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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/20360

DOI URL: <http://dx.doi.org/10.21474/IJAR01/20360>



RESEARCH ARTICLE

BIOCHEMICAL METABOLITE PROFILING IN ORAL POTENTIALLY MALIGNANT DISORDERS: A DIAGNOSTIC AND PROGNOSTIC APPROACH

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Manuscript Info

Manuscript History

Received: 06 December 2024

Final Accepted: 11 January 2025

Published: February 2025

Key words:-

Oral Cancer, Leukoplakia, Erythroplakia, Lichen Planus, OPMDs

Abstract

Cancer is characterised by the uncontrolled cell growth that disrupt surrounding tissues. Oral cancer (OC) typically starts as a tiny, unexplained growth or lesion of oral cavity extending to the oropharynx. Alterations in antioxidant defence, whether through an increase or decrease, can damage macromolecules, including proteins and other biochemical metabolites. Thus, protein, lipid profile and other biochemical metabolites can be potential biomarkers in oral premalignant. This research aims to determine and compare the levels of serum proteins, Lipid profile, urea, uric acid, and other metabolites in Oral Potentially Malignant Disorders (OPMDs) and Healthy Controls (HC). One hundred sixty males with healthy control were between 22 and 55. A substantial decrease in serum levels of uric acid, total protein, total cholesterol, HDL, VLDL and triglycerides (TG) was detected in subjects with oral precancerous lesions compared to controls. Additionally, oral cancer subjects exhibited elevated serum glucose, urea, and creatinine levels and reduced plasma lipid levels compared to precancerous subjects. The analysis revealed an inverse relationship between plasma lipid levels and the progression of oral diseases. Low serum levels of uric acid, total cholesterol, total protein, triglycerides, and HDL may serve as indicators of an increased risk for developing precancerous conditions, particularly in individuals with tobacco use. Elevated serum levels of creatinine and glucose and low lipid profile parameters could be potential diagnostic markers for assessing oral cancer and precancer, alongside other biochemical markers.

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Introduction:-

Cancer is categorised by the uncontrolled cell growth that invade and impairment of surrounding tissues. Oral cancer (OC) typically begins as a small, unexplained growth or lesion within the oral cavity, which includes the cheeks, lips, sinuses, hard and soft palates, tongue, and the mouth base scattering to the oropharynx. OC, a very common cancer worldwide in which India contributes nearly thirty percent of the global OC burden [1,2]. OC represents an important health challenge, especially in countries enduring economic transitions. The increasing prevalence of OC is a major public health concern, mainly because late-stage detection minimizes the chances of successful treatment with five-year survival rates typically around 20% [3,4].

Sholar, P.W (2014) stated that the World Health Organization (WHO) focused on a broad spectrum of conditions grouped in the term "Potentially malignant disorders of the oral mucosa (PMDs) in 2005, "which are now referred to as oral potentially malignant disorders (OPMDs) (Bhatia et al., 2013). OPMDs encompass a range of oral mucosal lesions with a high likelihood of progressing to cancer [5,6]. These conditions include leukoplakia (OLK), erythroleukoplakia (OLE), oral lichen planus (OLP), oral dysplasia and oral submucous fibrosis (OSF), all of which vary in their clinical presentation, histological types, and associated risk factors or causes. Oral squamous cell carcinoma (OSCC) occurs most prevalently, particularly in low- and middle-income countries (Chaiyarit, 2016). It is characterised by aggressive behaviour, a tendency for lymph node metastasis, and a generally poor prognosis. The overall five-year survival rate for OSCC is as low as 40%, but early detection (in stages I and II) can improve survival rates to over 80%. Early discovery of this illness aids in preventing premalignant lesions and diagnosing and treating this disease, which is required to transform premalignant lesions into OSCC and increase the 5-year survival rate [7].

Tobacco and alcohol are the two primary risk factors for patients across the world [8,9], accounting for around 75% of all OC [14]. Smoking and drinking together have a synergistic influence on the development of oral and oropharyngeal cancers, as well as lip and oral cavity malignancies [15]. According to a recent estimate by the International Agency for Research on Cancer, the ASR (age-standardized rate) crude rate affecting the global population will be 14.1 in 2024 [16]. Numerous risk factors have been associated with OPMDs, many of which overlap with those for OSCC, though the exact mechanisms and causes of malignant transformation remain unclear. The primary risk factors for OPMDs include lifestyle habits such as smoking, alcohol consumption, the use of betel nut derivatives [12,13].

Material Method:-

Study Design

The study was conducted at the Department of Oral Medicine, Kusum Debi Sundarlal Dugar Jain Dental College and Hospital, Cossipur, Kolkata, India, and received approval from the institutional ethical committee (Ref no. 08/IEC/RNLKWC/2024). It included 120 patients with oral potentially malignant disorders (OPMDs), divided into four groups: OLP (Group 1, n=40), OLK (Group 2, n=40), OLE (Group 3, n=40), and healthy controls (HC) (Group 4, n=40) for comparison. The analysis used a Student's t-test in Microsoft Excel 2024 (Version 16.89.1). Exclusion criteria included patients with abnormal clinical biochemistry/haematology, hepatitis B or C, AIDS, kidney, liver, lupus, lymphoproliferative, or heart disorders, and other cancers. Written informed consent was obtained from all participants, and venous blood samples were collected under sterile conditions for serum extraction.

Patients with abnormal clinical biochemistry or haematology results, as well as those diagnosed with acquired immune deficiency syndrome (AIDS) or hepatitis B or C, were excluded in the study. Written informed consent was acquired from all participants involved in the study. Individuals with kidney, liver, lupine, lymphoproliferative, heart disorders, and other cancers were excluded to prevent erroneous positive results. Venous blood samples were collected and serum extracted under sterile conditions.

Measurement Of Blood Biochemical Parameters:

Serum glucose levels were measured using an automated analyser, with 10 μ L of serum mixed with glucose reagent and compared to a glucose standard. After incubation for 10 minutes at room temperature or 5 minutes at 37°C, absorbance was measured at 500 nm using a Biosystem diagnostic kit [17-21]. Total cholesterol (TC) was assessed via an automated method, where 10 μ L of serum was mixed with a reagent and incubated for 10 minutes at 37°C. Absorbance was measured at 505 nm, and concentration was calculated using a standard curve [22-25]. Triglyceride (TG) levels were determined similarly by mixing 10 μ L of serum with a reagent and incubating for 10 minutes at

37°C. Absorbance was read at 505 nm using the Autospan liquid gold Triglyceride CHOD-PAP kit[26-29]. For VLDL cholesterol, 20 µL of serum was combined with a reagent and incubated at 37°C for 10 minutes, with absorbance measured at 340 nm using the VLDL BioAssay Systems kit [30-32]. LDL levels were evaluated by mixing 10 µL of serum with LDL reagent 1, incubating for 5 minutes, adding reagent 2, and incubating for another 5 minutes. Absorbance was measured at 550 nm using the Autospan liquid gold Direct LDL kit[33-42]. Serum creatinine (CEA) was determined by combining 100 µL of serum with a reagent and measuring absorbance at 510 nm after a 2-minute incubation at 37°C, using the Prietest™ creatinine test kit[43-45]. Total protein levels were measured by mixing 10 µL of serum with a reagent and incubating for 5 minutes at 37°C. Absorbance was measured at 578 nm using the Autospan liquid gold total protein kit[46-52]. Serum uric acid (UA) was evaluated by mixing 20 µL of serum with a reagent, incubating for 15 min at room temperature or 37°C(Clark, A. F. (1997), and measuring absorbance at 520 nm using a coral clinical system diagnostic kit[53,54]. Urea levels were estimated by mixing 10 µL of serum with a reagent, incubating in boiling water for 10 minutes, and measuring absorbance at 520 nm after a 5-minute incubation at 37°C, using a coral clinical system diagnostic kit(55-57).

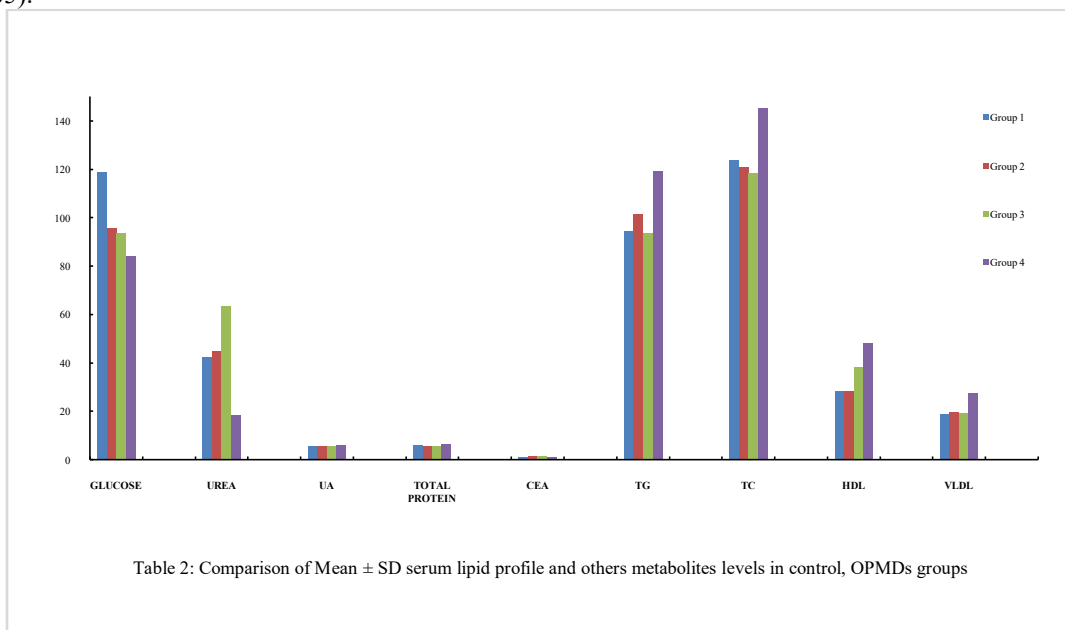
Result:-

In the present study, samples were selected in number for each disease group as compared to HC. The mean age of the OPMDs group 1 was 37.21 (range: 18–55 years), group 2 was 35.15 (range: 21–52 years), group 3 was 34.26, HC group was 26.03 (range: 24–50 years), and the mean difference between HC and OPMDs groups was not statistically significant [Table 1].

Table 1:- Age-wise distribution of participants in various disease groups.

Group	Age range	Mean±SD
Group 1 (OLP)	18-55	37.05±10.11
Group 2 (OLK)	21-52	35.37±8.88
Group 3 (OLE)	24-50	34.32±8.09
Group 4 (Control)	28-55	26.02±3.33

Table 2 and Graph 1 compare mean serum glucose values, lipid profile and other metabolite levels in the control and OPMD groups. The mean glucose level in the control group was 83.92, group 1 was 118.82, group 2 was 95.69 and group 3 was 93.52. serum glucose was raised in all the study groups. The alteration was statistically significant ($P \leq 0.05$). The mean TC level in the control group was 145.38, group 1 was 123.47, group 2 was 120.67 and group 3 was 118.52. The TC value in the OPMDs and Control groups was less than expected. P value came to OLP as 0.0029, OLE 0.0005 and 0.00003, respectively, for the OPMDs and HC groups, which was statistically significant ($P \leq 0.05$).



The mean serum TG level in the control group was 119.05, and OPMDs group 1 was 94.25, while for group 2, it was 101.02 and Group 3 was 93.22. The serum TG level in all the diseases was lower than usual. The mean serum HDL level in the control group was 48.21, with group 1 being 28.26, $P = 0.000$, group 2 being 28.27, $P = 0.000$, and group 3 being 38.37, $P=0.000$. All study groups' serum HDL cholesterol levels were below the ideal levels typical for healthy individuals. The P value was statistically significant for serum TG level and HDL in all the disease groups ($P \leq 0.05$). The mean serum VLDL level in the control group was 27.41, group 1 was 18.86, group 2 was 19.82, and group 3 was 19.32. Serum VLDL in OPMDs were statistically significant ($P \leq 0.05$).

Table 2:- Comparison of Mean \pm SD serum lipid profile and others metabolites levels in control, OPMDs groups.

Metabolites	Control	Group 1	Group 2	Group 3
Glucose	83.92 \pm 10.07	118.82 \pm 10.07 $P=0.00001$	95.69 \pm 17.55 $P=0.0004$	93.52 \pm 13.82 $P=0.0006$
Urea	18.18 \pm 3.21	42.24 \pm 3.21 $P=0.00004$	44.6 \pm 12.56 $P=0.00$	63.17 \pm 24.22 $P=0.000001$
UA	6.13 \pm 0.38	5.64 \pm 0.38 $P=0.01417$	5.62 \pm 0.59 $P=0.00002$	5.51 \pm 0.52 $P=0.000001$
Total protein	6.59 \pm 1.20	6.03 \pm 1.20 $P=0.02636$	5.46 \pm 0.88 $P=0.000009$	5.67 \pm 0.90 $P=0.00023$
CEA	0.82 \pm 0.08	1.03 \pm 0.08 $P=0.00010$	1.4 \pm 0.23 $P=0.000001$	1.17 \pm 0.24 $P=0.000001$
TG	119.05 \pm 17.58	94.25 \pm 18.05 $P=0.00340$	101.2 \pm 19.94 $P=0.00007$	93.22 \pm 21.16 $P=0.00001$
TC	145.38 \pm 30.83	123.47 \pm 30.83 $P=0.00293$	120.67 \pm 20.02 $P=0.00005$	118.52 \pm 23.74 $P=0.000038$
HDL	48.21 \pm 5.83	28.26 \pm 5.83 $P=0.00001$	28.27 \pm 4.81 $P=0.000001$	38.07 \pm 8.07 $P=0.000001$
VLDL	27.41 \pm 5.90	18.86 \pm 5.90 $P=0.000001$	19.82 \pm 1.89 $P=0.00001$	19.32 \pm 4.07 $P=0.00001$

The mean serum urea level in the control group was 18.18, and OPMDs group 1 was 42.24, while for group 2, it was 44.6 and Group 3 was 63.17. The serum urea value in all the disease groups was found to be increased than the typical value. P value was statistically significant in all the disease groups ($P \leq 0.05$).

The average serum UA level in the C group was 6.13, while in group 1 it was 5.64, in group 2 it was 5.62, and in group 3 it was 5.51. The overall protein level in the control group was 6.59, in group 1 it was 6.03, in group 2 it was 5.46, and in group 3 it was 5.67. The serum UA and total protein levels in OPMDs demonstrated statistical significance ($P \leq 0.05$). The average creatinine level in the HC group was 0.82, in group 1 it was 1.03, in group 2 it was 1.4, and in group 3 it was 1.17. The alteration was statistically significant ($P \leq 0.05$).

Discussion:-

Cholesterol, an amphipathic lipid, is necessary for the structure of cell membranes and the outer layers of plasma-lipoproteins((Grewal &Sankhyan, 2024)[58]. It has two forms: free cholesterol and cholesteryl ester; cholesterol merged with a long-chain fatty acid found in tissues and plasma lipoproteins. Synthesised from acetyl-CoA in various tissues, it is eventually removed from the body as cholesterol or bile salts. In the bloodstream, free cholesterol is transported by lipoproteins, exchanging with cholesterol in other lipoprotein membranes.

In the study of serum VLDL levels among precancer groups, including Group I (OLP), Group II (OLK), Group III (OLE), and Group IV(Controls), levels were reported as 18.86 \pm 5.90 mg%, 19.82 \pm 1.89 mg%, 19.32 \pm 4.07 mg%, and 27.4 \pm 5.90 mg%, respectively, in individuals with oral precancerous lesions. Head and neck cancer is a suggestive health concern, with tobacco product use being a major contributor to precancerous conditions. These conditions spur increased lipid use, including cholesterol, lipoproteins, and triglycerides, for creating new cell membranes. Individuals with oral precancerous lesions have a noteworthy predisposition to developing cancer, as tobacco carcinogens generate free radicals and ROS, quickening the oxidation of polyunsaturated fatty acids. Thus, there is higher lipid application for membrane formation, drawing lipids from the bloodstream or synthesising them through

metabolism or decomposition of significant lipoprotein fractions such as VLDL and HDL. This research showed a marked reduction in serum total cholesterol, VLDL, HDL, and triglycerides (TG) compared to controls in patients with oral precancer and cancer. A notable drop in serum TG and VLDL was explicitly met in oral cancer patients compared to those with precancerous lesions.

Uric acid, a catabolism product of purine nucleotides, is a significant antioxidant and potent free radical neutraliser in human fluids. Besides eliminating radicals, uric acid can bind metal ions like iron and copper, rendering them less reactive and unable to instigate free radical reactions (Glantzounis et al., 2005; Pasalic et al., 2012). Reports have highlighted uric acid's antioxidant role in various contexts (Waring et al., 2006; Hooper et al., 2000), with (Battino et al., 2008) finding notable differences in uric acid levels between patients and healthy controls, categorising it as a vital antioxidant in saliva (Sinbad, 2019). Our findings indicate reduced antioxidant uric acid levels, underscoring the need to investigate oxidative stress and free radicals in OLP, OLE, and OLK, involving larger patient groups and additional oxidative stress markers. The findings suggest a link between OLP, OLE, and OLK and decreased serum uric acid levels, marking uric acid as a valuable biomarker for assessing antioxidant status in precancerous treatment strategies and monitoring.

Urea and creatinine are nitrogenous byproducts of metabolism. Urea is primarily formed from dietary and tissue protein breakdown, while creatinine stems from muscle creatine breakdown. Urea can be expelled through saliva, with blood urea concentrations positively related to serum levels. In our study, patients exhibited elevated urea levels compared to healthy controls. High nitrogen compound levels in the blood may reveal underlying conditions, aiding in diagnosing halitosis and its root causes. Our findings indicate that serum CEA levels could be prospective tumour markers for screening oral precancer patients, with studies showing varied serum CEA activist rates among precancer patients, yet consistently higher than in healthy controls. Notably, smokers and drinkers showed elevated mean serum CEA levels compared to non-smokers and non-drinkers.

Free radicals are molecules with one or more unpaired electrons, allowing for independent existence. At high concentrations (Hassan et al., 2024) [60], they interact with intracellular macromolecules like DNA, proteins, carbohydrates, and lipids, promoting inflammation and carcinogenesis. Protein oxidation is significant in cancer pathogenesis, with reduced protein levels often seen in OPMDs (Curieses, 2021, p. 2600) [59]. In oral cancer, habits like tobacco and areca nut use considerably contribute to tissue damage, where free radicals play a crucial role. These habits are every day across all age groups. Serum protein levels decreased significantly in the precancerous stage, with results consistent with (Patidar KA et al., 2011). Intergroup serum protein comparisons were statistically significant ($p \leq 0.05$). Increased serum protein levels may result from inflammatory responses associated with oral malignancy. Our study found slight blood glucose level changes in precancerous conditions (leukoplakia/erythroplakia and lichen planus) compared to healthy controls, aligning with results of significantly high OLK and OLP prevalence, conflicting with this study's findings. Dietrich et al. noted that diabetic patients might face double the risk for OLK and OLP.

Conclusion:-

Precancerous cells can absorb lipids to support their growth and form phospholipid membranes. Identifying and understanding more enzymes in these metabolic pathways is crucial for accurately documenting these steps. Determining changes in metabolite levels through biochemical tests could be a key tool in evaluating precancerous conditions and other biochemical markers. The notably reduced serum cholesterol, triglycerides, and HDL cholesterol in patients with precancers, which was significant in our study, may be attributed to substantial alterations in cell integrity. The current study had limitations due to its small sample size.

In summary "Early detection is also known as secondary prevention," highlighting the need for further research with a larger sample size to gather epidemiological data and conduct long-term follow-up in precancerous and oral cancer patients. This will help us understand the initial changes and independence of the biochemical testing processes involved in carcinogenesis.

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