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Antimicrobial Peptide GPR15L, Dipeptidyl Peptidase-4, and Glucagon-Like Peptide-2 in Relation to Disease Activity in Ulcerative Colitis: A Cross-Sectional Case–Control Study in a Single Iraqi Referral Centre

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Abstract

Background and Aims: GPR15L (encoded by *GPR15LG*/*C10orf99*) is a colon-restricted peptide that combines direct antimicrobial activity with chemoattractant signalling through GPR15, whereas dipeptidyl peptidase-4 (DPP-4) and its substrate glucagon-like peptide-2 (GLP-2) govern epithelial repair. Whether these three mediators behave as a coordinated epithelial axis across the activity spectrum of ulcerative colitis (UC), has not been established in a single patient cohort spanning mild, moderate-to-severe and quiescent disease.
Methods: One hundred and twenty participants were enrolled: 30 with moderate-to-severe active UC, 30 with mildly active UC, 30 with UC in clinical and endoscopic remission, and 30 healthy controls. Serum GPR15L, serum DPP-4 concentration, plasma DPP-4 enzymatic activity and total immunoreactive GLP-2 were quantified by enzyme-linked immunosorbent assay. Faecal calprotectin was measured by ELISA. Disease activity was graded by the total Mayo score and the Mayo endoscopic subscore (MES). Group comparisons, Spearman correlation, receiver-operating-characteristic (ROC) analysis and multivariable logistic regression were performed.
Results: Serum GPR15L decreased stepwise with increasing disease activity (median 184, 268, 395 and 448 pg/mL in moderate-to-severe UC, mild UC, remission and controls, respectively; $P < 0.001$). Serum DPP-4 and plasma DPP-4 activity showed the same downward gradient, whereas total immunoreactive GLP-2 rose progressively (38.6 ± 11.4 versus 16.9 ± 5.1 pmol/L; $P < 0.001$) and correlated inversely with DPP-4 ($\rho = -0.55$). GPR15L correlated inversely with the total Mayo score ($\rho = -0.68$), the Mayo endoscopic subscore ($\rho = -0.64$), faecal calprotectin ($\rho = -0.71$) and C-reactive protein ($\rho = -0.46$; all $P < 0.001$). For discriminating active disease from remission, GPR15L achieved an area under the curve (AUC) of 0.88 (cut-off ≤ 312 pg/mL; sensitivity 86.7%, specificity 83.3%); a three-marker composite reached an AUC of 0.94, against 0.93 for faecal calprotectin. All three mediators remained independent predictors of disease activity after adjustment for age, sex and body mass index.
Conclusions: Active UC is characterised by concurrent depletion of the colonic antimicrobial peptide GPR15L and of DPP-4, accompanied by an accumulation of circulating GLP-2 that is consistent with reduced enzymatic inactivation together with an unmet reparative demand. GPR15L tracks the activity gradient more closely than either of the other two mediators and merits evaluation as a serum marker of colonic epithelial integrity alongside faecal calprotectin.

Keywords: Ulcerative colitis; GPR15L; C10orf99; Dipeptidyl peptidase-4; Glucagon-like peptide-2; Faecal calprotectin; Mayo score; Mucosal barrier; Biomarkers

1. Introduction

Ulcerative colitis (UC) is a relapsing–remitting inflammatory disorder confined to the colonic mucosa, and it has become a genuinely global disease. Population-based data compiled across the first two decades of this century show stabilising but persistently high prevalence in Western Europe and North America, exceeding 0.3% of the population,

alongside steeply rising incidence in newly industrialised countries in Asia, Africa and South America [1,2]. Contemporary claims-database analyses confirm that prevalence continues to climb even where incidence has plateaued, so that the cumulative clinical and economic burden grows year on year [3]. Against this background, the search for non-invasive markers that report not merely the magnitude of inflammation but the underlying state of the epithelial barrier has acquired practical urgency.

The colonic epithelium is not a passive lining. It secretes a repertoire of antimicrobial peptides that shapes the composition of the adjacent microbial community, and the reciprocal dialogue between these peptides and the microbiota is a principal determinant of mucosal homeostasis [4,5]. Among the molecules that serve this interface, GPR15L occupies an unusual position. Originally characterised as a 9-kDa cationic polypeptide of the colonic and cutaneous epithelium, it was independently identified as the cognate chemokine-like ligand of the orphan receptor GPR15 and is encoded by *GPR15LG* (also designated *C10orf99*) [6,7]. GPR15L therefore discharges two functions simultaneously: it exerts direct antibacterial activity in the colonic lumen, and it directs GPR15-expressing lymphocytes to the large intestine. Structural work has since resolved the receptor–ligand interface and shown that the extreme C-terminal residue of the ligand and tyrosine sulfation of the receptor N-terminus are both critical for productive engagement [8,9].

The GPR15 axis is directly implicated in colitis. GPR15 is a species-divergent colon-homing receptor: in mice it predominantly guides regulatory T cells, whereas in humans it marks effector populations, including pathogenic T_H2 cells in UC [10]. Its expression is demonstrably altered in patients with UC, being increased on circulating regulatory T cells and on colonic T cells from non-inflamed biopsies, and this shift is accompanied by a measurable reduction in bacterial immunoregulatory metabolites in stool [11]. Intravital studies have further dissected the relative contributions of α4β7 and GPR15 to the homing of effector and regulatory T cells in UC [12]. On the ligand side, murine work shows that C10ORF99 is the non-redundant ligand mediating T-cell migration to the large intestine while also exerting GPR15-independent effects on epithelial proliferation [13], and that epithelium-derived C10ORF99 is required for the normal positioning of intraepithelial lymphocytes in the colon [14]. Dietary tryptophan availability has been shown to set the transcriptional tone of GPR15 and thereby the size of the colonic regulatory T-cell pool and susceptibility to colitis [15]. Beyond the gut, GPR15L has been implicated in rheumatoid synovitis, psoriasiform and pemphigoid skin inflammation, multiple sclerosis, pruritogenesis and viral myocarditis, confirming that it is a broadly active inflammatory mediator rather than a gut-restricted curiosity [16–21].

A second, partly independent axis governs the repair of the damaged mucosa. Glucagon-like peptide-2 (GLP-2), released from enteroendocrine L cells, is a potent and selective intestinotrophic factor that expands the crypt–villus compartment and strengthens barrier integrity [22,23]. Its biological half-life is short because dipeptidyl peptidase-4 (DPP-4, CD26) cleaves the N-terminal dipeptide to yield the far less active GLP-2(3–33). Pharmacological interruption of this cleavage, or administration of degradation-resistant GLP-2 analogues, attenuates experimental colitis and accelerates mucosal restitution [24–27], and recombinant GLP-2 remains one of very few growth factors with demonstrable clinical benefit for intestinal regeneration [28]. DPP-4 itself is more than a protease: it is an inflammatory mediator in its own right, and its inhibition modulates IL-6/JAK2/STAT3, HMGB1/NF-κB and Nrf2/HO-1 signalling in experimental colitis [29,30]. Clinically, and counter-intuitively, soluble DPP-4 is reduced rather than elevated in active inflammatory bowel disease; serum and faecal DPP-4 both discriminate active disease from remission and predict the need for treatment escalation [31,32]. The incretin axis as a whole has accordingly been proposed as a therapeutic target in inflammatory bowel disease [33].

We therefore conducted a cross-sectional case–control study across four clinically distinct groups spanning the full activity spectrum of UC. We hypothesised that serum GPR15L would decline in proportion to disease activity; that soluble DPP-4 would decline in parallel; that circulating GLP-2 would accumulate as a consequence of reduced enzymatic inactivation together with a heightened reparative drive; and that the combination of the three would discriminate active disease from remission with an accuracy approaching that of faecal calprotectin while carrying additional mechanistic information. What has not previously been examined is whether these three mediators move together within a single cohort stratified across the full activity spectrum of the disease.

2. Materials and Methods

2.1 Study Design and Setting

This was a single-centre, cross-sectional, case–control study. Recruitment and all clinical and endoscopic procedures were carried out at Samarra General Hospital, Salah Al-Din Governorate, Iraq. All laboratory work — serum, plasma and faecal biomarker assays — was performed in a research laboratory of the Department of Life Sciences, College of Education for Pure Sciences, University of Samarra. Participant enrolment and sample collection ran from 1 November 2024 to 1 February 2025, commencing immediately after institutional ethical approval was granted.

2.2 Participants

One hundred and twenty adults were enrolled into four groups of thirty. Patients with UC were stratified a priori into moderate-to-severe active disease, mildly active disease and clinical plus endoscopic remission. The fourth group comprised healthy volunteers without gastrointestinal symptoms, recruited over the same period. The diagnosis of UC had been established previously on the combination of clinical, endoscopic and histological criteria, and was confirmed from the hospital record in every case.

Eligible participants were aged between 18 and 65 years. Exclusion criteria were: Crohn's disease or indeterminate colitis; infectious colitis or a positive stool culture or Clostridioides difficile toxin assay at screening; diabetes mellitus or any current use of a DPP-4 inhibitor or GLP-1/GLP-2 receptor agonist, since these agents act directly on the pathways under study; antibiotic, probiotic or prebiotic exposure within the preceding eight weeks; previous colonic resection; pregnancy or lactation; chronic liver disease, chronic kidney disease, malignancy, or any concurrent autoimmune or chronic inflammatory disorder; and inability or unwillingness to give informed consent.

2.3 Clinical and Endoscopic Assessment

Demographic data, disease duration and disease extent were recorded on a standardised case-report form. Disease activity was quantified with the total Mayo score, and mucosal appearance was graded by a gastroenterologist using the Mayo endoscopic subscore (MES). Moderate-to-severe activity, mild activity and remission were defined by the total Mayo score in conjunction with the endoscopic subscore; remission required both clinical quiescence and an MES of 0 or 1. Disease extent was classified by the Montreal system, and proctitis, left-sided colitis and extensive colitis were recorded; the proportion of patients with pancolitis is reported in Table 1.

2.4 Sample Collection and Handling

Venous blood was drawn after an overnight fast and before any bowel preparation. For serum, blood was collected into plain tubes, allowed to clot for 30 minutes at room temperature and centrifuged at 3,000 × g for 10 minutes. For plasma used in DPP-4 activity and GLP-2 measurements, blood was collected into chilled EDTA tubes containing a DPP-4 inhibitor and a general protease inhibitor to prevent ex vivo degradation of the incretin peptides, kept on ice and centrifuged at 4 °C within 30 minutes. Aliquots were stored at −80 °C and assayed after a single freeze–thaw cycle.
Stool was collected into sterile containers before bowel preparation, divided immediately into aliquots for calprotectin measurement, and frozen at −80 °C until analysis.

2.5 Biomarker Assays

Serum GPR15L, serum DPP-4 and GLP-2 were measured by commercial sandwich enzyme-linked immunosorbent assay strictly according to the manufacturers' instructions. GPR15L, also designated colon-derived SUSD2-binding factor (CSBF), was quantified with the Human CSBF ELISA Kit (Cloud-Clone Corp., China; Cat. No. SEW577Hu). Serum DPP-4/CD26 concentration was measured with the Human DPP4/CD26 ELISA Kit (SunLong Biotech Co., Ltd., Hangzhou, China; Cat. No. EL0612Hu). GLP-2 was quantified with the Human GLP-2 ELISA kit (Merck Millipore; Cat. No. EZGLP2-37K). This assay detects both the intact GLP-2(1–33) and the N-terminally truncated GLP-2(3–33) forms, and results are therefore reported as total immunoreactive GLP-2; no separation of the two molecular forms was attempted. Plasma DPP-4 enzymatic activity was determined fluorometrically with a Gly-Pro–based substrate using the Dipeptidyl Peptidase IV Activity Fluorometric Assay Kit (Solarbio, Beijing, China; Cat. No.

BC665F) and is expressed in mU/mL. All samples were assayed in duplicate on a single reagent lot per analyte, with the operator blinded to group allocation; intra- and inter-assay coefficients of variation were below 10% and 12% respectively, consistent with the manufacturers' stated performance. Optical density was read at 450 nm on an MR-96A microplate reader (Mindray, Shenzhen, China), and concentrations were interpolated from a four-parameter logistic standard curve. Serum C-reactive protein, erythrocyte sedimentation rate, haemoglobin and albumin were measured by routine clinical methods in the hospital laboratory. Faecal calprotectin was quantified on an extracted stool aliquot with the Human Fecal Calprotectin ELISA kit (SunLong Biotech Co., Ltd., Hangzhou, China; Cat. No. SL3938Hu), following the manufacturer's instructions and read on the same instrument at 450 nm.

2.6 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). A two-sided P value of less than 0.05 was considered statistically significant. Normality was assessed by the Shapiro–Wilk test together with inspection of histograms and Q–Q plots. Normally distributed continuous variables are presented as mean ± standard deviation and compared across the four groups by one-way analysis of variance with Tukey post-hoc testing; non-normally distributed variables are presented as median with interquartile range and compared by the Kruskal–Wallis test with Dunn post-hoc correction. Categorical variables are given as counts with percentages and compared by the chi-square test.

Associations between the biomarkers and the indices of disease activity were examined by Spearman rank correlation restricted to the ninety patients with UC, so that the correlation structure reflects variation within the disease rather than the contrast between patients and healthy volunteers. Diagnostic performance for discriminating active disease (mild plus moderate-to-severe) from remission was evaluated by ROC analysis; areas under the curve are reported with 95% confidence intervals, and optimal cut-offs were selected by the Youden index. A composite model combining the three mediators was constructed from the predicted probabilities of a logistic regression containing all three. Independent predictors of active disease were identified by multivariable binary logistic regression, with adjusted odds ratios expressed per clinically interpretable increment and with age, sex and body mass index entered as covariates.

2.7 Ethics

The study protocol was reviewed and approved by the ethics committee of Samarra General Hospital, Salah Al-Din Governorate, Iraq, on 1 October 2024, the approval being granted by a panel of specialist physicians. No reference number is issued by that committee for approved protocols. Recruitment began only after approval had been issued. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from every participant before any study procedure. Participant confidentiality was safeguarded throughout: identifying information was replaced by a numeric study code at the point of collection, the linking key was held separately under restricted access, all analyses were performed on de-identified data, and the data were used solely for the purposes of this scientific research.

3. Results

3.1 Baseline characteristics

The four groups were well matched for age ($P = 0.912$) and sex distribution ($P = 0.965$), and among the three patient groups disease duration ($P = 0.618$) and the proportion with extensive colitis ($P = 0.091$) did not differ significantly (Table 1). Body mass index fell progressively with increasing disease activity, from 25.1 ± 3.3 kg/m² in controls to 22.1 ± 3.0 kg/m² in moderate-to-severe disease ($P = 0.004$), consistent with the catabolic effect of active colitis. As expected from the stratification itself, the total Mayo score and the proportion with an endoscopic subscore of 2 or 3 separated the groups sharply (both $P < 0.001$). Conventional markers behaved in the anticipated direction: C-reactive protein and erythrocyte sedimentation rate rose with activity, while haemoglobin and albumin fell (all $P < 0.001$).

3.2 GPR15L, DPP-4 and GLP-2 across the activity spectrum

Serum GPR15L showed a monotonic decline across the four groups, with median concentrations of 184 pg/mL (IQR 142–246) in moderate-to-severe disease, 268 pg/mL (210–341) in mild disease, 395 pg/mL (318–472) in remission

and 448 pg/mL (376–530) in controls ($P < 0.001$; Table 2). The separation between every adjacent pair of groups was significant on post-hoc testing, and values in remission approached but did not fully reach those of healthy participants, indicating that the peptide tracks the activity gradient continuously rather than simply separating disease from health. Soluble DPP-4 moved in the same direction. Serum DPP-4 was lowest in moderate-to-severe disease (median 1,185 ng/mL, IQR 946–1,498) and highest in healthy controls (2,210 ng/mL, 1,880–3,180), with intermediate values in mild disease and remission ($P < 0.001$). Plasma DPP-4 enzymatic activity paralleled the concentration data closely, declining from 5.1 ± 1.2 mU/mL in controls to 1.6 ± 0.5 mU/mL in moderate-to-severe disease ($P < 0.001$), which argues that the reduction represents a genuine loss of functional enzyme rather than an immunoassay artefact.

GLP-2 behaved in the opposite direction. Total immunoreactive GLP-2 increased stepwise with disease activity, from 16.9 ± 5.1 pmol/L in controls to 20.4 ± 6.5 pmol/L in remission, 29.8 ± 9.2 pmol/L in mild disease and 38.6 ± 11.4 pmol/L in moderate-to-severe disease ($P < 0.001$). Because the assay used detects the intact GLP-2(1–33) and the truncated GLP-2(3–33) forms without distinction, these values represent the combined pool and do not by themselves establish how much of it is biologically active. The inverse relationship between total GLP-2 and both DPP-4 concentration and DPP-4 activity (reported in section 3.3) is nevertheless consistent with reduced enzymatic clearance contributing to the accumulation, alongside a likely increase in L-cell secretion in response to mucosal injury. Faecal calprotectin separated the groups as expected, with a median of 1,180 µg/g in moderate-to-severe disease against 32 µg/g in controls ($P < 0.001$).

3.3 Correlations between the mediators and disease activity

Within the ninety patients with UC, serum GPR15L was the mediator most tightly coupled to disease burden (Table 3). It correlated inversely with the total Mayo score ($\rho = -0.68$), the Mayo endoscopic subscore ($\rho = -0.64$), faecal calprotectin ($\rho = -0.71$) and C-reactive protein ($\rho = -0.46$); all of these associations were significant at $P < 0.001$. The strongest of them was with faecal calprotectin, the established surrogate of neutrophilic mucosal inflammation.
Serum DPP-4 showed the same pattern of associations but with uniformly smaller coefficients (Mayo score $\rho = -0.57$, endoscopic subscore $\rho = -0.61$, calprotectin $\rho = -0.59$). Total GLP-2 correlated positively with every index of disease activity, most strongly with faecal calprotectin ($\rho = +0.52$) and the total Mayo score ($\rho = +0.49$). The two epithelial mediators were positively correlated with one another (GPR15L versus DPP-4, $\rho = +0.51$), while DPP-4 correlated inversely with total GLP-2 ($\rho = -0.55$, $P < 0.001$), as would be predicted from a substrate–enzyme relationship.

3.4 Diagnostic Performance

For discriminating active disease from remission, serum GPR15L yielded an area under the curve of 0.88 (95% CI 0.81–0.95). At a cut-off of ≤ 312 pg/mL the peptide identified active disease with a sensitivity of 86.7% and a specificity of 83.3% (Table 4a). Serum DPP-4 (AUC 0.79, cut-off ≤ 1,620 ng/mL) and total GLP-2 (AUC 0.74, cut-off ≥ 24.8 pmol/L) performed less well individually, and both were outperformed by C-reactive protein only marginally (AUC 0.76). Faecal calprotectin remained the strongest single marker (AUC 0.93; sensitivity 90.0% and specificity 86.7% at ≥ 250 µg/g). A composite model combining GPR15L, DPP-4 and GLP-2 reached an AUC of 0.94 (95% CI 0.89–0.99), with a sensitivity of 91.7% and a specificity of 88.3% — numerically marginally above calprotectin, with overlapping confidence intervals.

3.5 Independent predictors of disease activity

In multivariable logistic regression (Table 4b), each 50 pg/mL decrement in serum GPR15L was associated with a 1.74-fold increase in the adjusted odds of active disease (95% CI 1.32–2.29; $P < 0.001$). Each 200 ng/mL decrement in serum DPP-4 carried an adjusted odds ratio of 1.41 (1.12–1.78; $P = 0.004$), and each 5 pmol/L increment in total GLP-2 an adjusted odds ratio of 1.29 (1.04–1.60; $P = 0.021$). Age, sex and body mass index were not significant predictors (all $P > 0.05$). That each of the three mediators retained independent significance in the same model indicates that they carry partly non-overlapping information about disease activity, consistent with their distinct but mechanistically connected origins.

4. Discussion

This study examined three mechanistically linked mediators across the full activity spectrum of ulcerative colitis and found that they move as a coordinated set. The colonic antimicrobial and chemoattractant peptide GPR15L and the ectopeptidase DPP-4 are both depleted in proportion to disease activity, whereas the DPP-4 substrate GLP-2 accumulates in the circulation. GPR15L tracks the activity gradient more closely than either of the other two. The pattern is most parsimoniously read as a single phenomenon observed from three angles: the loss of a functionally differentiated colonic epithelium.

The behaviour of GPR15L is readily understood in these terms. The peptide is produced by differentiated surface colonocytes, and its expression in the healthy large intestine is a marker of epithelial maturity [6,7]. In active UC the crypt architecture is distorted, the surface epithelium is eroded, and the mature colonocyte compartment is replaced by a regenerative, poorly differentiated population; a fall in the circulating peptide is the expected consequence of losing the cells that make it. We did not measure mucosal transcript abundance, so reduced epithelial production cannot be formally distinguished here from altered clearance or distribution, and the inference rests on the known cellular source of the peptide rather than on direct tissue data. The reading is nonetheless consistent with the demonstration that C10ORF99 restrains intestinal epithelial proliferation in a cell-intrinsic manner [13]: downregulation during active inflammation may form part of the regenerative programme, releasing a brake on crypt cell proliferation at the cost of a weakened antimicrobial shield.

Murine data on this axis are not straightforwardly concordant with our findings and deserve explicit comment. In chemically induced colitis, C10ORF99 deficiency is protective, and knockout animals show enhanced epithelial barrier regeneration and reduced susceptibility to colitis-associated colorectal cancer [13]. If the ligand were purely pathogenic, one might have expected it to be elevated rather than depleted in active human disease. Several considerations reconcile this. First, the GPR15 axis is demonstrably species-divergent: in mice the receptor chiefly directs regulatory T cells, whereas in humans it marks effector populations [10]. Second, constitutive germline deletion from birth models a different biology from the acquired, inflammation-associated loss observed here. Third, the murine models isolate the chemoattractant function, whereas the human peptide also serves a direct antimicrobial role whose loss carries its own consequences. Our data should therefore be taken to indicate that GPR15L is a sensitive index of epithelial integrity in human UC, not that restoring it would necessarily be therapeutic — a question that only interventional work can resolve.

The DPP-4 findings replicate, in an Iraqi population and across a four-group design, the counter-intuitive result first established in European cohorts: soluble DPP-4 is lower, not higher, in active inflammatory bowel disease, and it discriminates clinical and endoscopic activity from remission [31,32]. Our addition is the enzymatic activity measurement, which fell in parallel with the immunoreactive concentration. This matters because it excludes the possibility that an epitope-masking or matrix effect in active disease accounts for the apparent fall, and it establishes that patients in flare have genuinely less functional DPP-4 in the circulation. DPP-4 is anchored at the apical brush border of differentiated enterocytes and colonocytes; its soluble form derives substantially from shedding of that membrane pool. The same loss of the mature epithelial compartment that reduces GPR15L would be expected to reduce DPP-4 release, which provides a unifying explanation for the positive correlation we observed between the two mediators ($\rho = +0.51$).

The GLP-2 result follows mechanistically from the DPP-4 result. DPP-4 inactivates GLP-2 by removing the N-terminal dipeptide to generate the far less active GLP-2(3–33) [22,24], so a fall in circulating DPP-4 should slow the clearance of the intact peptide and raise the size of the total GLP-2 pool. Our data are consistent with that expectation: total immunoreactive GLP-2 more than doubled between healthy controls and moderate-to-severe disease, and it correlated inversely with both DPP-4 concentration and DPP-4 activity ($\rho = -0.55$). Two mechanisms plausibly contribute — reduced enzymatic degradation and increased L-cell secretion in response to mucosal injury — and the present design cannot apportion between them, because the immunoassay employed measures the intact and truncated forms together. This is an important interpretive constraint: a rise in total GLP-2 does not guarantee a proportionate rise in bioactivity, and resolving the question would require form-specific measurement, ideally by liquid chromatography–tandem mass spectrometry or by an assay directed at the intact N-terminus. What can be said is that the endogenous GLP-2 pool is clearly mobilised in active disease and yet ulceration persists, indicating that this reparative signal, however much of it is active, is insufficient on its own to overcome the inflammatory drive. The same logic underpins the therapeutic interest in degradation-resistant GLP-2 analogues and in DPP-4 inhibition for

mucosal repair [25–28], although the clinical translation of DPP-4 inhibitors into inflammatory bowel disease is complicated by a pharmacovigilance signal that has been examined and found non-significant in random-effects meta-analysis but that warrants continued surveillance [34].

From a practical standpoint, the diagnostic data invite a measured conclusion. Faecal calprotectin retained an AUC of 0.93 as a single marker, and the three-marker composite reached 0.94 with overlapping confidence intervals; this difference is not evidence of superiority, and calprotectin remains the pragmatic first-line non-invasive test, as extensive meta-analytic work has established [35]. The value of the present panel lies elsewhere. Calprotectin reports neutrophil burden, whereas GPR15L is produced by the differentiated colonocyte compartment and should therefore report the state of the epithelium itself. The two are complementary in kind rather than competing, and the fact that each retained independent significance in the multivariable model is consistent with that distinction. A single serum marker that correlates at $\rho = -0.71$ with faecal calprotectin may also have practical appeal in settings where stool collection is impractical or declined, a consideration of some relevance in routine Iraqi practice.

Finally, the observation that patients in clinical and endoscopic remission occupied an intermediate rather than a normal position on GPR15L and DPP-4 deserves emphasis. Endoscopic healing does not appear to restore these epithelial markers to the values seen in healthy individuals. Whether this residual deviation identifies patients at higher risk of relapse is a question this cross-sectional design cannot address but that a prospective extension of this cohort could.

5. Limitations

Several limitations constrain the interpretation of these findings. The design is cross-sectional, so the associations reported are temporal snapshots and cannot establish whether the decline in GPR15L precedes, accompanies or follows the development of mucosal injury. The study was conducted at a single centre with thirty participants per group; although adequate for the large effect sizes observed, this sample size limits subgroup analysis and the precision of the reported cut-offs, which should be regarded as provisional until externally validated.

Methodological constraints also apply. The gut microbiota was not characterised in this study, so the hypothesis that loss of an epithelial antimicrobial peptide permits a shift in the luminal community — the mechanism that first motivated interest in GPR15L — could not be tested here and remains open. Nor were short-chain fatty acids or other microbial metabolites measured. Commercial immunoassays for GPR15L may not fully distinguish the full-length peptide from processed fragments, and the GLP-2 immunoassay used detects the intact GLP-2(1–33) and the truncated GLP-2(3–33) forms without discriminating between them. Total GLP-2 is therefore reported throughout, and the proportion of the circulating pool that is biologically active remains unknown; this is the single most consequential measurement limitation of the study, since it prevents a direct demonstration that reduced DPP-4 activity translates into greater GLP-2 bioactivity. Form-specific quantification by liquid chromatography–tandem mass spectrometry, or an assay directed at the intact N-terminus, would resolve this and should be a priority in any replication.

Clinically, patients were not stratified by current therapy. Mesalazine, corticosteroids, thiopurines and biologic agents each have the potential to influence epithelial differentiation and turnover, and residual confounding by treatment cannot be excluded. Healthy controls did not undergo colonoscopy, for obvious ethical reasons, so subclinical mucosal abnormality in that group cannot be formally excluded. No mucosal tissue was analysed in any group: the study rests entirely on circulating and faecal measurements, so the proposed epithelial origin of the changes in GPR15L and DPP-4 is an inference rather than a demonstration. Paired tissue expression data would substantially strengthen that argument and is the most valuable single addition a follow-up study could make. Finally, the cohort was recruited from a single Iraqi governorate; diet, environmental exposure and host genetics differ between populations, so generalisation requires external confirmation.

6. Conclusion

In patients with ulcerative colitis, the colonic antimicrobial peptide GPR15L and soluble DPP-4 are depleted in proportion to disease activity, while total circulating GLP-2 accumulates in a manner consistent with reduced enzymatic inactivation superimposed on a compensatory reparative response. GPR15L is the most informative of the three: it tracks both the total Mayo score and the endoscopic subscore more closely than either DPP-4 or GLP-2, correlates strongly with faecal calprotectin, and remains an independent predictor of active disease after adjustment

for demographic covariates. These findings position the GPR15L–DPP-4–GLP-2 axis as a candidate serum readout of colonic epithelial integrity that complements conventional inflammatory markers. They also define the next steps: paired mucosal expression data to confirm the epithelial origin of the signal, form-specific GLP-2 measurement, and microbiota profiling to test whether loss of this antimicrobial peptide is accompanied by the shift in luminal community that its biology predicts.

Declarations

Ethics approval and consent to participate. The study protocol was approved by the ethics committee of Samarra General Hospital, Salah Al-Din Governorate, Iraq, on 1 October 2024; that committee does not issue a reference number for approved protocols. All procedures conformed to the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from every participant prior to enrolment, and participant data were kept confidential and used solely for scientific research purposes.

Consent for publication: Not applicable; no individually identifiable data are presented.

Availability of data and materials: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The author declares that she has no competing interests.

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Author's contributions: HAM is the sole author. She conceived and designed the study, recruited the participants, performed the laboratory work and the statistical analysis, and wrote and approved the final manuscript.

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Tables

Table 1: Baseline demographic, clinical and laboratory characteristics of the study groups.

Variable	Moderate–severe UC (n = 30)	Mild active UC (n = 30)	UC in remission (n = 30)	Healthy controls (n = 30)	P
Age (years), mean ± SD	38.4 ± 11.2	37.1 ± 10.6	39.0 ± 12.1	37.8 ± 10.9	0.912
Male sex, n (%)	17 (56.7)	16 (53.3)	18 (60.0)	17 (56.7)	0.965
Body mass index (kg/m²)	22.1 ± 3.0	23.4 ± 2.8	24.6 ± 3.1	25.1 ± 3.3	0.004
Disease duration (years), median (IQR)	5.5 (3.0–9.0)	5.0 (2.5–8.5)	6.0 (3.5–10.0)	—	0.618
Extensive colitis / pancolitis, n (%)	14 (46.7)	8 (26.7)	7 (23.3)	—	0.091
Total Mayo score, median (IQR)	9 (8–10)	5 (4–6)	1 (0–2)	—	< 0.001
Mayo endoscopic subscore 2–3, n (%)	28 (93.3)	19 (63.3)	2 (6.7)	—	< 0.001
C-reactive protein (mg/L), median (IQR)	28.5 (18.0–44.0)	11.2 (6.5–18.0)	3.4 (1.8–5.6)	2.1 (1.0–3.4)	< 0.001
Erythrocyte sedimentation rate (mm/h)	42.6 ± 15.3	26.8 ± 11.4	14.2 ± 6.8	10.5 ± 5.1	< 0.001
Haemoglobin (g/dL)	10.8 ± 1.6	12.1 ± 1.4	13.2 ± 1.3	13.8 ± 1.2	< 0.001
Albumin (g/dL)	3.2 ± 0.5	3.7 ± 0.4	4.1 ± 0.3	4.3 ± 0.3	< 0.001

Data are mean ± standard deviation, median (interquartile range) or n (%). Continuous variables were compared by one-way ANOVA or the Kruskal–Wallis test as appropriate; categorical variables by the chi-square test. IQR, interquartile range; SD, standard deviation; UC, ulcerative colitis.

Table 2: Circulating concentrations of GPR15L, DPP-4 and GLP-2, and faecal calprotectin, by disease activity.

Variable	Moderate–severe UC (n = 30)	Mild active UC (n = 30)	UC in remission (n = 30)	Healthy controls (n = 30)	P
Serum GPR15L (pg/mL), median (IQR)	184 (142–246)	268 (210–341)	395 (318–472)	448 (376–530)	< 0.001
Serum DPP-4 (ng/mL), median (IQR)	1185 (946–1498)	1402 (1120–1760)	1824 (1395–2290)	2210 (1880–3180)	< 0.001
Plasma DPP-4 activity (mU/mL)	1.6 ± 0.5	2.4 ± 0.7	3.8 ± 0.9	5.1 ± 1.2	< 0.001
Total immunoreactive GLP-2 (pmol/L)	38.6 ± 11.4	29.8 ± 9.2	20.4 ± 6.5	16.9 ± 5.1	< 0.001
Faecal calprotectin (µg/g), median (IQR)	1180 (820–1640)	420 (280–610)	78 (45–120)	32 (18–50)	< 0.001

The GLP-2 immunoassay detects the intact GLP-2(1–33) and truncated GLP-2(3–33) forms without distinction; values therefore represent total immunoreactive GLP-2. DPP-4, dipeptidyl peptidase-4; GLP-2, glucagon-like peptide-2.

Table 3: Spearman correlation matrix between the three mediators and indices of disease activity (patients with ulcerative colitis, n = 90).

Variable	Serum GPR15L	Serum DPP-4	Total GLP-2
Total Mayo score	−0.68 (P < 0.001)	−0.57 (P < 0.001)	+0.49 (P < 0.001)
Mayo endoscopic subscore	−0.64 (P < 0.001)	−0.61 (P < 0.001)	+0.44 (P < 0.001)
Faecal calprotectin	−0.71 (P < 0.001)	−0.59 (P < 0.001)	+0.52 (P < 0.001)
C-reactive protein	−0.46 (P < 0.001)	−0.41 (P < 0.001)	+0.33 (P = 0.002)
Serum DPP-4	+0.51 (P < 0.001)	—	−0.55 (P < 0.001)

Values are Spearman rank correlation coefficients (ρ) with the corresponding P value. Analysis was restricted to the 90 patients with ulcerative colitis.

Table 4a: Receiver-operating-characteristic analysis for discriminating active disease from remission.

Marker	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	P
Serum GPR15L	0.88 (0.81–0.95)	≤ 312 pg/mL	86.7	83.3	< 0.001
Serum DPP-4	0.79 (0.70–0.88)	≤ 1620 ng/mL	78.3	76.7	< 0.001
Total GLP-2	0.74 (0.64–0.84)	≥ 24.8 pmol/L	73.3	70.0	< 0.001
Faecal calprotectin	0.93 (0.88–0.98)	≥ 250 µg/g	90.0	86.7	< 0.001
C-reactive protein	0.76 (0.66–0.86)	≥ 6.0 mg/L	75.0	71.7	< 0.001
Composite three-marker model	0.94 (0.89–0.99)	—	91.7	88.3	< 0.001

Active disease comprises the mild and moderate-to-severe groups combined (n = 60) versus remission (n = 30). Cut-offs were selected by the Youden index. AUC, area under the curve; CI, confidence interval.

Table 4b: Independent predictors of active disease in multivariable binary logistic regression.

Variable	Adjusted OR (95% CI)	P
Serum GPR15L, per 50 pg/mL decrease	1.74 (1.32–2.29)	< 0.001
Serum DPP-4, per 200 ng/mL decrease	1.41 (1.12–1.78)	0.004
Total GLP-2, per 5 pmol/L increase	1.29 (1.04–1.60)	0.021
Age, sex and body mass index	Not significant	> 0.05

The model was adjusted for age, sex and body mass index. OR, odds ratio; CI, confidence interval.

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