE$
Error	$SSE = \Sigma\Sigma(X - \bar{X}_j)^2$	$df_2 = N - k$	$MSE = SSE / (N - k)$	
Total	$SST = SSB + SSE$	$df_3 = N - 1$		

F = MST/MSE

MST = SST/ p-1

MSE = SSE/N-p

SSE = $\sum$ (n−1)

s2

Where,

F = Anova Coefficient

MSB = Mean sum of squares between the groups

MSW = Mean sum of squares within the groups

MSE = Mean sum of squares due to error

SST = total Sum of squares

p = Total number of populations

n = The total number of samples in a population

SSW = Sum of squares within the groups

SSB = Sum of squares between the groups

SSE = Sum of squares due to error

s = Standard deviation of the samples

N = Total number of observations

Tukey's multiple comparison test

Tukey's multiple comparison test is a single-step multiple comparison procedure and statistical test. It can be used to find means that are significantly different from each other.

Tukey's test is based on a formula very similar to that of the *t*-test. In fact, Tukey's test is essentially a *t*-test, except that it corrects for family-wise error rate.

The formula for Tukey's test is:

$$q_s = \frac{Y_A - Y_B}{SE}$$

where,

Y_A - Larger of the two means being compared,

Y_B - Smaller of the two means being compared, and

SE - Standard error of the sum of the means.

The q_s value can then be compared to a q value from the studentized range distribution. If the q_s value is *larger* than the critical value q_α obtained from the distribution, the two means are said to be significantly different at level $\alpha : 0 \leq \alpha \leq 1$

CASE NO.	OPD NO.	NAME	AGE	SEX	OCCUPATION	MOUTH OPENING	IgG	IgM	IgA		
1	3766	BABITA SINGH	40	F	HOUSEWIFE	44MM	800	47.4	90		
2	10833	RAHUL	35	M	STUDENT	52MM	820	45.4	94		
3	14711	TAPASI	22	F	STUDENT	40MM	850	40	102.4		
4	15369	PRAKHAR	19	M	STUDENT	54MM	900	54.2	106.4		
5	17004	VINOD KUMAR	35	M	SALESMAN	58MM	920	52	92		
6	17010	GEETA PAL	20	F	HOUSEWIFE	42MM	856	45.1	76.5		
7	17710	UMA MISHRA	36	F	HOUSEWIFE	40MM	890	43	95		
8	18676	PUJA KUMARI	22	F	EMPLOYER	39MM	895	56	85.5		
9	17718	NEHA PAL	27	F	HOUSEWIFE	37MM	1,479	182.9	183.5		
10	22586	SURENDRA PRATAP	53	M	ENGINEER	58MM	950	46	88.2		
11	25528	MALA	32	F	EMPLOYER	42MM	856	56	97.2		
12	26483	RADHA	40	F	HOUSEWIFE	45MM	799	68.1	72.5		
13	28857	SHOAIB ALI	19	M	TECHNICIAN	56MM	834	39	98.4		
14	29094	MOHD ARIF	22	M	TECHNICIAN	55MM	886	44	82		
15	29331	RAJKAMAL	35	M	SELF EMPLOYED	52MM	792	56	78.6		
16	29339	ARCHANA YADAV	24	F	HOUSEWIFE	48MM	820	57.8	99.4		
17	29335	LAXMIKANT YADAV	32	M	EMPLOYER	56MM	932	56	90.2		
18	29345	DIVYANSH	20	M	STUDENT	54MM	943	48.5	82.3		
19	32944	ABDUL RAHEEM	17	M	EMPLOYER	52MM	876	47	75.5		
20	36669	MANISHA	35	F	HOUSEWIFE	48MM	788	56.2	86.6		

CASE NO.	OPD NO.	NAME	AGE	SEX	OCCUPATION	MOUTH OPENING	P/D	767-1590m	7.0-224.0m	1.0-356.0mg/dL)	
1	6306	NARESH JAISWAL	45	M	SELF EMPLOYED	21MM	OSMF GRADE 3	884	148	110	
2	6779	SUNIL PANDEY	46	M	VENDOR	51MM	SPECKLED LEUKOPLAKIA WRT RT BUCCAL MUCOSA, HOM LEUKOPLAKIA WRT LEFT BUCCAL MUCOSA WITH PSEUDOMEM CANDIDIASIS	1649	48.1	204.1	
3	6521	FATIMA	37	F	HOUSEWIFE	46MM	ORAL LICHEN PLANUS RETICULAR TYPE	1459	90.1	253	
4	6525	PAWAN TRIPATHI	42	M	SELF EMPLOYED	54MM	HOM LEUKOPLAKIA WRT BUCCAL MUCOSA BIL	1239	89.5	292.8	
5	7072	SHUBHASH	68	M	VENDOR	38MM	HOM LEUKOPLAKIA WRT BUCCAL MUCOSA, ANGULAR CHEILITIS	1562	48.1	183	
6	8163	SIYARAM	54	M	SELF EMPLOYED	42MM	SPECKLED LEUKOPLAKIA WRT LT BUCCAL MUCOSA, HOM LEUKOPLAKIA WRT RT BUCCAL MUCOSA	1205	98.5	203.6	
7	3918	MAHESH	28	M	COOK	41MM	OSMF GRADE 1	1345	148	350.3	
8	10843	RN SAHNI	48	M	JOURNALIST	42MM	HOM LEUKOPLAKIA WRT VENTRAL SURFACE OF TONGUE	1685	140.3	354	
9	13762	RAJ KISHOR	62	M	SELF EMPLOYED	51MM	HOM LEUKOPLAKIA WRT BUCCAL MUCOSA BIL	1100	49.5	253.8	
10	13780	SANTOSH K PANDEY	42	M	FARMER	40MM	OSMF GRADE 1	920	55.8	254	
11	18009	UMESH KUMAR	37	M	TECHNICIAN	55MM	ORAL LICHEN PLANUS RETICULAR TYPE	960	52.4	360.2	
12	17861	PRIYA	27	F	EMPLOYER	21MM	OSMF GRADE 3	1456	51.1	298.3	
13	18316	MANISH PANDEY	31	M	ENGINEER	37MM	OSMF GRADE 2	1185	55.8	342.6	
14	18670	SANJAY SINGH	49	M	BUSINESSMAN	48M	HOM LEUKOPLAKIA BIL	1335	47.8	334.5	
15	21275	RAMJI	28	M	SELF EMPLOYED	49MM	SPECKLED LEUKOPLAKIA WRT LT BUCCAL MUCOSA, HOM LEUKOPLAKIA WRT RT BUCCAL MUCOSA	1211	90.1	194.3	
16	22120	MANMOHAN DAS	62	M	SELF EMPLOYED	52MM	HOM LEUKOPLAKIA WITH SUP CANDIDIASIS	1100	48	253.2	
17	26025	RASHID ZAMAL	25	M	SELF EMPLOYED	50MM	HOM LEUKOPLAKIA WRT BUCCAL MUCOSA BIL	1050	110.2	102.5	
18	26492	KUNJ BIHARI	48	M	SELF EMPLOYED	45MM	HOM LEUKOPLAKIA BIL, ANGULAR CHEILITIS	980	98.7	282.8	
19	3152/22	NEERAJ	45	M	GOVT EMPLOY	51MM	NON-HOM LEUKOPLAKIA IRT RT BUCCAL MUCOSA	880	87.6	99.2	
20	31480	DHARMENDRA KUMAR	50	M	SELF EMPLOYED	48MM	HOM LEUKOPLAKIA IRT LABIAL MUCOSA	1394	208	184	

CASE NO.	OPD NO.	NAME	AGE	SEX	OCCUPATION	MOUTH OPENING	FINAL DIAGNOSIS	IgG	IgM	IgA
1	13303	RAMLOT	50	M	UNEMPLOYED	30MM	OSCC LEFT BUCCAL MUCOSA	1216	85.1	239.9
2	13559	SUBEDAR	65	M	FARMER	25MM	OSCC RT LAT BORDER OF TONGUE	1658	161.1	330.4
3	13766	INDRAPRASAD RAJARAM	69	M	UNEMPLOYED	28MM	OSCC RT BUCCAL MUCOSA	1236	98.5	269.5
4	15128	RAM SUBHAG	57	M	FARMER	19MM	CA TONGUE RT BORDER	1656	164.4	230.4
5	17449	RAM AVTAR	60	M	FARMER	25MM	CA LEFT BUCCAL MUCOSA	1116	88.5	219.5
6	18241	DHIRENDRA	44	M	VENDOR	30MM	OSCC RT SIDE ALVEOLAR MUCOSA	1919	251.5	157.7
7	21032	VIJAY	45	M	SELF EMPLOYED	29MM	OSCC RT SIDE ALVEOLAR MUCOSA	1459	241.8	188.6
8	21642	RAMAKANT YADAV	25	M	VENDOR	28MM	OSCC RT BUCCAL MUCOSA	1216	85.1	239.9
9	3709	SHEETLA PRASAD	37	M	TECHNICIAN	26MM	OSCC LT SIDE ALVEOLAR MUCOSA	1819	211.5	197.7
10	23274	NAVJOT	35	M	TECHNICIAN	28MM	OSCC RT BUCCAL MUCOSA	1319	80.7	245.8
11	23812	MANOJ	32	M	VENDOR	32MM	CA TONGUE RT BORDER	1453	118	209
12	29606	AYUB AHMAD	40	M	SELF EMPLOYED	27MM	CA TONGUE LEFT BORDER	1668	161.1	348.4
13	31483	SURYAMADAN SINGH	65	M	SELF EMPLOYED	28MM	OSCC RT SIDE ALVEOLAR MUCOSA	1619	231.8	188.6
14	31746	SOMNATH	52	M	TECHNICIAN	26MM	CA TONGUE LEFT BORDER	1561	209.5	198.2
15	32095	CHANDRAKANT	70	M	UNEMPLOYED	29MM	CA LEFT BUCCAL MUCOSA	1556	228	187.5
16	32459	SOUKAR SINGH	65	M	SELF EMPLOYED	27MM	CA TONGUE RT BORDER	1165	119.4	167.4
17	33631	JAI PRAKASH	65	M	UNEMPLOYED	26MM	OSCC RT SIDE ALVEOLAR MUCOSA	1254	123.2	110
18	33658	CHOTU LAL	48	M	DRIVER	27MM	CA LEFT BUCCAL MUCOSA	1119	90.7	260.6
19	4241	MAHESH	35	M	EMPLOYER	22MM	CA TONGUE RT BORDER	1090	201.2	159.3
20	37355	ANIL YADAV	40	M	EMPLOYER	30MM	OSCC RT BUCCAL MUCOSA	1139	85.7	265.4

10. PLAGIARISM REPORT

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