Original Research Paper

Volume - 16 | Issue - 07 | July - 2026 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Physiology

THE NEXUS OF OXIDATIVE STRESS AND HISTOLOGICAL TRANSFORMATION IN ORAL POTENTIALLY MALIGNANT DISORDERS

Nabarun Sasmal	Research Center for Natural & Applied Sciences, Raja Narendralal Khan Women's College [Autonomous], Dept. of Physiology, West Bengal, Medinipur, Paschim Medinipur;721102
Rashmi Mukherjee	Assistant Professor Dept. of Botany, Raja Narendralal Khan Women's College [Autonomous], West Bengal, Medinipur, Paschim Medinipur, Medinipur;721102
Surajit Bose	Associate Professor Department of Oral and Maxillofacial Pathology and Microbiology Kusum Devi Sunderlal Dugar Jain Dental College and Hospital, Kolkata, West Bengal, India;700002
Dilip Kr. Nandi	Associate Professor Dept. of Physiology, Raja Narendralal Khan Women's College [Autonomous], West Bengal, Midnapore, Paschim Medinipur, Medinipur; 721102

ABSTRACT Oral potentially malignant disorders (OPMDs) comprise a variety of conditions that carry a higher risk of turning into malignant forms. Early detection and proper intervention are vital to prevent the development of oral squamous cell carcinoma (OSCC). Oxidative stress (OS) significantly contributes to the emergence of OPMDs by causing cellular damage and genetic mutations. This study explores the connection between histological changes and oxidative stress markers in OPMDs. A retrospective clinicopathological study was performed on patients diagnosed with OPMDs. Histological assessments were done to evaluate the severity of dysplasia and related pathological features. Immunohistochemical analysis was used to examine OS indicators, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and lipid peroxidation products (LPO).The findings showed a strong link between OS markers and the severity of dysplasia, with higher marker levels found in severe dysplasia cases compared to mild dysplasia and normal tissue. These results emphasize the important role of OS in the malignant transformation of OPMDs. The study highlights the need for early diagnosis and treatment approaches, stressing oxidative stress markers as potential diagnostic tools for assessing risk.

KEYWORDS : OPMDs, ROS, Oxidative Stress, Histopathology

INTRODUCTION

Oral cancer (OC) is a substantial global health concern, with an annual mortality rate of 3.8 deaths per 100,000 individuals. Many cases of OC originate from asymptomatic lesions classified as OPMDs. The World Health Organization (WHO) expresses OPMDs as "clinical conditions associated with an increased risk of oral cancer development, occurring either in a defined precursor lesion or in normal oral mucosa." Commonly recognized conditions include leukoplakia (OLK), erythroplakia(OLE), and palatal lesions from reverse cigar smoking, while the malignant potential of OLP residues a topic of debate[1,2].

Pathological Features

OLP is a long-lasting inflammatory condition with an unknown cause, marked by episodes of flare-ups and relief. It features white, web-like lesions that may appear in conjunction with areas that are atrophic, erosive, ulcerative (see Figure 1), or in plaque form. These lesions often appear symmetrically on both sides. Desquamative gingivitis might also be observed [3].

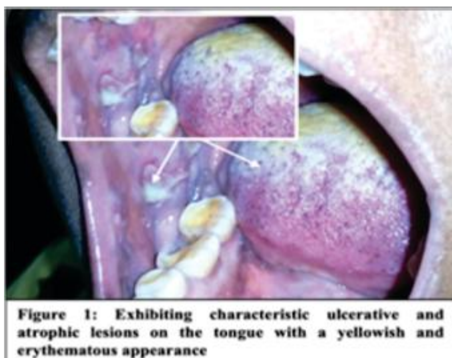

Figure 1: Exhibiting characteristic ulcerative and atrophic lesions on the tongue with a yellowish and erythematous appearance

Leukoplakia presents as white plaques of uncertain risk, marked by hyperkeratosis of the stratified squamous epithelium. It can be categorized as homogenous (flat, uniformly white) or non-homogenous (nodular, verrucous, or erythron leukoplastic) (Figure 2). In contrast, erythroplakia manifests as red patches (Figure 3), often exhibiting severe epithelial dysplasia. Histopathological analysis of leukoplakia commonly reveals hyperkeratosis, acanthosis, and varying degrees of dysplasia. Erythroplakia, characterised by epithelial atrophy and lack of keratinisation, has a higher malignant potential. WHO categorises oral epithelial dysplasia into mild, moderate, and severe dysplasia based on architectural and cytological abnormalities. Severe dysplasia exhibits pronounced nuclear pleomorphism, hyperchromatism, increased mitotic activity, and architectural disarray, making it highly prone to malignant transformation [4].

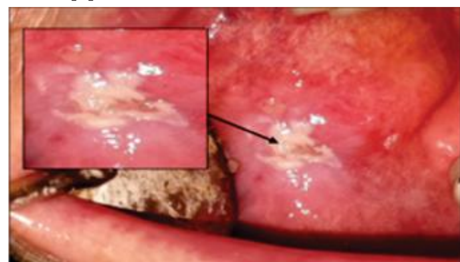

Figure 2: Characterized by a white patch on the buccal mucosa. mix of white and red patches with Small, raised white nodules.

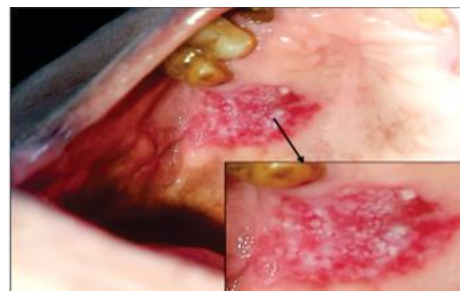

Figure 3: Erythroleuplakia on the buccal mucosa in a patient with submucosal fibrosis. A biopsy of the lesion showed severe dysplasia on histology.

A binary scoring method has been suggested to enhance classification precision, separating high-risk lesions (displaying four or more structural changes and five or more cellular alterations) from low-risk lesions (having fewer than four structural or five cellular changes). While WHO acknowledges the potential of this system, further

validation is needed for widespread adoption [5].

Oxidative Stress and Its Role in OPMDs

OPMDs are seen early indicators of OS, making it essential thoroughly grasp their fundamental processes. A key element in this progression is OS , which arises while there's an odds amid reactive oxygen species (ROS) and the body's antioxidant resistances. Damage caused by ROS impacts proteins, lipids, and nucleic acids, leading to histopathological changes [6].

The oral cavity is particularly susceptible to oxidative stress due to exposure to external factors like tobacco smoke and pollutants. Enzymatic antioxidants such as SOD, GPX, and CAT help neutralize ROS, counteracting oxidative harm. However, excessive ROS production can overpower these antioxidant routines, leading to OS, which causes lipid peroxidation and inflammation. This contributes to cellular disturbances and the onset of cancer [7].

MATERIALS AND METHODS

This study was conducted by the Department of Oral Medicine, Kusum Devi Sunderlal Dugar Jain Dental College and Hospital, Cossipur, Kolkata, India, following ethical approval (Ref No. 08/IEC/RNLKWC/2024). The study included 90 OPMDs patients categorized into three groups: Group II (OLP, n=30), Group III (OLK, n=30), Group IV (OLE, n=30), and (HC) Group I (n=30). Patients were excluded if they had abnormal biochemical or haematological profiles, systemic conditions such as hepatitis B/C, acquired immunodeficiency syndrome (AIDS), renal or hepatic disorders, systemic lupus erythematosus, lymphoproliferative malignancies, or any active cancer. Prior to the collection of samples, informed consent was obtained from all participants.

Measurement of Oxidative Stress Parameters

OS in patients with OPMDs was measured by estimating key enzymatic and non-enzymatic antioxidants in their serum samples. The following biochemical parameters were evaluated.

To evaluate OS in patients with OPMDs, key enzymatic and non-enzymatic antioxidants—specifically Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), and Lipid Peroxidation (MDA)—were measured in serum samples using commercial assay kits from Thermo Fisher Scientific and Sigma-Aldrich [8-12]

Histopathology

Tissue samples underwent fixation, processing, afterwards embedded in paraffin. The pieces were stained with hematoxylin & eosin (H&E) for microscopic examination to evaluate cellular and structural alterations.

Statistical Analysis of Oxidative Stress Parameters

Statistical analyses were performed using two sample unequal variance t-test compare group differences, with significance set at $p \leq 0.05$. Data were offered as Mean ± standard deviation(SD) and studied using statistical software (Version 16.89.1). The statistical analysis was performed (Table 1).

Table 1: Test of normality for Oxidative Stress parameters across the different OPMDs Groups.

Parameters	Group I (HC) Mean ±SD	OPMDs Group Mean ± SD	P value
SOD (Unit/ml)	5.73±0.40	OLP: 03.78 ±0.97 OLK: 02.93±0.54 OLE: 02.94±0.33	0.001
GPX (Unit/ml)	43.24±1.71	Group II : 34.23 ± 02.10 Group III : 32.08±02.36 Group IV : 31.96±01.37	0.002
CAT (Unit/ml)	17.46±0.68	OLP: 13.91±02.29 OLK: 10.77±02.05 OLE: 10.24±01.40	0.001
LPO (µg/ml)	1.51±0.13	Group II : 2.12±0.19 Group III : 2.17±0.15 Group IV : 2.16±0.13	0.001

RESULT

Histological Features

At the time of diagnosis, all patients showed Acanthosis. The epidermis appears thickened with elongated rete ridges. There is an increased thickness of the stratum corneum, the outermost layer of the epidermis, which is called Hyperkeratosis. A dense band of lymphocytes is seen at the dermo-epidermal junction (DEJ). This is a hallmark feature of OLP. The Zone of Civatte is a band of eosinophilic degeneration in the basal layer of the epidermis, (Pranay et al., 2013) just above the lymphocytic infiltrate (Figure 4).

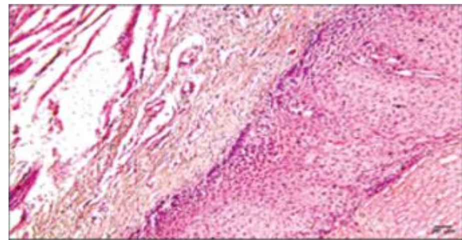

Figure 4: Biopsy of OLP show a dense band-like lymphocytic infiltrate at the interface between the epithelium and the connective tissue, focal areas of hyperkeratinized epithelium.

Acanthosis shows that the epidermis appears thickened with elongated rete ridges. Hyperkeratosis is an increased thickness of the stratum corneum, the outermost layer of the epidermis [13]. The basal cells show some change in size and shape, with enlarged nuclei and increased mitotic activity, which is known as cellular atypia. There is a mild to moderate infiltrate of chronic inflammatory cells, mainly lymphocytes, in the lamina propria. Based on the observed features, there is a mild to moderate dysplasia associated with OLK (Figure 5)[14].

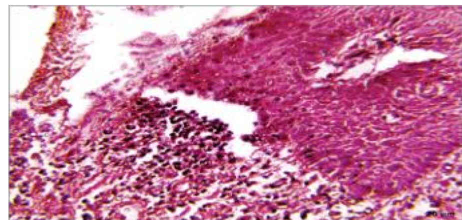

Figure 5: Biopsy of Oral leukoplakia revealing mild dysplasia characterised by increased cellularity of the lower third of the squamous epithelium accompanied by dyskeratosis, nuclear irregularities (anisonucleosis), and suprabasal mitoses.

Severe dysplasia is noted by abnormal growth of the basal layer extending into the superior third of the epithelium (refer to Figure 6). There are noticeable changes in both the cells and their structure, and all forms of abnormalities might be existing in severe dysplasia. This classification is quite subjective and often results in significant disagreement among observers, indicating that binary scoring must be validated before it is consistently used for the oral cavity (see Figure 6).

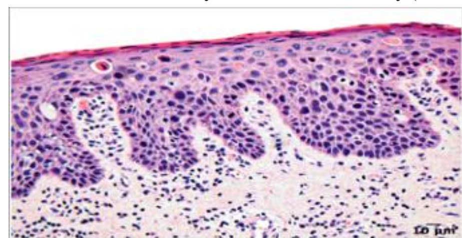

Figure 6: This biopsy of oral erythroplakia shows severe squamous dysplasia. The sample reveals cellular abnormalities affecting the entire thickness of the epithelium, which include dyskeratosis, frequent abnormal nuclei with variation of size and shape, and frequent mitoses.

Statistical Analysis of Oxidative Stress Parameters

Table:1 compare mean serum SOD values, GPX and other parameters of OS levels in the HC and OPMDs groups. The mean SOD level in the Group I was 5.73, Group II was 3.78, Group III was 2.93 and Group IV was 2.94, serum SOD was low in all the study groups. The alteration was statistically significant ($P \leq 0.05$). The mean GPX level in Group I was 43.24, Group II was 34.23, Group III was 32.08 and Group IV was 31.96. The GPX value in the OPMDs and HC groups was less than expected. Respectively, for the OPMDs and Group I, which was statistically significant ($P \leq 0.05$). The mean serum CAT level in Group I was 17.46, and OPMDs Group II, it was 13.91, while for Group III, it was 10.77, and in Group IV was 10.24. The serum CAT level in all the diseases was lower than usual. The mean serum LPO level in Group I was 1.51, with Group II being 2.12, $P = 0.001$, Group III being 2.17, $P = 0.001$, and Group IV being 2.16, $P = 0.001$.

DISCUSSION

The present study evaluates oxidative stress parameters in patients with OPMDs, including OLP, OLK and OLE in comparison to HC. The results provide compelling proof that oxidative stress is crucial in

the pathophysiology of these conditions, potentially contributing to their malignant transformation.

OS results from an imbalance amid ROS and antioxidant cover mechanisms, leading to cellular and molecular damage. The observed reduction in enzymatic antioxidants, such as SOD, GPX, and CAT, in OPMDs patients indicates an impaired antioxidant defense system. The significantly lower SOD levels (Group II: 3.78 ± 0.97, Group III: 02.93 ± 0.54, Group IV: 02.94±0.33) compared to Group I (05.73±0.40, P = 0.001) suggest that patients with OPMDs are unable to adequately neutralize superoxide radicals. A similar trend was observed in GPX and CAT levels, which were markedly lower in OPMDs patients corresponding to healthy controls ($p < 0.05$). These findings align with previous studies that have demonstrated reduced antioxidant enzyme activity in patients with oral premalignant lesions and OSCC.

LPO, a vital indicator of OS related cellular damage, was notably increased in patients with OPMDs (Wu et al., 2012)[15]. The average serum LPO levels were significantly higher in all OPMDs groups (Group II: 02.12 ± 0.19, Group III: 02.17 ± .15, Group IV: 02.16 ± 0.13) when compared to (HC) (1.51 ± 0.13, P = 0.001). These elevated malondialdehyde (MDA) levels among these patients suggest intensified oxidative damage to cell membranes, which could lead to genetic mutations and cancer development (Waris & Ahsan, 2006)[16].

The histopathological findings corroborate these biochemical observations. OLP cases exhibited acanthosis, hyperkeratosis, and a dense band of lymphocytic infiltration at the dermo-epidermal junction, consistent with its inflammatory nature. OLK demonstrated varying degrees of dysplasia, with severe dysplasia (Speight, 2007)[17] characterized by basal cell hyperplasia, increased mitotic activity, and nuclear pleomorphism. OLE, known for its high malignant potential, exhibited pronounced epithelial atrophy and loss of keratinization, making it more susceptible to neoplastic changes.

The significant alterations in oxidative stress biomarkers in OPMD patients underscore the need for early diagnosis and management to prevent malignant transformation. Antioxidant therapy, lifestyle modifications, and regular monitoring of oxidative stress markers may serve as potential strategies to mitigate disease progression (Marrocco et al., 2017)[18]. Moreover, given the strong association between oxidative stress and oral carcinogenesis, salivary biomarkers could be explored as non-invasive tools for early detection and risk assessment.

CONCLUSION

This analysis emphasizes the significant role of OS in the development of OPMDs, besides their potential progression to OSCC. The notable changes in antioxidant enzyme levels, heightened lipid peroxidation, and increased inflammatory markers suggest that oxidative stress is a crucial element in the disease's progression. The findings underscore the importance of using oxidative stress biomarkers in assessing risk and detecting early malignant transformation in OPMDs.

REFERENCES

1. Tranby, E. P., Heaton, L. J., Tomar, S. L., Kelly, A. L., Fager, G. L., Backley, M., & Frantsve-Hawley, J. (2022). Oral Cancer Prevalence, Mortality, and Costs in Medicaid and Commercial Insurance Claims Data. *Cancer Epidemiology, Biomarkers & Prevention*, *31*(9), 1849. https://doi.org/10.1158/1055-9965.EPI-22-0114
2. Sasmal, N., Mukherjee, R., Nandi, D. K., & Bose, S. (2025). Biochemical Metabolite Profiling In Oral Potentially Malignant Disorders: A Diagnostic And Prognostic Approach. *International Journal of Advanced Research*, *13*, 127–134. https://doi.org/10.21474/ijar01/20360
3. Kumari, P., Debta, P., & Dixit, A. (2022). Oral Potentially Malignant Disorders: Etiology, Pathogenesis, and Transformation Into Oral Cancer. *Frontiers in Pharmacology*, *13*, 825266. https://doi.org/10.3389/fphar.2022.825266
4. Pindborg, J. J. (1980). *Oral cancer and precancer*. Butterworth-Heinemann.
5. Ku Kujan, O., Oliver, R. J., Khattab, A., Roberts, S. A., Thakker, N., & Sloan, P. (2006). Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. *Oral Oncology*, *42*(10), 987-993. https://doi.org/10.1016/j.oraloncology.2005.12.014
6. Ionescu, C., Kamal, F. Z., Ciobica, A., Halitchi, G., Burlui, V., & Petroaie, A. D. (2024). Oxidative Stress in the Pathogenesis of Oral Cancer. *Biomedicines*, *12*(6), 1150. https://doi.org/10.3390/biomedicines12061150
7. Bhattacharyya, A., Chattopadhyay, R., Mitra, S., & Crowe, S. E. (2014). Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases. *Physiological Reviews*, *94*(2), 329. https://doi.org/10.1152/physrev.00040.2012
8. Superoxide dismutase (SOD) Colorimetric Activity kit. (n.d.). https://www.thermofisher.com/order/catalog/product/EIASODC
9. Catalase Colorimetric Activity Kit. (n.d.). https://www.thermofisher.com/order/catalog/product/EIACATC
10. Glutathione peroxidase (GSH-PX) Activity Kit. (n.d.). https://www.thermofisher.com/order/catalog/product/EEA010
11. *Lipid Peroxidation (MDA) Assay Kit sufficient for 100 colorimetric or fluorometric tests TBARS Assay*. (n.d.). https://www.sigmaaldrich.com/IN/en/product/sigma/mak085
12. Rifai, N., Tracy, R. P., & Ridker, P. M. (1999). Clinical efficacy of an automated high-sensitivity C-reactive protein assay. *Clinical Chemistry*, 45(12), 2136–2141.
13. Seborrheic Keratosis (and Variants). Diagnostic Pathology: Neoplastic Dermatopathology (Second Edition), 44-49. https://doi.org/10.1016/B978-0-323-44310-4.50023-3
14. Wenig, B. M. (2002). Squamous Cell Carcinoma of the Upper Aerodigestive Tract: Precursors and Problematic Variants. Modern Pathology, 15(3), 229-254. https://doi.org/10.1038/modpathol.3880520
15. Wu, C., Huang, C., Chang, K., & Chen, C. (2012). Corrigendum to 'Continuous intra-arterial infusion chemotherapy as a palliative treatment for oral squamous cell carcinoma in octogenarian or older patients' [Oral Oncology 46/7 (2010) 559–563]. *Oral Oncology*, *48*(6), 567. https://doi.org/10.1016/j.oraloncology.2012.04.008
16. Waris, G., & Ahsan, H. (2006). Reactive oxygen species: role in the development of cancer and various chronic conditions. Journal of Carcinogenesis,5(1),14. https://doi.org/10.1186/1477-3163-5-14
17. Speight, P. M. (2007). Update on Oral Epithelial Dysplasia and Progression to Cancer. Head and Neck Pathology, 1(1), 61. https://doi.org/10.1007/s12105-007-0014-5
18. Marrocco, I., Altieri, F., & Peluso, I. (2016). Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. Oxidative Medicine and Cellular Longevity, 2017(1), 6501046. https://doi.org/10.1155/2017/6501046