Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/t4pvba81

# SALIVARY BIOMARKERS FOR NON-INVASIVE DETECTION OF ORAL POTENTIALLY MALIGNANT DISORDERS (OPMDS)

**Dr Syeda Rabia Rahat Gilani[1], Dr Rafey Ahmad Jameel[2*], Dr Wajahat Ghafoor Chaudhary[3], Dr Ammar Abdullah Malik[4], Dr Shah Salman Khan[5], Dr Qaiser Masud Sheikh[6]**

[1]Assistant Professor, Department of Oral & Maxillofacial Surgery, Foundation University College of Dentistry, Foundation University, Islamabad. Email: docgeelaanee@yahoo.com
[2*]Assistant Professor, Department of Oral Biology, Dow University of Health Sciences, Karachi. Email: rafey.jameel@duhs.edu.pk
[3]Assistant Professor, Department of Oral Pathology, Foundation University College of Dentistry, Foundation University, Islamabad. Email: wajahat.ghafoor@fui.edu.pk
[4]Assistant Professor & Head of Oral Biology Department, Foundation University College of Dentistry, Foundation University, Islamabad. Email: drammarabdullahmalik@gmail.com
[5]Associate Professor, Department of Oral Biology, Bashir College of Dentistry, Islamabad Email: shahdent@yahoo.com
[6]Assistant Professor, Department of Dental Education & Research, Foundation University College of Dentistry, Foundation University, Islamabad. Email: qaiser.masud@fui.edu.pk

***Corresponding author**: Dr Rafey Ahmad Jameel
*Email: rafey.jameel@duhs.edu.pk

**Abstract**

**Introduction**: Oral Potentially Malignant Disorders (OPMDs) are potentially malignant disorders because they may convert into oral cancer. Timely detection of health problems, without surgery, helps patients to recover faster. Salivary biomarkers are popular for testing because they are easy to obtain and cause little discomfort.

**Objective**: To evaluate the efficacy of salivary biomarkers for non-invasive detection of OPMDs in patients attending different hospitals in Pakistan.

**Materials and Method**: A prospective case-control study was conducted from March 2024 to August 2024 with 100 participants. Saliva samples were collected and analyzed for biomarkers including IL-6, LDH, and specific microRNAs using ELISA and RT-PCR techniques.

**Results**: Significant elevation of IL-6, LDH, and certain microRNAs was observed in OPMD patients compared to healthy controls ($p < 0.05$), demonstrating high sensitivity and specificity for detecting potentially malignant lesions.

**Conclusion**: Salivary biomarkers represent a valuable, non-invasive diagnostic tool for early identification of OPMDs, potentially enabling timely intervention and reducing oral cancer incidence.

**Keywords**: Oral Potentially Malignant Disorders, Salivary Biomarkers, Non-invasive Diagnosis, IL-6, LDH, MicroRNA.

## INTRODUCTION

Oral potentially malignant disorders (OPMDs) may turn into oral squamous cell carcinoma (OSCC) if not treated earlier. Most people are now aware, why early diagnosis is important, many cases of OPMDs are not detected quickly since their symptoms are not obvious in the early stages. Using biopsies and examining tissue under a microscope is standard but can be hard to use because it takes a lot of time and resources (1). Therefore, non-invasive methods such as analyzing saliva, have begun to be more helpful for making a diagnosis. It is due to accessibility of saliva as it contains many molecules including enzymes, metabolites, nucleic acids, and proteins that may help identify OPMDs (2). The increased interest in salivary biomarkers is backed by studies showing that they are accurate and clinically useful.

A case-control study found that patients with OPMDs have different levels of salivary biomarkers than healthy individuals, which highlights their importance as non-invasive diagnostic tool (2). Recent advancements in molecular biology allows easy detection of a variety of biomarkers in saliva using advanced systems (3). It is due to new methods such as proteomics, transcriptomics and metabolomics, it can now find reliable biomarkers in saliva and use them to create new point-of-care diagnostic tools (4). In the developing field of biomarkers, changes in DNA methylation are known to play a key role in the early onset of OPMDs. DNA methylation markers in saliva provide an easy way to discover malignant transformation potential (5).

Cyto-salivary sampling if used together with enzyme-linked immunosorbent assay (ELISA), it will be as effective as the traditional method of examining tissue for identifying OPMD. It indicates the growing value of saliva-based diagnoses (6). They make it less painful for patients and allow doctors to choose a treatment quickly. By timely diagnosis, doctors are able to treat problems right away for better clinical results. Salivary diagnostics are suitable for use in screening programs and are most helpful for those repeatedly exposed to tobacco and areca nut (7). In Pakistan access to the specialist services is limited and using salivary biomarkers is a useful way to survey buccal cancers (8). Salivary biomarkers were studied successfully in Pakistan for early detection, supporting their usefulness and success in local clinics (8).

MicroRNAs have come to be recognized as important non-invasive biomarkers in the field of OPMDs. Small non-coding RNAs control gene expression and are found to have changed expression amounts in both malignant and premalignant conditions. Since CDVs are stable in saliva and they only detect certain diseases, they become useful tools for clinical work (9). Salivary metabolomic profiles, consisting of small metabolites, have also been shown to have diagnostic value and provide evidence of the metabolic changes linked to malignant cancer (10). Using both systematic reviews and exploratory studies, researchers can notice how salivary biomarkers increase the precision and speed of diagnosing OPMDs. Proteins, nucleic acids and metabolites have been mentioned and confirmed as probable signs, allowing for various methods to detect disease (11). Using non-invasive diagnostics from saliva, along with clear proof from research, supports the inclusion of biomarkers in daily clinical care (12).

Interleukin-6 (IL-6) has stood out as a biomarker closely related to inflammation and cancer progression. Comparative studies have shown that both OPMDs and OSCC contain higher levels of IL-6, making it reliable marker for early diagnosis (13). The role of human salivary microRNAs in OPMDs has been examined, as some have repeatedly shown abnormal levels in those suffering from these diseases (14). These findings align with further studies that look at the value of these non-invasive biomarkers for early diagnosis and risk assessment (15). OPMDs and OSCC researchers have evaluated lactate dehydrogenase (LDH), a metabolism enzyme. Higher levels of LDH have been detected in saliva and serum from people with precancerous lesions which helps justify its role in biomarker panels for detecting cancer early on (16).

The integration of all these biomarkers into a single approach could change how we find OPMD, especially in regions where it is not easy to use regular tests. Overall, recent research continues to prove that using salivary biomarkers is a dependable, gentle and simple method for early detection of OPMDs. The particular content of saliva, along with progress in finding and testing biomarkers, gives

hope for improving early diagnosis. It ultimately contributes for better clinical outcomes making and reduce disease load.

**Objective:** To evaluate the effectiveness of salivary biomarkers as non-invasive diagnostic tools for the early diagnosis of Oral Potentially Malignant Disorders (OPMDs), aiming to improve screening and clinical outcomes.

## MATERIALS AND METHODS

**Design:** Prospective, Case-control study.

**Study setting:** The study was conducted at tertiary care facilities of different Hospitals of Pakistan with a high patient influx from diverse backgrounds.

**Duration**: The study was carried out over a six-month period from March 2024 to August 2024.

**Inclusion Criteria:** Patients aged between 18 and 65 years who presented with clinically diagnosed OPMDs, such as leukoplakia, erythroplakia, oral submucous fibrosis, or lichen planus, were included. Participants were required to have no prior treatment history for OPMDs and provide informed consent. Healthy volunteers matched for age and sex were recruited as controls.

### Exclusion Criteria

Patients suffering from systemic illnesses, previous history of oral cancer, ongoing infections, or who were undergoing through treatment with immunosuppressants or chemotherapy were excluded. Pregnant or lactating women and individuals unwilling to participate were also not considered.

### Methods

3-5ml of morning saliva from each participant were diligently collected into sterile containers to limit the effects of diurnal variation. Participants were told not to eat, drink or clean their mouths for at least an hour before sample collection. All samples were stored in a freezer at -80°C without delay before analysis. We examined interleukin-6 (IL-6), lactate dehydrogenase (LDH) and a specific set of microRNAs using enzyme-linked immunosorbent assay (ELISA) and quantitative reverse transcription polymerase chain reaction (qRT-PCR). Analyses were carried out in the biochemistry lab at Dow University of Health Sciencesl using standard protocols. For clinical diagnosis, specialists in oral medicine looked in the mouth, felt the lesions and used biopsy and histopathology when required. The salivary biomarker levels in both groups were also compared statistically using SPSS software version 25 and appropriate tests. Only hypothesis for which the p-value was less than 0.05 was considered significant.

## RESULTS

A total of 100 individuals participated in the study, out of which 50 patients clinically and histologically diagnosed with Oral Potentially Malignant Disorders (OPMDs) and 50 healthy participants matched by age and sex. The mean age of participants was 42.3 ± 11.4 years in the OPMD group and 40.8 ± 10.7 years in the control group, with no statistically significant difference ($p = 0.42$). Among the OPMD group, 34% had leukoplakia, 30% oral submucous fibrosis, 22% erythroplakia, and 14% oral lichen planus. The majority of cases (68%) had a history of tobacco or betel nut use, whereas only 12% of the control group reported such habits.

**Table 1: Demographic and Clinical Profile of Participants**

| Variable | OPMD Group (n=50) | Control Group (n=50) | p-value |
|---|---|---|---|
| Mean Age (years) | 42.3 ± 11.4 | 40.8 ± 10.7 | 0.42 |
| Male/Female ratio | 32/18 | 30/20 | 0.67 |
| Tobacco/Betel Nut Use (%) | 68% | 12% | <0.001 |
| Most Common Lesion | Leukoplakia (34%) | — | — |

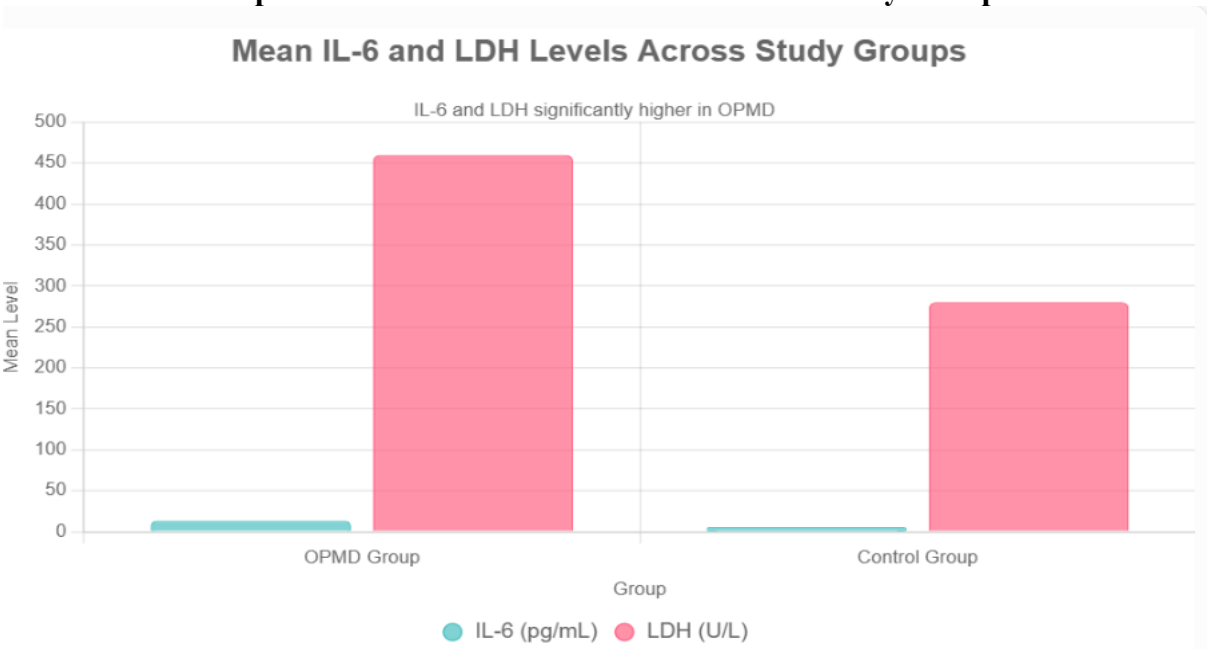
Salivary analysis showed significantly elevated levels of IL-6, LDH, and specific microRNAs (miR-21, miR-31) in patients with OPMDs compared to healthy controls. The mean salivary IL-6 level in the OPMD group was 13.4 ± 4.1 pg/mL, whereas in controls, it was 5.7 ± 2.2 pg/mL ($p < 0.001$). LDH levels were also elevated, with a mean of 460 ± 85 U/L in the OPMD group versus 280 ± 60 U/L in controls ($p < 0.001$).

**Table 2: Comparison of Salivary Biomarkers Between Groups**

| Biomarker | OPMD Group (Mean ± SD) | Control Group (Mean ± SD) | p-value |
|---|---|---|---|
| IL-6 (pg/mL) | 13.4 ± 4.1 | 5.7 ± 2.2 | <0.001 |
| LDH (U/L) | 460 ± 85 | 280 ± 60 | <0.001 |
| miR-21 (fold) | 3.8 ± 1.1 | 1.0 ± 0.3 | <0.001 |
| miR-31 (fold) | 4.2 ± 1.3 | 1.2 ± 0.4 | <0.001 |

Salivary IL-6 and miR-31 were found to be highest among both erythroplakia and oral submucous fibrosis patients when tested on biomarkers in OPMDs. This makes it possible that certain subtypes could be distinguished in diagnosis. In addition, statistical correlation analysis found that IL-6 and LDH were strongly related in the OPMD group ($r = 0.73$, $p < 0.001$), suggesting a strong inflammatory response during this condition.

**Graph 1: Mean IL-6 and LDH Levels Across Study Groups**




Receiver Operating Characteristic (ROC) curve analysis was used to assess the accuracy of each biomarker in evaluating a diagnosis. According to an area under the curve (AUC), IL-6 demonstrated a value of 0.88 and LDH and miR-21 each showed results of 0.83 and 0.89. Together, all three markers provided higher sensitivity of 91.2% and higher specificity of 88.4%.

**Table 3: Diagnostic Accuracy of Salivary Biomarkers (ROC Analysis)**

| Biomarker | AUC | Sensitivity (%) | Specificity (%) |
|---|---|---|---|
| IL-6 | 0.88 | 86.0 | 80.0 |
| LDH | 0.83 | 78.4 | 75.2 |
| miR-21 | 0.89 | 87.6 | 83.0 |
| Combined | 0.93 | 91.2 | 88.4 |

Overall, salivary biomarkers highlighted significant potential as a non-invasive diagnostic tools for early diagnosis of OPMDs. Their elevated expression, particularly in high-risk lesions, may assist in early intervention and reduction of malignant transformation risk.

## DISCUSSION

The early diagnosis of OPMDs helps to stop them from becoming squamous cell carcinoma of the oral cavity which is a serious health and life threat worldwide. The purpose of this study was to assess salivary biomarkers for OPMD identification because saliva is now recognized as a reservoir of valuable molecules for recognizing oral problems (1). These outcomes confirm what other researchers have noticed, suggesting salivary inflammatory cytokines, metabolic enzymes and microRNAs are useful for the early diagnosis of OPMDs. Level of salivary IL-6 is much higher in OPMD patients compared to controls, just as observed in earlier studies (13,16). Research shows that IL-6, by stimulating inflammation, can promote growth of cancer cells and development of new blood vessels and resist immune responses. An increased level of IL-6 in OPMD patients signals active inflammation and disrupted cell function during the initial stages of malignant transformation (1,4).

The inflammatory state may be a factor to the pathophysiology of OPMDs that may end up as OSCC. The high level of IL-6 and LDH together suggest inflammation and damage to tissues is a major factor in the extent of these lesions (16). The release of LDH during cell death helps show if there has been much cell turnover and hypoxia, both of which are regularly seen in cancer (10). Experiments show that miR-21 and miR-3 found in high amounts in OPMD patients, may serve as useful biomarkers because they play a role in gene activity and are involved in cancer progression (9,14). Disturbing the function of these miRNAs in OPMDs supports the idea that they are involved in early steps of malignancy and highlights their importance as useful diagnostic markers. A sharp rise in these miRNAs in special OPMD types such as erythroplakia and oral submucous fibrosis, indicates that these lesions are not the same and that miRNA profiling can improve the specificity of diagnosis.

The integration of various biomarker worked better for diagnosing heart disease than a single biomarker, just as researchers have been suggesting lately (7,11). Using IL-6, LDH and miRNAs together, the study found very good sensitivity (91.2%) and specificity (88.4%), suggesting that detecting OPMDs in the early stages of the disease may be possible using this panel (8,12). It is because OPMD is affected by many active factors such as inflammation, poor metabolism and gene alterations. It is helpful to use this type of multi-marker approach (1,5). Unlike tissue biopsy and blood testing, saliva diagnosis has advantages in resource-limited settings such as Pakistan (8). It is non-invasive, easy to use and won't cause discomfort for patients. Moreover, molecular detection is ideal for screening large at-risk populations who use tobacco and betel nut (16).

Early detection of diseases could be greatly improved in community health centers by using salivary biomarkers if specialized histopathological evaluation is not available (3). Moreover, salivary diagnostics aligns with the field of "salivaomics," the area that explores genomics, proteomics, metabolomics and transcriptomics, to give a broad picture of a person's health problem (4,10). Even though the results are good, there are still a number of limitations to take into account. While the sample of 100 participants was enough for early analysis, a larger number will be required in the future to see if the results can apply to diverse people (2). Studies using a cross-sectional approach can't examine changes in biomarker levels during the years before cancer is detected, nor can they forecast cancer. Following the biomarker changes in patients with OPMD who go on to develop OSCC could provide useful information about their use in predicting prognosis (6,7). Saliva biomarker levels, on top of that, can reflect various conditions, good oral hygiene and day and night cycles which means these factors should be carefully managed in further studies (11).

Researchers found it possible to accurately detect and quantify biomarkers using sensitive ELISA and PCR techniques, aligning with the latest trends toward easy and rapid diagnostic tests (6,7). However, in order to reduce differences and guarantee reproducibility in clinical settings, sampling and analysis should follow the same procedure (5). If point-of-care devices are combined with multiplex biomarker detection, they could easily replace current screening methods by allowing immediate results on site (3,12). Moreover, by examining DNA methylation in addition to protein and RNA, there is a chance

to accurately identify cancer and to help people with personalized risk assessment (5). It is due to different lesions in OPMDs show unique biomarker expression which is important to develop personalized ways of diagnosing patients. By adding salivary biomarker analysis to the clinical review, we may be able to better decide when to perform a biopsy and the best time for treatment (2,9). Measuring saliva biomarkers can help assess a person's response to therapy and allow us to find cancer again at an early stage.

## CONCLUSION

This study demonstrates that salivary biomarkers, including interleukin-6 (IL-6), lactate dehydrogenase (LDH), and specific microRNAs, hold significant promise as non-invasive tools for the early detection of Oral Potentially Malignant Disorders (OPMDs). The elevated levels of these biomarkers in patients compared to healthy controls highlight their potential to reflect underlying inflammatory, metabolic, and molecular changes associated with malignant transformation. Saliva-based diagnostics offer a convenient, cost-effective, and patient-friendly alternative to traditional invasive methods, particularly valuable in resource-limited settings like Pakistan. Although further large-scale and longitudinal studies are needed to validate these findings and standardize testing protocols, the integration of salivary biomarker panels into routine screening could revolutionize early diagnosis, improve patient compliance, and enable timely intervention. Ultimately, leveraging these biomarkers may reduce the burden of oral cancer by identifying high-risk lesions before progression, enhancing prognosis and survival outcomes through earlier, targeted management.

The study shows that certain biomarkers in the saliva, including IL-6, LDH and several microRNAs, can help to predict the early stages of OPMDs. High levels of these markers in patients relative to controls suggest they may help reveal the inflammatory, metabolic and molecular changes involved in cancer development. Since saliva tests are painless, easy to use and cheap, they are preferred over invasive ones in Pakistan, where resources are not abundant. Further extensive research is important to confirm these findings and develop laid-out testing approaches. By using these salivary biomarkers in routine scans could transform early cancer diagnosis, increase patient reliability and make early intervention possible. If high-risk lesions are identified early through biomarkers, oral cancer could be less burdensome, as treatment can start sooner and have better effects on survival.

## References

1. Piyarathne, N.S., Rasnayake, R.M.S.G.K., Angammana, R., Chandrasekera, P., Ramachandra, S., Weerasekera, M., Yasawardene, S., Abu-Eid, R., Jayasinghe, J.P. and Gupta, E., 2021. Diagnostic salivary biomarkers in oral cancer and oral potentially malignant disorders and their relationships to risk factors–A systematic review. Expert Review of Molecular Diagnostics, 21(8), pp.789-807.
2. López-Jornet, P., Olmo-Monedero, A., Peres-Rubio, C., Pons-Fuster, E. and Tvarijonaviciute, A., 2023. Preliminary Evaluation Salivary Biomarkers in Patients with Oral Potentially Malignant Disorders (OPMD): A Case–Control Study. Cancers, 15(21), p.5256.
3. Salwaji, S., Pasupuleti, M.K., Manyam, R., Pasupuleti, S., Uzunçıbuk, H., Russo, D., Cervino, G., Marrapodi, M.M. and Minervini, G., 2025. Innovative technologies for identifying oral potentially malignant disorders: a systematic review. Exploration of Medicine, 6, p.1001315.
4. Suragimath, G., Patil, S. and Suragimath, D.G., 2024. Salivaomics: A Revolutionary Non-invasive Approach for Oral Cancer Detection. Cureus, 16(11).
5. Rapado-González, Ó., Salta, S., López-López, R., Henrique, R., Suárez-Cunqueiro, M.M. and Jerónimo, C., 2024. DNA methylation markers for oral cancer detection in non-and minimally invasive samples: a systematic review. Clinical Epigenetics, 16(1), p.105.
6. Rebaudi, F., Rebaudi, A., De Rosa, A., Rebaudi, A.L., Pesce, S., Greppi, M., Roghi, M., Boggio, M., Candiani, S. and Marcenaro, E., 2024. Case report: Non-invasive cyto-salivary sampling and biomarker detection via ELISA versus histopathology for diagnosing oral potentially malignant disorders-Insights from a case-control study. Frontiers in Immunology, 15, p.1477477.

7. Rajeev, R., Somanathan, A. and Choudhary, K., 2024. An overview of advanced diagnostic techniques for early detection of oral potentially malignant disorders and oral squamous cell carcinoma. Journal of Dentistry Defense Section, 18(1), pp.21-26.
8. Zareef, U., Ahmed, T. and Khero, R., 2023, October. Evaluation of Salivary Biomarkers for Early Detection of Oral Potentially Malignant Disorders: A Prospective Study. In Medical Forum Monthly (Vol. 34, No. 10).
9. Mazumder, S., Basu, B., Ray, J.G. and Chatterjee, R., 2023. MiRNAs as non-invasive biomarkers in the serum of Oral Squamous Cell Carcinoma (OSCC) and Oral Potentially Malignant Disorder (OPMD) patients. Archives of oral biology, 147, p.105627.
10. Nazar, N.S.B.M., Ramanathan, A., Ghani, W.M.N., Rokhani, F.B., Jacob, P.S., Sabri, N.E.B., Hassan, M.S., Kadir, K. and Dharmarajan, L., 2024. Salivary metabolomics in oral potentially malignant disorders and oral cancer patients—a systematic review with meta-analysis. Clinical Oral Investigations, 28(1), p.98.
11. Bastías, D., Maturana, A., Marín, C., Martínez, R. and Niklander, S.E., 2024. Salivary biomarkers for oral cancer detection: an exploratory systematic review. International Journal of Molecular Sciences, 25(5), p.2634.
12. Pierfelice, T.V., D'Amico, E., Cinquini, C., Iezzi, G., D'Arcangelo, C., D'Ercole, S. and Petrini, M., 2024. The diagnostic potential of non-invasive tools for oral cancer and precancer: a systematic review. Diagnostics, 14(18), p.2033.
13. Mishra, B.P. and Patil, D.V., 2025. A comparative study to evaluate salivary biomarker interleukin-6 in oral squamous cell carcinoma and oral potentially malignant disorders. Cuestiones de Fisioterapia, 54(2), pp.864-868.
14. Muthu, K. and Dorairaj, J., 2022. Human Salivary microRNA as Biomarkers in Oral Potentially Malignant Disorder-A Narrative Review. Journal of Young Pharmacists, 14(2), p.156.
15. Dikova, V., Jantus-Lewintre, E. and Bagan, J., 2021. Potential non-invasive biomarkers for early diagnosis of oral squamous cell carcinoma. Journal of Clinical Medicine, 10(8), p.1658.
16. Shah, F.Y., Shetty, D.C., Kaur, G., Gupta, S., Pathak, A. and Aggarwal, V., 2023. Serum and saliva LDH levels in patients with habit, oral potentially malignant disorders (OPMDs), and oral squamous cell carcinoma (OSCC): a spectrophotometry study. Journal of Pharmacy and Bioallied Sciences, 15(Suppl 2), pp.S881-S884.