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Assessment of LGR5 Expression in Colonic Biopsies of Patients with Chronic Diarrhea of Unknown Origin

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ABSTRACT

Background: Chronic diarrhea of unknown origin (CDUO) presents a diagnostic challenge due to the absence of clear histological or inflammatory findings. Emerging evidence suggests that intestinal stem cell dysfunction may contribute to mucosal abnormalities. This study aimed to assess the expression of LGR5 in colonic biopsies of patients with CDUO compared to healthy controls.

Methods: A comparative cross-sectional study was conducted at Khalifah Gul Nawaz Teaching Hospital, Bannu, from January to June 2025. A total of 120 participants were enrolled, including 60 cases of CDUO and 60 age-matched controls. Colonic biopsies were obtained during colonoscopy, and LGR5 expression was evaluated using immunohistochemistry and scored semi-quantitatively (0 to 3+). Data were analyzed using SPSS version 26, with a significance level of $p \leq 0.05$.

Results: Among cases, 66.7% had low LGR5 expression (score 0–1+), compared to only 16.7% in controls. High LGR5 expression (score 2+–3+) was observed in 33.3% of cases versus 83.3% of controls. Strong staining (>50% cells) was seen in only 10% of cases compared to 50% of controls. The difference in LGR5 expression between groups was statistically significant ($p < 0.0001$).

Conclusion: LGR5 expression was significantly reduced in patients with CDUO, suggesting potential intestinal stem cell dysfunction. LGR5 may serve as a useful biomarker for identifying subclinical mucosal defects in unexplained chronic diarrhea.

Keywords: Chronic Diarrhea, LGR5 Protein, Intestinal Stem Cells, Colonic Mucosa, Biomarkers.

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INTRODUCTION

Chronic diarrhea, defined as the passage of loose or watery stools lasting more than four weeks, remains a prevalent and often debilitating condition, particularly when no definitive cause can be identified despite a comprehensive diagnostic evaluation[1, 2]. Known etiologies include infectious, inflammatory, malabsorptive, secretory, and functional disorders; however, a significant proportion of patients are classified under chronic diarrhea of unknown origin (CDUO) when routine clinical, endoscopic, and histologic assessments fail to establish a diagnosis[3, 4]. This diagnostic uncertainty not only compromises clinical decision-making but also imposes a substantial psychological and financial burden on affected individuals[5].

The colonic epithelium undergoes rapid and continuous regeneration, driven by a specialized population of intestinal stem cells (ISCs) located at the base of the crypts of Lieberkühn[6]. These ISCs play a critical role in maintaining epithelial integrity, regulating fluid and electrolyte balance, and ensuring mucosal healing following injury[7]. Among the markers identified for ISCs, LGR5 (Leucine-rich repeat-containing G-protein coupled receptor 5) stands out as a definitive and well-characterized marker of active intestinal stem cells[8, 9]. First identified by Barker et al. in 2007, LGR5-expressing crypt base columnar (CBC) cells are now recognized as key drivers of epithelial self-renewal in both the small intestine and colon[10].

LGR5 functions as a receptor in the Wnt signaling pathway, which is essential for ISC maintenance and proliferation[11]. It is known to be highly expressed in homeostatic epithelium and has also been implicated in tissue repair following epithelial injury[12]. Importantly, aberrant expression of LGR5 has been observed in various gastrointestinal pathologies, including inflammatory bowel disease (IBD), colorectal adenomas, and carcinoma, highlighting its potential diagnostic and prognostic significance[13, 14]. Despite this, its role in non-inflammatory, non-neoplastic conditions like CDUO remains poorly understood.

In patients with chronic diarrhea lacking overt mucosal damage or inflammation on biopsy, subtle defects in epithelial turnover or barrier function may contribute to symptoms[15]. Given the central role of ISCs and LGR5 in particular, in epithelial regeneration and barrier maintenance, exploring LGR5 expression in CDUO may reveal novel insights into mucosal dysfunction that are invisible on routine histology[7]. The evaluation of LGR5 could also aid in identifying early epithelial alterations and may even have implications for future stem cell–targeted therapies.

By comparing LGR5 expression in CDUO patients to that of normal colonic tissue, this research seeks to uncover early molecular changes in the crypt architecture that may contribute to chronic

diarrhea in the absence of identifiable histopathologic abnormalities. This study may also provide a basis for considering LGR5 as a potential molecular marker in the diagnostic workup of unexplained chronic gastrointestinal symptoms. The present study aimed to assess LGR5 Expression in Colonic Biopsies of Patients with Chronic Diarrhea of Unknown Origin.

METHODS

This was a comparative cross-sectional study conducted at the Histopathology and Gastroenterology Departments of Khalifah Gul Nawaz Teaching Hospital, Bannu. The study was conducted over a six-month period, from January 1, 2025, to June 30, 2025. Ethical approval for this study was obtained from the Institutional Review Board (IRB) of Bannu Medical College, which is affiliated with Khalifa Gul Nawaz Teaching Hospital, Bannu, (Ethical Approval No: 389/Dir&MJ/BMC/2024: Dated: 11/12/2024.

The sample size was calculated using the OpenEpi Software (Version 3.01). The prevalence of chronic diarrhea of unknown origin was considered as 13%,[16] a 95% confidence level, 5% margin of error, and power of 80%, the estimated minimum sample size was 113. To account for potential data attrition or inadequate biopsy specimens, the sample size was rounded up to 120, with 60 cases (patients with chronic diarrhea of unknown origin) and 60 controls (normal colonic biopsies from individuals undergoing colonoscopy for screening or non-diarrheal indications).

A non-probability consecutive sampling technique was used for the recruitment of both cases and controls. Cases (n = 60) were the patients presenting with chronic diarrhea (>4 weeks), with no identifiable cause after clinical, laboratory, stool, and endoscopic evaluations. Controls (n = 60) were the individuals undergoing colonoscopy for screening (e.g., anemia, cancer screening) with normal findings and no gastrointestinal symptoms.

Participants were divided into two groups: cases and controls. For the case group, individuals aged between 18 and 65 years with a history of chronic diarrhea of unknown origin, defined as persistent diarrhea lasting more than four weeks with no identifiable cause after thorough clinical, laboratory, and endoscopic evaluation, were included. Only those with normal or non-diagnostic findings on colonoscopy and routine histology were eligible. Written informed consent was obtained from all participants before inclusion in the study. For the control group, age-matched individuals undergoing colonoscopy for reasons unrelated to gastrointestinal symptoms, such as routine screening or evaluation for anemia, were included. These individuals had no history of diarrhea or chronic gastrointestinal complaints, and their colonic biopsies showed normal histology. All controls provided written informed consent before participation.

Patients were excluded from the study if they had a known cause of diarrhea, such as inflammatory bowel disease, gastrointestinal infections, malabsorption syndromes, or celiac disease. Those with systemic conditions that could affect bowel function, such as diabetes mellitus with autonomic neuropathy, were also excluded. Additional exclusion criteria included recent use (within the past four weeks) of antibiotics, immunosuppressive drugs, or probiotics, as these could alter gut flora or mucosal behavior. Biopsy samples that were inadequate for molecular or immunohistochemical analysis were excluded from the final evaluation. Pregnant or lactating women were also excluded due to ethical and physiological considerations.

Eligible participants were identified from both the outpatient and inpatient departments of gastroenterology. Once patients were screened for eligibility based on the inclusion and exclusion criteria, written informed consent was obtained. Detailed demographic and clinical data, including age, gender, presenting symptoms, duration of diarrhea (for cases), and relevant medical history, were recorded in a structured data collection form.

During colonoscopy, four mucosal biopsies were obtained from the sigmoid colon of each participant, both from cases and controls. These biopsy specimens were immediately placed in sterile cryovials. A portion of the tissue was fixed in 10% neutral-buffered formalin for histopathological examination, while the remaining tissue was preserved in RNA stabilization solution for molecular analysis, if required.

Regular histological analysis was performed on all biopsy samples using hematoxylin and eosin (H&E) staining. This was done to make sure there was no neoplasia, inflammation, or other structural abnormalities. The final immunohistochemistry study only included tissues from both groups that were histologically normal.

Utilizing a validated anti-LGR5 monoclonal antibody, immunohistochemistry for LGR5 expression was performed. A citrate buffer was used to retrieve the antigen, endogenous peroxidase activity was blocked, the primary antibody was incubated, and DAB (3,3'-diaminobenzidine) chromogen was used for detection. Hematoxylin was then used as a counterstain for the slides. The LGR5 staining data were evaluated by two separate and skilled histopathologists who were not aware of the samples' clinical grouping.

LGR5 expression was evaluated using a semi-quantitative scoring system that accounted for both the proportion of crypt base cells showing positivity and the intensity of staining. The scoring was defined as follows: a score of 0 indicated no staining, 1+ represented weak staining involving less than 25% of cells, 2+ indicated moderate staining involving 25–50% of cells, and 3+ denoted strong

staining involving more than 50% of crypt base cells[10]. This standardized approach ensured consistency and objectivity in the interpretation of immunohistochemical results.

Data were entered and analyzed using 'IBM SPSS Statistics version 26.0'. Descriptive statistics were used to summarize clinicopathological characteristics. Continuous variables, such as age, were presented as 'means ± standard deviation' (SD), while categorical variables, such as gender and LGR5 expression scores, were presented as frequencies and percentages. To compare LGR5 expression between cases and controls, a Chi-square test was used, and a p-value ≤ 0.05 was considered statistically significant.

RESULTS

Table 1: Clinico-Pathologic Parameters of Study Participants (n=120)

Parameter	Cases (n = 60)	Controls (n = 60)	Total (n = 120)
Gender			
Male	32 (53.3%)	30 (50%)	62 (51.7%)
Female	28 (46.7%)	30 (50%)	58 (48.3%)
M: F Ratio	1.1:1	1:1	
Age			
< 60 years	40 (66.7%)	42 (70%)	82 (68.3%)
≥ 60 years	20 (33.3%)	18 (30%)	38 (31.7%)
Mean Age (years)	42.3 ± 11.2	41.6 ± 10.7	

A total of 120 participants were included in the study, comprising 60 cases with chronic diarrhea of unknown origin and 60 healthy controls. Among the cases, 53.3% were male and 46.7% were female, while in the control group, males and females were equally represented, each constituting 50% of the group. Overall, the study population consisted of 51.7% males and 48.3% females. With respect to age distribution, 66.7% of the cases were below 60 years of age and 33.3% were aged 60 years or above. Similarly, in the control group, 70% were under 60 years and 30% were aged 60 or above. The mean age of participants was comparable between the groups **(Table 1).**

Table 2: Expression of LGR5 in Cases and Controls of the Study participants (n-120)

LGR5 Expression Score	Cases (n = 60)	Controls (n = 60)
0 – No staining	18 (30%)	2 (3.3%)
1+ – Weak (<25%)	22 (36.7%)	8 (13.3%)
2+ – Moderate (25–50%)	14 (23.3%)	20 (33.3%)
3+ – Strong (>50%)	6 (10%)	30 (50%)

The expression of LGR5 was notably different between cases and controls. Among the cases, 30% showed no staining, and 36.7% exhibited weak staining (<25% positive crypt cells). Moderate staining (25–50%) was observed in 23.3% of the cases, while only 10% demonstrated strong expression of LGR5 (>50%). In contrast, only 3.3% of the controls had no staining, and 13.3% showed weak staining. A higher proportion of controls exhibited moderate (33.3%) and strong (50%) LGR5 expression compared to the cases **(Table 2)**.

Table 3: Frequency of LGR5 Expression in Study Participants (n=120)

LGR5 Score Category	Cases (n = 60)	Controls (n = 60)	P-value
Low (0–1+)	40 (66.7%)	10 (16.7%)	<0.0001
High (2+–3+)	20 (33.3%)	50 (83.3%)	

Low: <25% crypt cells positive; High: ≥25% crypt cells positive. The chi-square test is applied to calculate the p-value p-value ≤ 0.05 is considered significant.

When categorized into low and high expression levels, LGR5 expression showed a significant difference between cases and controls. Among the cases, 66.7% exhibited low LGR5 expression (<25% crypt cells positive), while only 33.3% showed high expression (≥25% crypt cells positive). In contrast, the control group had a much lower frequency of low expression (16.7%) and a substantially higher frequency of high expression (83.3%). This difference was found to be statistically significant with a *p*-value of <0.0001, indicating a strong association between reduced LGR5 expression and chronic diarrhea of unknown origin **(Table 3)**.

DISCUSSION

In this study, patients with chronic diarrhea of unknown origin (CDUO) demonstrated significantly reduced LGR5 expression in colonic crypt base cells compared to control subjects, with 66.7% of cases exhibiting low expression (<25% positive crypt cells) versus just 16.7% in controls

($p < 0.0001$). These results align with emerging evidence that intestinal epithelial disorders, including inflammatory and degenerative conditions, may involve impairment of stem cell populations.

In healthy human colon tissue, LGR5-expressing cells are tightly localized to the crypt base and maintain epithelial homeostasis. In contrast, premalignant lesions show expansion and mislocalization of LGR5+ cells toward the luminal surface[13]. Our findings deviate in that CDUO patients exhibit reduced LGR5 in crypt bases, suggesting a potential depletion of active stem cells rather than expansion.

In inflammatory bowel disease (IBD), especially Crohn's disease, single-cell analysis has revealed a notable reduction in both the percentage and expression level of LGR5+ intestinal stem cells, alongside an increased proportion of LGR5-low subtypes (ISC-II/III).[17] While our study focused on non-inflammatory diarrhea, the pattern of decreased LGR5 mirrors IBD findings, hinting that epithelial injury, even subclinical, may suppress LGR5+ stem cell activity.

Experimental models show that Lgr5+ stem cells can regenerate the crypt after injury, either through de-differentiation of progenitors or activation of reserve stem cell pools (ISC-II/III, +4 cells)[18]. In our CDUO cases, reduced LGR5 may indicate exhausted or non-responsive stem cell fractions unable to regenerate adequately, which could underpin symptoms of chronic diarrhea.

A recent study identified quiescent LGR5+ human colonic stem cells (marked by p27) capable of forming crypts post-injury in xenotransplant models[19]. If such quiescent pools are compromised or diminished in CDUO, this could explain reduced regenerative capacity despite normal histology on H&E.

In colitis-associated cancers, a large subset lacks LGR5 expression compared to sporadic colorectal carcinoma, highlighting that chronic inflammation can alter ISC phenotypes and suppress LGR5 expression[13]. Our study supports the notion that chronic mucosal stress, even without histologic inflammation, can diminish LGR5 expression.

LGR5 is a Wnt target gene and forms a positive feedback loop via R-spondin signaling[20, 21]. Disruption of Wnt signaling, due to altered niche factors, could down-regulate LGR5 and impair epithelial renewal. This may be operative in CDUO where subtle mucosal barrier dysfunction exists.

In models of chronic epithelial injury, prolonged loss of Lgr5+ cells leads to persistent crypt dysfunction, despite activation of reserve populations[22]. Our cases might mirror early stages of such exhaustion, where reserve activation is inadequate or absent.

Organoid cultures derived from LGR5+ cells can reconstruct functional crypts and repair mucosal damage[23]. Reduced LGR5 in CDUO likely impairs this regenerative potential, suggesting a mechanistic basis for sustained symptoms.

Paneth and stromal niche factors are essential for maintaining LGR5+ ISC function. Studies emphasize role of Paneth-secreted factors and microenvironment in ISC regulation[24]. Alterations in niche signaling, undetectable on routine histology, might underlie LGR5 downregulation in CDUO. While most ISC-focused research centers on cancer or inflammatory disorders, our study is among the first to document *functional* epithelial stem cell impairment in CDUO. This echoes broader reviews that highlight ISC dysregulation as a contributor to non-inflammatory gut dysfunctions[25].

Overall, our results demonstrate a significant decrease in LGR5 expression in patients with chronic diarrhea of unknown origin compared to controls. This reduction is consistent with findings in inflammatory and neoplastic conditions where ISC function is altered. It supports a hypothesis that *stem cell exhaustion or suppression* may underlie chronic diarrhea even in the absence of visible histopathologic change. Future studies should explore whether functional restoration of LGR5+ ISCs via Wnt modulation or organoid-based therapies could benefit this patient subgroup.

CONCLUSION

In contrast to healthy controls, this study showed that colonic biopsies of patients with chronic diarrhea of unexplained cause showed considerably lower expression of the intestinal stem cell marker LGR5. The predominance of low LGR5 expression among cases suggests a potential impairment in epithelial stem cell function and mucosal regeneration, despite normal histological appearance. These findings highlight the possibility of underlying molecular dysfunction in the intestinal crypts that may contribute to persistent diarrheal symptoms in the absence of overt pathology. Assessing LGR5 expression may offer a novel diagnostic insight into unexplained chronic diarrhea and pave the way for future research on stem cell–targeted therapies in functional gastrointestinal disorders.

LIST OF ABBREVIATIONS

CDUO: Chronic Diarrhea of Unknown Origin

LGR5: Leucine-rich Repeat-containing G-protein Coupled Receptor 5

ISC: Intestinal Stem Cell

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None

CONFLICT OF INTEREST

None

ETHICAL APPROVAL

The ethical approval was obtained from the Institutional Review Board (IRB) of Bannu Medical College, which is affiliated with Khalifa Gul Nawaz Teaching Hospital, Bannu, (Ethical Approval No: 389/Dir&MJ/BMC/2024: Dated: 11/12/2024.

AUTHORS' CONTRIBUTION

All authors contributed equally as per ICMJE policy

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