

**Systematic Review****Diagnostic and Prognostic Potential of Biochemical and Hematological Markers in Tobacco Users with Oral Pre-Cancer Lesions****Muhammad Rafique Tagar<sup>1</sup>, Arsalan Ahmed<sup>2</sup>, Muhammad Mustafa<sup>3</sup>, Muzaffar Qayum Khan Ghauri<sup>4</sup>, Muhammad Aqeel Aslam<sup>5</sup>, Shakeel Ahmed Tagar<sup>6</sup> and Shaharyar Ahsan<sup>7</sup>**<sup>1</sup>Department of Biochemistry, Bibi Aseefa Dental College, Shaheed Mohtarma Benazir Bhutto Medical University, Larkana, Pakistan<sup>2</sup>Department of Periodontics, Muhammad Dental College, Mirpurkhas, Pakistan<sup>3</sup>Department of Periodontics, Shahida Islam Dental College, Lodhran, Pakistan<sup>4</sup>Department of Orthodontics, Bhitai Dental and Medical College, Mirpurkhas, Pakistan<sup>5</sup>Department of Oral Medicine, Muhammad Dental College, Mirpurkhas, Pakistan<sup>6</sup>Institute of Pharmacology, Shaheed Mohtarma Benazir Bhutto Medical University, Larkana, Pakistan<sup>7</sup>Department of Research and Development, Scientia Nova, Islamabad, Pakistan**ARTICLE INFO****Keywords:**

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Department of Research and Development, Scientia Nova, Islamabad, Pakistan  
[shaharyarhasanofficial@gmail.com](mailto:shaharyarhasanofficial@gmail.com)Received Date: 16<sup>th</sup> May, 2024Acceptance Date: 27<sup>th</sup> June, 2024Published Date: 30<sup>th</sup> June, 2024**ABSTRACT**

Oral Pre-Cancer Lesions (OPLs) including leukoplakia, erythroplakia, and submucous fibrosis denote biochemical and histopathologically altered changes in the oral mucosa marked by subcellular and structural anomalies evocating of potential for a malignant transformation, which is primarily caused by tobacco exposure. Early diagnosis is of paramount importance to halt the progression of premalignant lesions to high-grade dysplasia and even oral cancer.

**Objective:** To find the diagnostic and prognostic potential of biochemical and haematological markers in Tobacco Users (TU) with OPL. **Methods:** PRISMA guidelines were followed to perform this systematic review. After retrieving 170 epidemiological studies published from 2013 to 2023, through multiple databases (PubMed, Google Scholar, Sci-hub, and Science Direct), 21 were included to determine the potential of biochemical and haematological markers in risk stratification and early detection of OPL. **Results:** According to the following systematic review, extracted data showed specific biochemical and haematological indicators that could serve as markers in risk stratification and early detection of OPL. The OPL group exhibited significantly higher levels of biochemical markers IL-6, IL-8, TNF- $\alpha$ , HCC-1, PF-4, FRR, TP, MDA, MMP-12, and Ceruloplasmin and hematological markers NLR, PLR, CRP, ESR, WBC, and low Hb as compared to the control group. Following risk stratification, a group with older age, tobacco association with OPL, and elevated levels of markers were categorised as a higher-risk group. **Conclusions:** The biochemical and haematological markers are potential promising markers in the early detection of OPL from malignant lesions with diagnostic and prognostic significance.

**INTRODUCTION**

Oral Premalignant Lesions (OPLs) of the oral cavity span a diverse array of pathology. OPL is defined as a morphologically altered or abnormal change in the tissue of the oral mucosa that exhibits potential for malignant transformation [1]. It was proposed as an Oral Potentially Malignant Disorder (OPMD) by the World Health Organization in 2005 [2]. These precancerous lesions include leukoplakia, submucous fibrosis and erythroplakia

[3]. The oral cavity is lined with stratified squamous epithelium which is sensitive to potential carcinogens [2]. Epithelium anomaly accompanied by exposure to carcinogens such as tobacco, alcohol, Human Papillomavirus (HPV) and betel nut might produce a cellular microenvironment that leads to the formation of dysplastic epithelium. It clinically manifests as premalignant oral lesions, leukoplakia, lichen planus, erythroplakia and has

diverse rates of progression to carcinoma. The presence of dysplastic epithelium in any of these entities underscores the necessity of histopathological assessment [4, 5]. OPL affect 1.5% to 4.5% of the global population with a higher prevalence in men compared to women [67]. The incidence was higher in Asian, South American and Caribbean populations, reflecting geographical variations attributable to different rates of alcohol and tobacco consumption [2]. Out of every 100th cancer case reported worldwide, 17 to 35 are of oral cancers. The Malignant Transformation Rate (MTR) of OPLs accounts for 0.7% to 2.9% annually [8, 9]. Globally, HPV is also a contributing factors for oral premalignant lesions [10]. However, tobacco use either smokeless or smoking, has emerged as one of the most significant factors among the various contributors to the development of all types of OPL and is composed of alkaloid nicotine and other harmful substances which are deadly carcinogens and toxic [9, 11, 12]. Leukoplakia develops as white patches or plaques on the oral mucosa; erythroplakia materializes as red patches; clinical manifestations of OSMF associated with fibrotic changes in the oral submucous lead to restricted mouth opening and chewing [13]. The progression of premalignant lesions to malignant oral cancer such as Oral Squamous Cell Carcinoma (OSCC) is accompanied by several stages, varying types of dysplasia and clinically prominent variable states of the oral mucosa [14, 15]. The malignant transformation rate of OPL to OSCC varied based on factors, population, gender, habits and dysplasia severity. Effective management and diagnosis of premalignant lesions at early stages aids in halting the progression to oral cancer and is a preeminent priority to reduce mortality and morbidity [16]. Histopathological moderate to severe degree of Oral Epithelial Dysplasia (OED) is a conventionally utilized cue to determine the risk of malignant transformation by inspection and palpation. However, this histologic method is sparse and results in inaccurate outcomes. As a substantial number of lesions that lack dysplastic alternation microscopically before advancement into oral cancer, OED and early OSCC appear as minor lesions of normal mucosa, whereas Leukoplakia and leukoedema are clinically similar to high-risk OPL. It indicates that the traditional approach of oral examination performed by an oral oncologist is unable to precisely detect and distinguish OED, early OSCC, other lesions and classify OPL as high risk or low risk and lead to diagnosis at advanced disease stages [17, 18]. The non-invasive approach involves markers that can identify premalignant oral lesions and may be effective for proactive intervention in patient groups at high risk [19]. Therefore, biochemical and haematological markers show significant potential in

mitigating diagnosis limitations and could serve as gold standards [20]. The biochemical markers include proinflammatory cytokine Matrix Metalloproteinase (MMP) Lactate Dehydrogenase (LDH) that can detect early cellular changes and inflammation associated with OPL [21-23]. Hematological markers include Complete Blood Count (CBC), differential count (DC), Neutrophil to Lymphocyte Ratio (NLR) and Erythrocyte Sedimentation Rate (ESR) which can indicate cellular dysregulation inflammation abnormalities in blood cell count associated with OPL [24]. To date, there are limited comprehensive studies on these markers. Therefore, this study is conducted with the objective of finding the diagnostic and prognostic potential of biochemical and haematological markers in Tobacco Users (TU) with OPL.

The systematic review aimed to augment existing literature by providing a comprehensive examination to assess the potential of biochemical and hematological markers in risk stratification and early detection of OPL in TU that would help clinicians and researchers to optimize their approach to the early detection of OPL and to arrest progression into oral cancer.

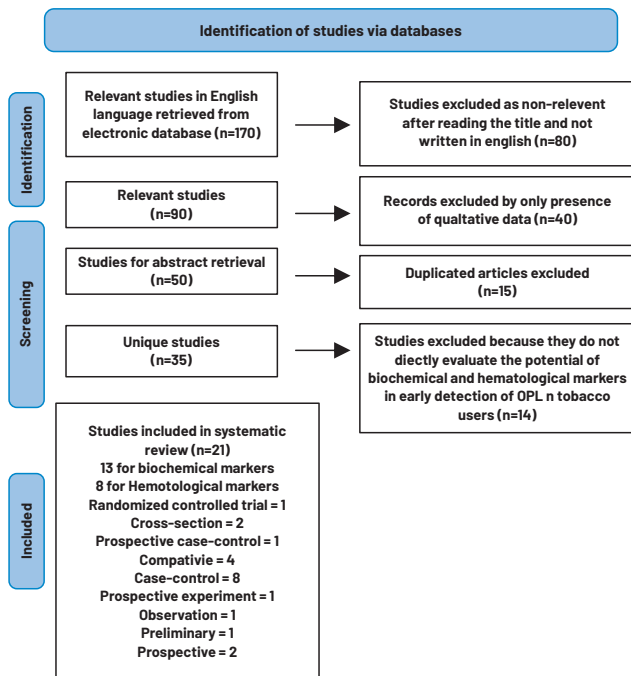
## METHODS

Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines were followed to perform this systematic review. The data for the last (omit this word) OMITTED ten years 2014-2023 was collected using several databases (PubMed, Google Scholar, Sci-hub and Science Direct) using Boolean logic "AND" and "OR" and searching through Medical Subject Headings (MeSH Terms) and keywords. Different terminologies were used to explore the literature "Potential Biochemical markers" and "Hematological markers" combined with "Premalignant oral lesions". A total of 170 articles were retrieved from the included databases. Out of all these studies, 21 articles were considered eligible after applying inclusion/exclusion criteria and deleting the duplicates and irrelevant articles (Figure 1). To determine the association of tobacco with OPL, the statistical test of Chi-square using Microsoft Excel 365 was applied to 9 studies included based on homogeneity in data. Other studies were excluded due to variances in characteristics like methodologies. With degree of freedom as 1 and a P-value less than 0.05 was used to determine the significant ( $p < 0.05$ ) association was found between TU and the group of subjects with OPL as compared to the control group (CL). The risk stratification was performed based on TU association with OPL or OPM, Age, biochemical and haematological markers level, and tobacco type and placed into different risk groups (Higher Risk Group, Moderate Risk Group, and Lower Risk Group). The group with OPL and OML with the use of tobacco and elevated levels of biochemical and hematological markers

was identified as a higher risk group, a group with TU considered as a moderate risk group and CL, non-tobacco user and without OPL designated low-risk group. To assess specificity, the studies chose a reference standard through histopathological examination like a biopsy, blood tests, or saliva tests, and cut-off values are established for each marker based on existing literature. They involved CL groups without OPL and evaluated multiple markers in combination. The endpoints of the study included potential and levels of biochemical and haematological markers, and discrimination between precancerous and cancerous lesions. To make sure that the respective marker was against the cancer and not against any other inflammatory disease or cause, studies generally included healthy control groups without oral lesions and no evidence of inflammation in the mouth. This allowed comparing marker levels between the OPL group and both control groups which differentiated markers specifically elevated in OPLs from those that might be elevated due to general inflammation. Furthermore, studies reviewed existing literature on potential markers. Established markers with documented associations with OPL progression provided a stronger foundation for further investigation. In figure 1, PRISMA flowchart depicting the study selection process for determining the potential of biochemical and haematological markers in risk stratification and early detection of Oral Pre-Cancer Lesions in tobacco users.

determine the potential of biochemical markers in risk stratification and early detection of OPL by comparing the patients who consumed tobacco with OPL and malignant lesions with CL [21-34]. The endpoints of the study included potential and levels of biochemical markers, and discrimination between precancerous and cancerous lesions. The pro-inflammatory cytokines IL-6, IL-8, TNF- $\alpha$ , HCC-1, PF-4, TNF- $\alpha$  and IL-6 and combined Proteomics, IL-8, IL 1 $\beta$ , transcriptomic, DUSP1 distinguished the OPL from malignant lesions and serve as potential markers in early detection of OPL and have diagnostic and prognostic significance [21, 27, 30]. Punyani et al., reported IL-8 alone as non-conclusive for OPL detection whereas IL-6 was found as a potential marker [29, 32]. FRR and TP are potential biochemical salivary markers for OPL with a sensitivity and specificity of 54.4% to 82.3% [26]. Nimal et al., reported low levels of GSH and serum albumin in TU, and TU with OPL as compared to CL [33]. The risk stratification was done considering the level of biochemical markers. They were correlated with the TU and presence of OPL or OPM. The group with OPL and OML with the use of tobacco and elevated levels of biochemical markers was identified as a higher risk group, a group with TU considered as a moderate risk group and CL, Non-TU and without OPL designated low-risk group [21-34].

Abbreviations: OPL: Oral Premalignant Lesions; CL: Control; Tu: Tobacco User; PM: Premalignant; Lk: leukoplakia OSF: Oral Submucous Fibrosis; OL: Oral Lichen; LDA: Lactate Dehydrogenase; IL: Interleukin; ADA: Adenosine Deaminase; FRR: Ferritin; TP: Total Protein; MMP; Matrix Metalloproteinase; GSH: Glutathione; MDA: Malondialdehyde; TNF: Tumor Necrosis Factor ; OSCC: Oral Squamous Cell Carcinoma ; PF4: Platelet Factor; HCC-1: Human CC Chemokine; HRG: Higher Risk Group; MRG: Moderate Risk Group; LRG: Lower Risk Group; SLT: Smokeless Tobacco.



**Figure 1:** Prisma Flowchart Depicting the Study Selection Process

## RESULTS

The available data evaluates the potential of biochemical and hematological markers in risk stratification and early detection of OPL. In the table 1, all of the identified studies

**Table 1:** Summary of Biochemical Markers and their Outcomes in risk Stratification and Early Detection of Premalignant Oral Lesions Human Samples

S.No	Groups	OPL	Tobacco User/PM	Sample (All Studies on Humans)	Chemical Markers	Outcomes	Risk Stratification	Study	Reference
1	CL Subjects (St): 26 TU St: 26 TU+OPL St: 26	PM	yes	Saliva	LDH	Increased LDH levels in TU with OPL compared to TU alone and CL LDH as potential /promising marker in early stages of PM progression to oral cancer	Subjects with OPML and TU are classified as high -risk group (HRG), TU only as moderate-risk (MRG), and CL as low-risk group (LRG)	Comparative	Javaraiah et al (2020)[23]
2	OPL St: 100 OSCC St: 100 CL St: 100	PM	yes	Saliva	TNF-α	Increased TNF-α levels in TU with OPL compared to TU alone and CL Potential /promising marker in detection of OPML	HRG: OSCC subjects LRG: OPML and CL	Prospective experimental	Krishnan et al (2020)[25]
3	OPML St: 33 OSCC St: 33 CL St: 33	OL	9	Saliva	IL-1α,IL-6, IL-8,IP-10 MCP-1, TNF-α, HCC-1, and PF-4	IL-6,IL-8,TNF-α, HCC-1, and PF-4 were discriminated OL, OSCC, and CL/serves as potential markers in early detection No correlation was found among tobacco	HRG: OL subjects LRG: CL subjects	Casecontrol	Dikova et al (2021)[21]
4	OPL St: 57 CL St: 32	OL	9.90%	Saliva	ADA,FRR, TP	FRR and TP are potential salivary markers for OPL with sensitivity and specificity 54.4% to 82.3%	HRG: OPL subjects LRG: OSCC and CL subjects	Casecontrol	López-Jornet et al (2023)[26]
5	OPL St: 60 OSCC St: 60 CL St: 60	PM	58/96.7%	Saliva	Proteomics, IL-8, IL 1β, transcrip-tomic, DUSP1	Combined Proteomics and transcriptomic markers discriminated OPL and OSCC and from CL/Potential for OPML detection Tobacco consumption was higher in the OPL group	HRG: OPL subjects LRG: OSCC and CL subjects	Casecontrol	Gleber-Netto et al (2016)[27]
6	OPL St: 30 OSCC St: 30 CL St: 30	Lk	17/56.7%	Saliva	TNF-α	Increased TNF-α levels in OSCC compared to OPL and CL TNF-α can be used as a marker for predicting premalignant oral lesions and distinguishing premalignant from malignant oral cancer	HRG: OPL subjects LRG: OSCC and CL subjects	Comparative	Ameena et al (2019)[28]
7	OPL St: 25 OSCC St: 25 CL St: 25	PM	25	Saliva	IL-8	IL-8 was found as non-conclusive for premalignant lesions /requires further research with large sample sizes	HRG: OPL subjects LRG: OSCC and CL subjects	Preliminary	Punyani et al (2013)[29]
8	OPL St: 19 OSCC St: 19 CL St: 19	PM	NR	Saliva	TNF-α and IL-6	Increased TNF-α and IL-6 levels in OSCC compared to OPL and CL Discriminated OSCC, OPL from CL /have diagnostic and prognostic significance	HRG: OPL subjects LRG: OSCC and CL subjects	Casecontrol	Jureti et al (2013)[30]
9	OPL St: 30 CL St: 30	PM	19	Blood	MDA	Increased MDA levels in OPL compared to CL Potential biomarker for early detection	HRG: OPL subjects LRG: CL subjects	Casecontrol	Mohideen et al (2021)[31]
10	OSF St: 30 OSCC St: 30 CL St: 30	OSF	19	Blood	MDA	OSCC>OSF>CL Increased MMP 12 levels in OSCC compared to OSF and CL MMP-12 markers serve as a non-invasive early diagnostic tool for premalignant oral lesions	HRG: OSF subjects LRG: OSCC and CL subjects	Casecontrol	Saleem et al (2019)[22]
11	OPL St: 100 OSCC St: 100 CL St:100	PM	100	Saliva	IL-6	Proinflammatory cytokines IL-6 have diagnostic and prognostic significance	HRG: OPL subjects LRG: OSCC and CL	Casecontrol	Dineshkumar et al (2016)[32]

12	CL St: 60 TU St: 60 TU+OPL St: 60 TU+OML: St: 60	PM	yes/60	Blood	GSH/Serum albumin/TP	GSH and serum albumin decrease with increased use of tobacco TP was found as a weak marker/GSH and serum albumin were reliable markers	HRG: TU and OPL subjects MRG: TU and OML subjects LRG: TU and CL subjects	Cross-sectional	Nimbal et al (2024)[33]
13	Lk St: 25 OSF St: 25 OSCC St: 25 CL St:25	Lk/ OSF	Yes	Sera	Cerulo-plasmin	Higher levels in three groups compared to CL. Potential markers for OPML and OSCC An association was found between gutkha and smoking tobacco	HRG: Lk and OSF subjects LRG: CL	Observational	Patil et al (2021)[34]

In the table 2, all of the identified studies evaluate the potential of hematological markers in risk stratification and early detection of OPL by comparing the patients who consumed tobacco with OPL and malignant lesions with CL [24, 33, 35, 36-41]. The endpoints of the study included potential and levels of hematological markers, and discrimination between precancerous and cancerous lesions. Vankadara et al., and Salema et al., reported CRP as a potential marker used to discriminate and gauge premalignant lesions and malignant transformation [35, 36]. The hematological markers NLR with sensitivity and specificity of 92%, PLR, HB, ESR, PLC DLC, and WBC were found as valuable prognostic indicators for OPL [41]. However, further research is required to claim these as reliable diagnostic markers [24, 33, 35-40]. The risk stratification is done considering the level of hematological markers. The group with premalignant and malignant lesions with the use of tobacco and elevated levels of CRP, NLR, PLR, ESR, and WBC was identified higher risk group, a TU group considered as a moderate risk group and CL, Non-TU and without OPL deemed low-risk group [24, 33, 35-41]. Abbreviations: OPL: Oral Premalignant Lesions; CL: Control; Tu: Tobacco User; PM: Premalignant; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; NLR: Neutrophil-Lymphocyte Ratio; PLC: Platelets Count; Hb; Hemoglobin; TLC: T-Lymphocytes Count; WBC: White Blood Cells; PLR: Platelet-To-Lymphocyte Ratio; OML: Oral Malignant Lesions; HRG: Higher Risk Group; MRG: Moderate Risk Group; LRG: Lower Risk Group Statistical Chi-square test and Risk stratification. In Table 3 attached as a supplementary file, nine studies were specifically included based on homogeneity in data as the studies shared similar characteristics according to the chi-square principle [21, 22, 26, 27, 30-34]. Other studies were excluded as including studies with different characteristics like methodologies could lead to misleading results. Statistical test Chi-square was applied using 'Microsoft Excel' to determine the association of tobacco with oral premalignant lesions. Overall, a statistically significant p value < 0.05 (with D.F =1) association was found between TU and the group of patients with OPL as compared to the CL group, as individual analysis might lead to inconclusive or weak results due to limited data [22, 26, 27, 31-34]. A non-significant association was observed in the two studies possibly due to the small sample size or the influence of confounding factors [21, 30]. The risk stratification was done based on tobacco use association with OPL, Age, biochemical markers level, and tobacco type. All types of tobacco correlated with increased risk of OPL in which smoking is common. The group of patients with old age TU association with OPL elevated level of biochemical markers was categorized as a higher risk group, whereas as CL group younger age than the older group and without association of tobacco with OPL and low level of biochemical markers was deemed low risk group [21, 22, 26, 27, 30-34] (Table 3: Supplementary information).

**Table 2:** Summary of Hematological Markers and their Outcomes in Risk Stratification and Early Detection of Premalignant Oral Lesions

S.No	Groups	OPML	Tobacco User/PM	Sample	Hematological Markers	Outcomes	Risk Stratification	Study	Reference
1	OPML Subjects (St): 30 CL St: 30	PM	Yes	Blood	CRP	Increased CRP levels in OPL compared to CL subjects Potential marker	Subjects with OPL are classified as high-risk group (HRG), and CL as low-risk group (LRG)	Comparative	Vankadara et al (2018)[35]
2	OPL St:14 OML St: 39	PM	NR	Blood	CRP	Potential marker used to gauge premalignant lesions and malignant transformation	HRG: OPL and OML subjects	Comparative	Salema et al (2024)[36]
3	POML St: 50 OSCC St: 50 CL St: 50	PM	NR	Blood	NLR/PLR/ HB/ESR	Increased NLR/PLR/HB/ESR levels in OSCC compared to OPL and CL Valuable markers for OPL	HRG: OPL and OSCC subjects LRG: CL subjectsects	Comparative	Ram et al (2023)[24]
4	OPL St: 50 CL St: 50	PM	Yes	Blood	NLR	OPL>CL Increased NLR levels in OPL compared to CL Valuable diagnostic adjunct for OPML	HRG: OPL subjects LRG: CL subjects	Prospective case-control	Singh et al [40]
5	OPML St: 30 CL St: 30	PM	NR	Blood	WBC/TLC and DLC	OPL>CL Larger sample sizes are required to determine the significance of these markers	HRG: OPL subjects LRG: CL subjects	Prospective	Narang et al [38]



6	OPL St: 100 CL St: 100	OL/Lk	NR	Blood	RBC, WBC, Platelets, Hb, Hematocrit	Minimal variations were observed among groups/ Further research is required to claim these as reliable diagnostic markers	HRG: OPL subjects LRG: CL subjects	Randomized trial	Shanthi et al [39]
7	OPL St: 50 CL St: 0SSC St: 50	PM	NR	Blood	Hb, TLC, DLC	TLC neutrophil count and lymphocyte count showed significant differences among three groups/Used as markers for OPML	HRG: OPL subjects LRG: CL subjects	Case-control	Singh et al (2023)[40]
8	OPL St: 14 CL St: 29	PM	Yes	Saliva	NLR	Elevated NLR levels in OPL than CL /Potential prognostic indicator / sensitivity and specificity of 92%ML	HRG: OPL subjects LRG: CL subjects	Prospective	Magdum et al (2024)[41]
9	CL St: 60 TU St: 60 TU+OPL St: 60 TU+OML: 60	PM	Yes	Sera	WBC/PLC/ HB/CRP/ESR	WBC, PLC, HB levels were decrease in three groups compared to CL CRP/ESR levels were higher in three groups compared to CL	HRG: TU and OPL subjects MRG: TU and OML LRG: TU and CL	Cross-sectional	Nimbal et al [33]

## DISCUSSION

Our systematic review based study indicated specific biochemical and haematological indicators that could serve as markers in risk stratification and early detection of OPL. The OPL group exhibited significantly higher levels of biochemical markers IL-6, IL-8, TNF- $\alpha$ , HCC-1, PF-4, FRR, TP, MDA, MMP-12, and Ceruloplasmin and hematological markers NLR, PLR, CRP, ESR, WBC, and low Hb as compared to the control group. Following risk stratification, a group with older age, tobacco association with OPL, and elevated levels of markers were categorised as a higher-risk group. Similar to our study, to determine the potential of biochemical markers in the early detection of OPL, Dikova et al., undertook a 3-year study at the Oncology laboratory of the University General Hospital of Valencia (HGU) Spain [21]. The study analyzed the panel of cytokines IL-1 $\alpha$ , IL-6, IL-8, IP-10, MCP-1, TNF- $\alpha$  and HCC-1, and PF-4 among three groups of TU 330SCC/330PL/33CL. The findings suggest that the panel of five markers IL-6, IL-8, TNF- $\alpha$ , HCC-1, and PF-4 discriminate between OSCC OLP and CL and serve a useful role in early disease detection. The results are similar to our study analysis and to a 2-year study carried out by Jureti et al., at the Department of Oral and Maxillofacial Surgery of the University of Rijeka Croatia [30]. The study includes three groups' 190PML/190SCC/19CL to determine the levels of proinflammatory cytokines IL-6 and TNF $\alpha$ . Elevated levels of proinflammatory cytokines were found in the OSCC and OPL group as compared to the CL group. To assess the potential of TNF- $\alpha$ , a 3-year experimental study was carried out by Krishnan et al., in dental clinics in Chennai [25]. The study involved 100 OPML and 100 OSCC who consumed tobacco and 100 CL and reported that the proinflammatory cytokine TNF- $\alpha$  marker discriminated the premalignant lesions from malignant ones with higher specificity and sensitivity. The results were similar to a 1-year comparative study by Ameena et al., at Azeezia Dental College of India [28]. TNF- $\alpha$  level was higher in LK and in OSCC who were TU

as compared to CL. A P value  $\leq 0.01$  was found in the TNF- $\alpha$  level between the different histopathological grades of OPL and OSCC. Another comparative study with promising results for LDA as a potential and promising marker for the detection of OPL was undertaken by Javaraiah et al., in the Department of Sagar College of Dental Sciences India to detect the potential of LDA as a biochemical marker in the early detection of OPL [23]. There was a significantly elevated level of LDH in a group of TU with OPL ( $706.1 \pm 1.99$  U/L) as compared to TU without OPL ( $319 \pm 80.53$  U/L) and CL ( $267 \pm 27.64$  U/L). Our study found that it is necessary to investigate the potential biochemical markers capable of assessing the risk of malignant transformation in OPL. Similarly, López-Jornet et al., conducted a study to evaluate the potential of biochemical markers ADA, FRR and TP [26]. Among study groups, 9.9% were active smoking TU in the OPL group, whereas 42% of the OPL group and 70% of participants in the CL group had never consumed tobacco. There was no significant difference in ADA levels between the two groups. Though levels of Ferritin (FFR) which plays a key role in cancer progression, ( $12.66 \pm 10.50$ ) and TP ( $23.41 \pm 17$ ) were significantly higher in the OPL group as compared to the CL; FRR ( $7.19 \pm 4.44$ ); TP ( $14.15 \pm 15.19$ ) with sensitivity and specificity of 54.3, indicated FRR and TP as potential markers for OPL [42]. As Matrix Metalloproteinase (MMP) plays a role in the modification of extracellular matrix, a study was conducted by Saleem et al., among TU groups 300SF/300SCC/30CL to assess the potential of salivary biochemical MMP-12 marker in precancerous lesions [22]. Higher expression of MMP-12 was observed in oral submucous fibrosis as compared to CL, indicating a noninvasive and early diagnostic marker of OSF. The study reported that MMP-12 expression was higher among a group of TU. To examine the potential of biochemical markers GSH, TP and Serum Albumin (SA) levels in SLT consumers with OPL and malignant lesions, a study was

undertaken by Nimbale et al., at the Dental College of India. The findings suggest a higher level of GSH and SA in CL as compared to other groups and TP was found as a weak marker as no significant difference was found between groups [33]. To assess the potential of Hematological markers in the early detection of OPL, a comparative study was executed by Vankadara et al., at Dental College and Hospital of India to detect CRP marker of inflammation [35]. The CRP levels were higher in Group 1, ranging from 0.8 to 53.9 mg/l with a mean SD of  $5.59 \pm 9.86$  mg/l compared to CL with CRP levels ranging from 0.1 to 18.3 mg/l with a mean SD of  $3.88 \pm 4.50$  mg/l considered CRP as a potential marker for assessment of severity of disease. The findings are similar to a study carried out by Salema et al., at Dental College and Hospital of India to assess the potential of CRP markers in the detection of OPL [36]. Another study was carried out by Ram et al., to examine the potential of hematological markers NLR, PLR, HB, and ESR in early detection of precancerous and cancerous oral lesions [24]. The mean NLR was higher in OPL (3.12) and OSCC groups (3.67) as compared to CL (2.16). The mean Hb content was decreased in the OPL (13.77) and OSCC (12.76) group than CL (14.8). Whereas the ESR was lower in CL (9.65) as compared to OPL (17.2) and OSCC (27.4). These markers can be used for the early detection of OPL and OSCC. A study was undertaken by Nimbale et al., at the Dental College of India to evaluate the potential of hematological markers WBC, PLR, HB, CRP and ESR in TU with premalignant and malignant lesions [33]. According to the findings, CRP, total Red Blood Cell counts (RBC) and ESR levels were significantly higher in TU and OPL groups than in CL group. Whereas the Hb levels, total platelet and leukocyte count, were decreased more in TU and OPL exposed subjects than CL group indicating chronic inflammation and impaired pulmonary function due to TU. The limitations of the systematic review include variations in study designs, risk factors such as tobacco exposure and patient population, and small sample sizes, across studies included. Future research should focus on longitudinal studies with large sample sizes to validate the reliability and efficacy of these markers in risk stratification and early detection of OPL.

## CONCLUSIONS

The biochemical and hematological markers in Tobacco users are potential markers in the early detection of OPL from malignant lesions with diagnostic and prognostic significance. The OPL group exhibited significantly higher levels of biochemical markers IL-6, IL-8, TNF- $\alpha$ , HCC-1, PF-4, FRR, TP, MDA, MMP-12 and ceruloplasmin and hematological markers NLR, PLR, CRP, ESR, WBC, and low Hb as compared to the control group. Following risk stratification, a group with older age, tobacco association with OPL, and elevated levels of markers were categorised

as a higher-risk group. Integrating these findings into clinical protocols leads to robust assessing methods, ultimately improving patient outcomes.

## Authors Contribution

Conceptualization: MRT

Methodology: MM

Formal analysis: SA

Writing, review and editing: MRT, AA<sup>1</sup>, MQKG, MAA<sup>2</sup>, SAT, SA

All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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