RESEARCH ARTICLE

Editorial Process: Submission:01/12/2023 Acceptance:06/23/2023

Role of Confocal Laser Endomicroscopy in Early Detection of Upper Gastrointestinal Malignancy in High Risk Patients

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Abstract

Background: Upper gastrointestinal malignancies are a major global health burden. Early diagnosis of upper gastrointestinal premalignant and malignant lesions is crucial for improving prognosis and reducing morbidity and mortality. The purpose of this study was to investigate the diagnostic accuracy of confocal laser endomicroscopy (CLE) in detecting upper gastrointestinal premalignant and early malignant lesions in high-risk patients, as well as diagnosing patients with inconclusive white light endoscopy (WLE) and histopathology results.**Methods:** It was a cross-sectional study that included ninety (n = 90) high-risk patients with inconclusive diagnoses of upper gastrointestinal lesions on WLE and WLE-based biopsy histopathology. These patients underwent CLE, and the definitive diagnosis was confirmed using CLE and CLE-target biopsy histopathology. Diagnostic accuracy was determined by comparing the sensitivity, specificity, predictive values, and accuracy between the procedures. **Result:** The mean patient age was 47.43 ± 11.18 years. CLE and target biopsy confirmed that 30 (33.3%) patients had normal histology, while 60 (66.7%) patients were diagnosed with gastritis, gastric intestinal metaplasia, high-grade dysplasia, adenocarcinoma, Barrett's esophagus, and squamous cell carcinoma of the esophagus. The results of CLE were superior to those of WLE in terms of diagnostic parameters. Additionally, CLE demonstrated nearly similar results in sensitivity (98.33%), specificity (100%), positive predictive value (100%), negative predictive value (96.77%), and accuracy (98.89%) when compared to CLE-target biopsy. **Conclusion:** CLE showed higher diagnostic accuracy in differentiating normal, premalignant and malignant lesions. It effectively diagnosed patients who initially had inconclusive WLE and/or biopsy results. Furthermore, early detection of upper gastrointestinal premalignant or malignant lesions may improve prognosis and reduce morbidity and mortality.

Keywords: Biopsy- CLE- malignant- premalignant- WLE

Asian Pac J Cancer Prev, **24 (6)**, 1949-1954

Introduction

Upper gastrointestinal malignancies are a major global health burden and the incidence varies greatly by geographic region, race, and socioeconomic status (Abengozar et al., 2021). In Pakistan, gastrointestinal malignancies account for 14.87% of all malignancies (Ali et al., 2022). Endoscopy is the gold standard procedure for early detection of premalignant and malignant gastrointestinal lesions, typically requiring a biopsy for a definitive diagnosis. Techniques like chromoendoscopy and magnifying endoscopy combined with narrow band imaging (M-NBI) have shown promise in enhancing the early identification and diagnosis of upper gastrointestinal malignancies (Costamagna and Marchese, 2010; Gong et al., 2015). However, optimal diagnostic methods have yet to be established (Jang, 2015).

Gastric carcinoma, an aggressive disease, is the third leading cause of cancer-associated deaths globally, often diagnosed at advanced stages (Johnston and Beckman, 2019). The pathogenesis of gastric cancer involves a progressive sequence from chronic atrophic gastritis to intestinal metaplasia, intraepithelial neoplasia, and ultimately, adenocarcinoma. Intestinal metaplasia is widely recognized as a precancerous lesion in the progression of gastric cancer (Dhondrup et al., 2022). The prognosis for advanced gastric carcinoma is poor, highlighting the need for early detection to improve 5-year survival rates (Hohenberger and Gretschel, 2003; Sobrino-Cossío et al., 2018). Investigating the association between

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premalignant lesions and the progression of gastric carcinoma is therefore crucial for the prompt detection and treatment (Ge et al., 2018). The intestinal metaplasia is typically diagnosed through a pathological examination of a biopsy specimen using white light endoscopy (WLE). However, it is time-consuming, fails to identify mucosal changes, and increases the likelihood of random biopsies (Sobrino-Cossío et al., 2018).

Barrett's esophagus, which develops as a result of long-standing gastroesophageal reflux disease, requires vigilant surveillance due to a significant 30-50 fold increase in the risk of esophageal adenocarcinoma. WLE with random biopsies may overlook certain lesions in Barrett's esophagus, as intraepithelial neoplasia or early adenocarcinoma can present in patchy segments (Neumann et al., 2012). Recently, a latest endoscopic procedure, Confocal Laser Endoscopy (CLE), has emerged for diagnosing various gastrointestinal disorders (Tomizawa et al., 2022). Early detection of Barrett's esophagus, gastric cancer, and other upper gastrointestinal malignancies using CLE significantly improves prognosis, reduces healthcare costs, and has a substantial impact on mortality reduction (Canakis et al., 2022).

This study aims to determine the diagnostic accuracy of CLE in detecting premalignant and early malignant lesions in the upper gastrointestinal tract among high-risk patients. Additionally, it aims to assess the utility of CLE in diagnosing patients with inconclusive results from white light endoscopy (WLE) and pathology results.

Materials and Methods

Study design and patients selection

This prospective cross-sectional study was conducted at the PNS Diagnostic and Research Institute of Gastroenterology and Hepatology, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, Pakistan, from January to December 2021. The minimum sample size of n=87 was calculated using the online sample size calculator OpenEpi (https://www.openepi.com/SampleSize/SSPropor.htm) considering the reported prevalence of 6% for upper gastrointestinal malignancies missed during endoscopy (Januszewicz et al., 2022), a 95% confidence level, and a design effect of 1. Ninety (n = 90) patients undergoing CLE were recruited through non-probability consecutive sampling. These patients had previously undergone white light endoscopy (WLE) with clinical suspicion of premalignant or early malignant lesions, but had negative histopathological results from WLE-based biopsy. Written informed consent was obtained from all participants, and a comprehensive medical history, clinical examination, and investigations were conducted. The study adhered to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines (Bossuyt et al., 2015), and a study flow diagram is presented in Figure 1.

Inclusion criteria

- Male or female, aged 12-60 years.
- High-risk patients with alarming symptoms and/or family history with symptoms suggestive of upper gastrointestinal malignancies, but a negative or failed biopsy result of WLE and a strong suspicion of disease.

Exclusion criteria

- Allergic to the fluorescein dye used in the procedure.
- Patients with acute gastrointestinal bleeding, gastrectomy, advanced gastrointestinal malignancies, diagnosed cases of premalignant and malignant lesions of the upper gastrointestinal tract.
- Patients with bleeding disorders, serious cardiopulmonary disorders, and renal insufficiency.
- Pregnant and lactating females.
- Patients not willing to participate.

Procedure

Initially, all patients were given Inj. fluorescein 0.5 cc I/V as a test dose to check for an allergic reaction to the injected dye. In the absence of any allergic reaction, a complete dose of contrast agent, fluorescein 2.5 cc to 5 cc I/V, was administered. After sedation (Inj. Midazolam 2 cc I/V), a probe CLE (Cellvizio; Mauna Kea Technologies, Paris, France) was performed by a highly skilled gastroenterologist, and endoscopic diagnosis was established through real-time images. Biopsies were taken from the targeted lesions and sent for histopathology. The histopathologists, with over 5 years of experience, were blinded to the clinical information and results of the index tests. Digital images of different sites were recorded and reviewed by an endoscopic expert who was blinded to the patient's medical history and diagnostic information. Patients were kept under observation for an hour after the procedure to check vitals and ensure stability. The lesions on CLE were diagnosed according to the Miami classification (Wallace et al., 2011).

Stastical analysis

The data was analyzed using SPSS v.23. Baseline characteristics of patients are presented as frequency/percentage or mean ± SD for categorical and continuous variables, respectively. To determine the diagnostic accuracy, the procedure results were compared and analyzed for sensitivity, specificity, predictive values, and accuracy.

Sensitivity and specificity were used to analyze the accuracy of the index test in comparison with the reference standard test. Sensitivity was defined as the test's ability to correctly identify patients with upper gastrointestinal lesions, while specificity was defined as the test's ability to correctly identify patients without lesions. Based on the results of the index test compared to the reference standard test, patients were categorized as true positive (correctly identified with upper gastrointestinal lesions), true negative (correctly identified without upper gastrointestinal lesions), false positive (incorrectly identified with upper gastrointestinal lesions), or false negative (incorrectly identified without upper gastrointestinal lesions).

Positive predictive value (PPV) was used to indicate the accuracy of a positive test result, calculated by dividing true positives by the sum of true positives and false positives. Negative predictive value (NPV) measures the accuracy of a negative test result, obtained

by dividing true negatives by the sum of true negatives and false negatives. The diagnostic accuracy of the test was defined by its ability to correctly identify or rule out upper gastrointestinal lesions. The calculations were performed using MedCalc statistical software.

First, diagnostic parameters were calculated to compare the diagnostic accuracy of the index test WLE with the reference standard test CLE. Second, the results of WLE combined with WLE-based biopsy were taken as the index test, and diagnostic accuracy parameters were calculated by comparing them with the reference standard test of CLE combined with CLE-target biopsy. Third, the results of CLE as an index test were compared with CLE-target biopsy as the reference standard test.

Results

Patient Characteristics

Baseline characteristics are presented in Table 1. The mean patient age was 47.43 ± 11.18 years. In total, there were 60 males (66.7%) and 30 females (33.3%). Eighty nine (98.8%) patients had a history of proton pump inhibitor (PPI) medication, whereas 87 (96.6%) patients

Table 1. Baseline Characteristics of Patients

Variables		Frequency (%) n = 90
Gender	Male	60 (66.7)
	Female	30 (33.3)
Epigastric/abdominal pain		87 (96.7)
Vomiting		54 (60)
Hemetemesis		0 (0)
Weight loss		54 (60)
Dysphagia		12 (13.3)
H. pylori positive		21 (23.3)
Treatment with PPI		89 (98.8)
Duration of treatment with PPI	4-8 weeks	2 (2.2)
	>4-8 weeks	87 (96.7)
Family history of gastrointestinal malignancy		48 (53.3)

PPI, Proton pump inhibitors

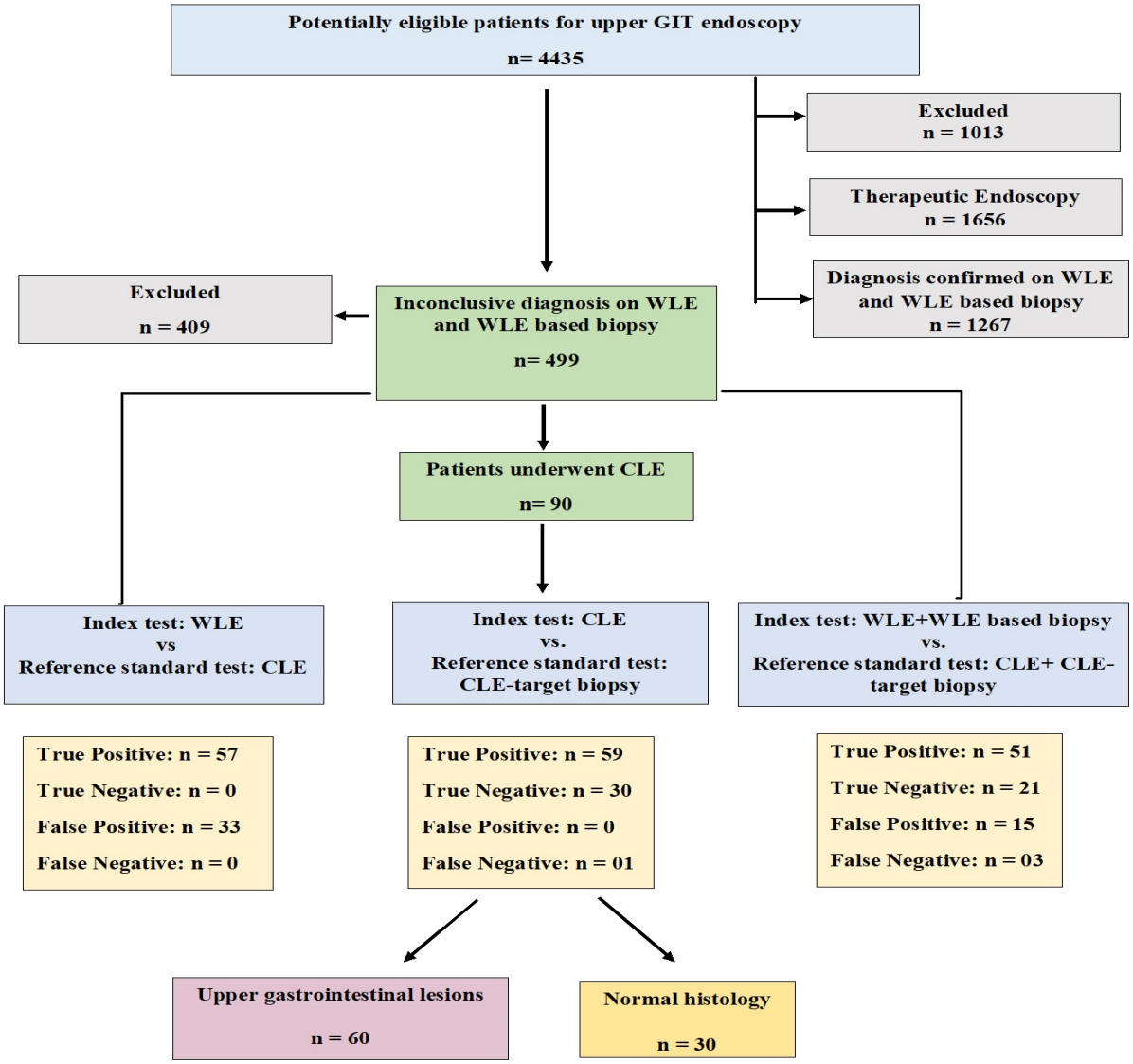

Figure 1. Study Flow Diagram. Abbreviations: CLE, Confocal laser endomicroscopy; GIT, Gastrointestinal; WLE, White light endoscopy

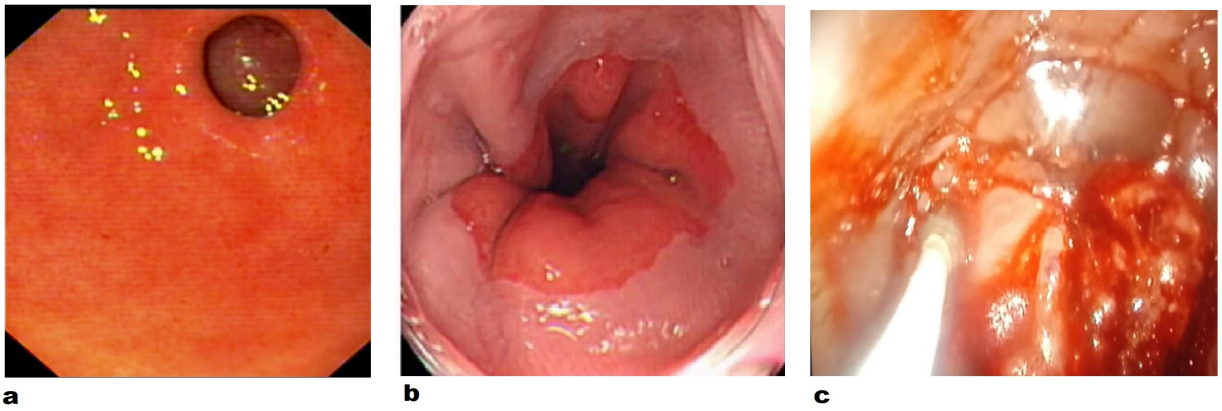

Figure 2. White Light Endoscopy Showing (a) Gastritis (b) Irregular Z line at gastroesophageal Junction (c) Visible growth

Table 2. The Diagnosis Results of Upper Gastrointestinal Lesions under CLE and CLE-Target Biopsy Histopathology

Diagnosis of upper gastrointestinal lesions	Frequency (%) n = 90
Normal epithelium	30 (33.3)
Gastritis	30 (33.3)
Gastric Intestinal Metaplasia	12 (13.4)
Gastric high grade dysplasia	03 (3.3)
Gastric adenocarcinoma	03 (3.3)
Barrett's oesophagus with high grade dysplasia	06 (6.7)
Squamous cell carcinoma of oesophagus	06 (6.7)

CLE, Confocal laser endomicroscopy

were on long term PPI medication. Twenty one patients (23.3%) tested positive for H. pylori, and more than half had a family history of gastrointestinal malignancy. The mean duration of symptoms was 9.23 ± 6.59 months. The mean hemoglobin levels were 10.93 ± 1.07 g/dL, while the total leukocyte count was 8.9 ± 1.93 x 10^9/L, and the platelet count was 225.1 ± 40.54 x 10^9/L. Figures 2 and 3 display images of various lesions detected on WLE and CLE, respectively.

CLE and Histopathological Diagnosis

Among the 90 patients, CLE and target biopsy histopathology results confirmed that 30 (33.3%) patients had normal histology, while 60 (66.7%) patients were diagnosed with gastritis, gastric intestinal metaplasia, high-grade dysplasia and gastric adenocarcinoma, Barrett's and squamous cell carcinoma of the esophagus. The confirmed diagnosis after CLE and target biopsy histopathology are presented in Table 2. The comparison between real-time diagnosis through CLE and post-diagnosis using recorded images and videos from other endoscopic experts showed agreement. The findings of stand-alone CLE in 89 patients were consistent with CLE-targeted biopsy histopathology results. However, there was one case of misdiagnosis where the CLE results (showing normal epithelium) did not match the histopathology results (indicating inflammation) (Figure 3).

Accuracy of WLE, CLE, and Histopathology

We compared the accuracy between (i) WLE and CLE, (ii) WLE combined with WLE-based biopsy and CLE combined with CLE-target biopsy, and (iii) CLE and CLE-target biopsy. As shown in Table 3, with CLE as the standard test, WLE sensitivity was 100%; however, the test was not specific and had the lowest accuracy. When comparing WLE and CLE along with specific biopsy histopathology reports, the sensitivity, specificity, and accuracy increased. With WLE combined with WLE-based biopsy, there were 27 (30%) misdiagnosed

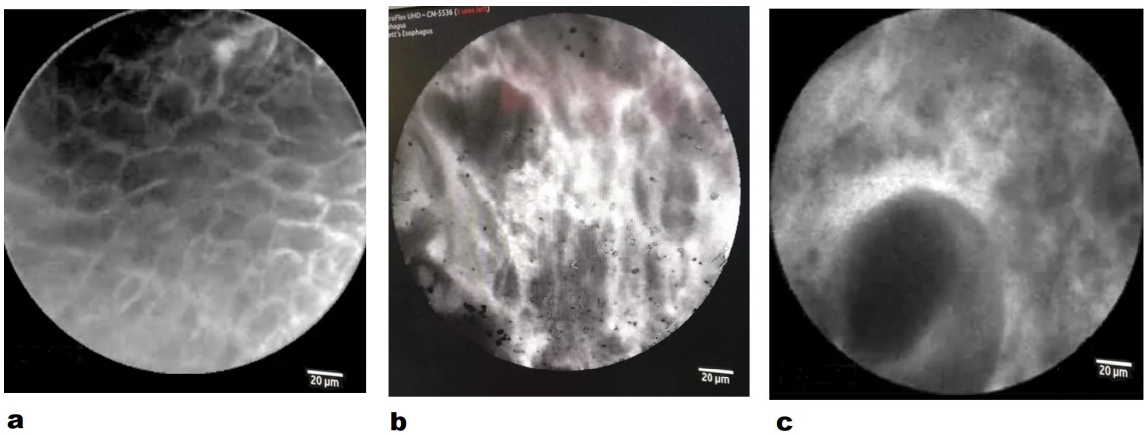

Figure 3. Confocal Laser Endomicroscopy Showing (a) Normal oesophageal epithelium (b) Barrett's oesophagus with high dysplasia (c) Gastric intestinal metaplasia

Table 3. Comparison of Diagnostic Parameters for the Detection of Upper Gastrointestinal Lesions

Diagnostic parameters	WLE vs. CLE	WLE+WLE based biopsy vs. CLE+ CLE-target biopsy	CLE vs. CLE-target biopsy
True Positive (n)	57	51	59
True Negative (n)	0	21	30
False Positive (n)	33	15	0
False Negative (n)	0	3	1
Sensitivity (%)	100	94.4	98.33
(95% CI)	(93.73-100)	(84.61-98.84)	(91.06-99.96)
Specificity (%)	----	58.33	100
(95% CI)		(40.76-74.49)	(88.43-100)
Positive predictive value (%)	-----	77.27	100
(95% CI)		(65.30-86.69)	(93.94-100)
Negative predictive value (%)	-----	87.5	96.77
(95% CI)		(67.64-97.34)	(83.30-99.92)
Accuracy (%)	63.33	80	98.89
(95% CI)	(52.51-73.25)	(70.25-87.69)	(93.96-99.7)

CI, Confidence interval; CLE, Confocal laser endomicroscopy; WLE, White light endoscopy

cases that were identified on CLE. CLE showed higher specificity, predictive values, and accuracy compared to WLE and exhibited almost similar results compared to CLE-target biopsy histopathology.

Discussion

Advanced endoscopic imaging technologies enhance the ability to detect dysplastic lesions with high accuracy; however, they do not replace the conventional histological diagnosis (Maione et al., 2022). Even though the time and cost required to use the new CLE technology must be considered, its impact on the differential diagnosis of benign and malignant conditions is substantial (Kim et al., 2022). In our study, we found that the CLE is an appropriate diagnostic approach since it is far superior to conventional WLE in terms of diagnostic accuracy. In addition, random biopsies through WLE may not accurately identify all dysplastic regions, as it samples only 4–5% of the mucosa (Maione et al., 2022). CLE was designed to acquire higher resolution and magnification imaging, referred to as "optical biopsies," of the gastrointestinal mucosa's histopathology. The significance of CLE lies in its ability to precisely and accurately identify normal tissue architecture. Moreover, CLE requires fewer samples as it utilizes microscopically targeted "smart" biopsies, resulting in a higher yield. CLE can be employed to visualize luminal structures such as the esophagus, stomach, and colon, as well as ductal structures like the pancreatic and bile ducts. This optimization of endoscopic diagnosis through CLE leads to a reduction in unnecessary resections, avoids repeated biopsies, and ultimately mitigates the risks and financial burden associated with repetitive indiscriminate endoscopic exams (Lerner et al., 2022). The most significant use of CLE in the esophagus is for surveillance and interpretation of suspicious lesions in Barrett's esophagus. In a meta-analysis of 14 studies involving 843 individuals, CLE was found to improve the diagnostic yield for dysplasia/cancer by 34% in Barrett's esophagus (Qumseya et al., 2013). In contrast, conventional WLE has been shown to frequently overlook early malignancies in Barrett's esophagus (Tang et al., 2021). Consistent with our finding, Kim Y et al. (2022) found that probe-based CLE exhibited significantly higher sensitivity, accuracy, and negative predictive value compared to WLE/M-NBI. Moreover, CLE demonstrated superior diagnostic and predictive capabilities for residual gastric carcinoma when compared to WLE/M-NBI.

In another study, conventional biopsies misclassified 12 out of 54 lesions. However, CLE accurately diagnosed 11 of these 12 lesions. The accuracy of conventional biopsy for diagnosing adenocarcinoma was 85.2%, and 91.7% for cancer differentiation. However, 8 out of the 32 adenocarcinomas were misclassified. On the other hand, CLE achieved an accuracy of 90.7% for diagnosing adenocarcinoma and 75.9% for cancer differentiation. Three of the 32 adenocarcinomas were misclassified by CLE. Notably, there was no difference in adenocarcinoma diagnosis between real-time and offline images (Bok et al., 2013). In our study, CLE alone proved to be sufficient for detecting lesions and distinguishing between normal tissue and premalignant/malignant lesions. It yielded diagnostic test results that were comparable to CLE-targeted biopsy histopathology. On the other hand, isolated WLE demonstrated higher sensitivity, but when combined with WLE-based biopsy reports, it exhibited improved specificity, predictive values, and accuracy. We were unable to report the specificity of isolated WLE due to our stringent patient selection criteria, which only included high-risk patients with initial negative biopsies. Besides the small sample size, another limitation of this study was its single study center design.

In conclusion, CLE showed higher diagnostic accuracy in differentiating normal, premalignant and malignant lesions. It effectively diagnosed patients who initially had inconclusive WLE and/or biopsy results. Furthermore, early detection of upper gastrointestinal premalignant or malignant lesions may improve prognosis and reduce morbidity and mortality.

Author Contribution Statement

The authors confirm contribution to the paper as follow: Nand Lal Seerani: conceptualization, study design, patients assessment and paper review; Hira Laghari: enrolled patients, performed clinical examination, study design and literature review; Feriha Fatima Khidri: Manuscript writing, literature review and statistical analysis; Sajan Sawai: Interpretation of results; Akram Bajwa: Interpretation of results, verified data and critical review of manuscript; Jalpa Devi: enrolled patients and collected clinical data..

Acknowledgements

The authors would like to thank LUMHS and technical staff for providing assistance and patients for their participation in the study.

Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval

The study was approved by the Ethical Review Committee of LUMHS, Jamshoro (Approval No. LUMHS/REC/-880, Dated: 12-03-2020). The study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki.

Consent to participate

Written informed consent was obtained from all individual participants included in the study.

Consent for publication

Written informed consent was obtained from all individual participants in the study for publication of their data.

Availability of data and material

The data of the manuscript will be provided on the request, by the corresponding author.

Conflict of interest

The authors have no conflicting interests to declare that are relevant to the content of this article.

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