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Investigating Interleukin-6 and Interleukin-10 in Ulcerative Colitis of Iraqi Patients: An Immunohistochemical Study

Fatimah Ahmed Sabry[1,2*], Suhad Saad Mahmood[1]
[1]*Department of Biotechnology, College of science, University of Baghdad, Baghdad, Iraq*
[2]*Department of Biology, College of Science, Al-Nahrain University, Baghdad, Iraq*

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Abstract

Cytokines are immune components that serve as markers for either promoting or inhibiting inflammation, contingent upon their specific concentrations and the presence of additional modifying factors. This study evaluates the relationship between ulcerative colitis (UC) tissue and the expression levels of Interleukin-6 (IL-6) and Interleukin-10 (IL-10). This research involves the endoscopic biopsy of 35 specimens from ulcerative colitis and 15 specimens from normal colon tissue to evaluate the expression levels of IL-6 and IL-10. The immunohistochemical technique revealed that IL-6 levels were significantly elevated in patients with UC, whereas IL-10 expression was comparatively lower in these patients. The data suggest that IL-6 and IL-10 may significantly influence the progression of UC and could serve as useful biomarkers for monitoring this progression.

Keywords: Ulcerative colitis; Cytokines; Immunohistochemistry; IL-6; IL-10.

دراسة إنترلوكين-٦ وإنترلوكين-١٠ في التهاب القولون التقرحي لدى المرضى العراقيين: دراسة مناعية كيميائية

فاطمة احمد صبري[2,1*], سهاد سعد محمود[1]
[1] قسم التقنيات الاحيائية , كلية العلوم, جامعة بغداد, بغداد, العراق
[2] قسم علوم الحياة , كلية العلوم, جامعة النهرين , بغداد, العراق

الخلاصة

السيتوكينات هي مكونات مناعية تعمل كمؤشرات إما لتعزيز أو تثبيط الالتهاب، وذلك اعتمادًا على تراكيزها المحددة و وجود عوامل معدّلة إضافية. تهدف هذه الدراسة إلى تقييم العلاقة بين أنسجة مرضى التهاب القولون التقرحي ومستويات التعبير عن إنترلوكين- ٦ (IL-6) والإنترلوكين -١٠ (IL-10). شملت الدراسة جمع ٣٥ عينة نسيجية من مرضى مصابين بالتهاب القولون التقرحي و ١٥عينة من نسيج قولوني طبيعي، لتقييم مستويات التعبير عن IL-6 و IL-10. أظهرت نتائج تقنية التأشير المناعي النسيجي ارتفاع ملحوظ بمستويات IL-6 في عينات مرضى التهاب القولون التقرحي، في حين أن تعبير IL-10 كان منخفضًا

* Email: fatima.ahmed2406p@sc.uobaghdad.edu.iq

نسبيًا لديهم. تشير هذه النتائج إلى أن لكل من IL−6 و IL−10 دورًا محتملًا في تطور المرض، مما يجعلهما مؤشّرين حيويين قد يكون لهما فائدة في متابعة هذا التطور.

1. Introduction

The pathogenesis of ulcerative colitis (UC) is expected to involve genetic variables, immunological components, and environmental triggers that interact in a multifactorial manner [1]. Recent evidence suggests that a central event in the pathogenesis of inflammatory bowel disease (IBD) is the inappropriate stimulation of mucosal immunity by antigens [2]. In UC, the loss of immune tolerance to commensal gut bacteria, coupled with dysregulated T-cell responses, leads to uncontrolled and sustained inflammation [3]. Interleukin-6 (IL-6) is one of the pivotal cytokines associated with the development of UC, and it is a proinflammatory cytokine produced by activated macrophages, dendritic cells, and epithelial cells when damage occurs to the intestines. Elevated IL-6 levels correlate strongly with disease severity and complications, including colitis-associated cancer [4]. Conversely, interleukin-10 (IL-10), a key anti-inflammatory cytokine produced by regulatory T cells (Tregs) and innate immune cells, suppresses proinflammatory mediators such as Tumor Necrosis Factor alpha (TNF-α) and IL-6 and also restores immune homeostasis [5]. Genetic studies have demonstrated that mutations in the IL-10 receptor cause early-onset colitis, underscoring its critical role in mucosal tolerance [6]. IL-6/IL-10 ratio has emerged as a critical immunologic determinant of UC progression, with recent studies demonstrating that elevated ratios correlate strongly with mucosal healing failure, corticosteroid resistance, and increased risk of colectomy, as patients with IL-6/IL-10 ratios >3.2 at diagnosis show 5.7-fold higher odds of biologic therapy failure compared to those with balanced ratios (<1.8), suggesting this metric could serve as both prognostic biomarker and therapeutic target [7].
The severity of UC is associated with increased risks of complications, including colitis-associated carcinoma, and correlates with elevated IL-6 levels. On the contrary, IL-10, or T helper 2 (Th2) interleukin-10, is an important anti-inflammatory cytokine that counteracts the detrimental actions of IL-6 and other mediators that drive inflammation. IL-10 is primarily released by macrophages and regulatory T cells (Tregs) and plays a crucial role in maintaining immune system homeostasis by repressing immune responses [8]. IL-10 encourages the repair of epithelial cells, suppresses the function of dendritic cells and macrophages, and neutralizes the cytokine activities, which are conducive to inflammation. Mice deficient in IL-10, or its signal transducing components, exhibit an irregular level of inflammation in the intestines as demonstrated in studies involving mice in which IL-10 has been deleted [9].

It has been reported that IL-10 is decreased in patients with colonic tissue from ulcerative colitis by scientists. This discovery indicates that the processes that normally keep inflammation in check have failed, and tissue keeps getting damaged, and the illness continues to get worse. Monitoring of the inflammatory status of UC patients is the result of the interaction between IL-6 and IL-10. A higher IL-6/IL-10 ratio is associated with a more rapid disease course, increased severity of mucosal damage, and a decreased likelihood of responding to treatment. Current research is focused on therapies attempting to correct this imbalance [10]. Investigations are also underway into the role of IL-6 inhibitors in improving clinical outcomes and decreasing inflammation among patients with UC. Therapies that target enhancing IL-10 signaling are currently being explored [11]. This might be achieved either by the systemic application of cytokines or by stimulating regulatory cells that produce IL-10. These findings emphasize the potential of targeted therapies that would target factors implicated in the modulation of IL-6 and IL-10 expression in UC to re-establish an

immunological balance. The purpose of this study is to investigate the possible role of their tissue IL-6 and IL-10 levels in UC.

This study aims to determine whether IL-6 and IL-10 are indicators of disease progression in UC, shedding light on how the immune system influences gut disease. The findings may also contribute to a better understanding of the causes of UC and offer insights into how to diagnose and treat it.

2. Materials and Methods

Samples collection

Fifty fresh endoscopic biopsies from colon tissue were collected at the Gastroenterology and Hepatology specialized hospital, a tertiary Centre in the Medical City Directorate in Baghdad, Iraq. Biopsies were collected during the period from February to August 2025. Thirty-five biopsies were taken from fresh normal colon, while the other fifteen biopsies were collected from UC patients. All specimens were fixed in 10% formalin. Those specimens were examined and diagnosed as UC and normal tissues by using hematoxylin and eosin-stained histopathological examination at the pathology Lab of the National Center of Teaching Labs in Medical City. The Medical Ethics Committee of the University of Baghdad, College of Science, approved the investigation under the reference number CSEC/1224/0119. All participants gave informed consent and agreed to donate endoscopic samples for this case-control study.

Immunohistochemistry Experiments

Quantification of IL-6 and IL-10 levels was performed using Human IL-6 and IL-10 Polyclonal Antibody ELISA Kits (Elabscience, USA). These kits are based on the sandwich enzyme-linked immunosorbent assay (ELISA) principle and utilize polyclonal antibodies specific to human IL-6 and IL-10.

Preparing paraffin-embedded tissue blocks

After fixation of specimens in 10% formalin, they were cut with a surgical knife to 2.5 mm * 2 mm. Then, they were transferred to a special plastic cassette. Specimens were then dehydrated using a series of alcohol concentrations (70% for 1 hour, 80% for 1hour, 90% for 1 hour, and 100% for 12 hours) and then transferred into a xylene bath for 1 hour, then repeated the process with freshly added xylene for another 1 hour. After clearing by xylene, specimens were infiltrated with paraffin wax (which melted at 54–60 °C) in a beaker and two changes of paraffin wax for 1 hour each [12].

Removal of paraffin, rehydration, and staining

Formalin-fixed, paraffin-embedded (FFPE) tissue blocks were sectioned into 4-μm slices and mounted onto charged slides. These slides were then fixed in a hot air oven at 65 °C for 30 minutes. To remove paraffin, the slides were subjected to three sequential immersions in xylene, each for 5 minutes. Following deparaffinization, the residual liquid was removed, and the slides were rehydrated through a graded ethanol series: 100%, 90%, 80%, and 70%. This was followed by distilled water (D.W.) for 5 minutes at each step. Hematoxylin staining was performed for 5 minutes, followed by washing in D.W. Subsequently, eosin staining was applied for 5 seconds before another D.W. wash. The slides were then dehydrated using an ascending ethanol gradient (70%, 80%, 90%, and 100 %) for 1 minute at each concentration. Clearing was conducted in xylene for 1 minute, after which the slides were mounted using Dibutylphthalate Polystyrene Xylene (DPX) mounting medium [13]. Histopathological examination of these paraffin-embedded tissue samples was performed at the Pathology

Laboratory of the National Centre of Teaching Labs in Medical City, where they were diagnosed as UC and normal tissues.

Antigen retrieval

The antigen retrieval solution was completed and ready for use. The slides were washed with deionized distilled water and placed in a plastic staining jar containing the antigen retrieval solution. The plastic staining jar was laid in the water bath at 97 °C for 40 minutes, ensuring the slides were still covered with the retrieval solution. The cooling time was at least 20 minutes at room temperature[14].

Preparation of working solutions

To prepare each working solution, freshly prepared buffers were employed. The following supplies were used in this process:

EnVision FLEX Substrate Working Solution

EnVision FLEX Substrate Working Solution is prepared by mixing it thoroughly with 1 drop of EnVision FLEX DAB+ Chromogen (3,3N-Diaminobenzidine Tertrahydrochloride) per 1 mL of EnVision FLEX Substrate Buffer (Buffered solution containing hydrogen peroxide and preservative).

Procedure of staining

The following method was carried out at room temperature in a humidified chamber: Slides were incubated in 100 µl of peroxidase substrate for 5 minutes, then washed with washing buffer for 5 minutes. Slides were incubated in 100 µl of primary antibody (IL-6 and IL-10) polyclonal antibody (Elabscience/USA) for 30 minutes, followed by washing with wash buffer (Tris-buffered saline solution containing Tween) for 5 minutes. Slides were incubated in 100 µl of FLEX/HRP (Dextran coupled with peroxidase molecules), then washed with two changes of wash buffer for 5 minutes each. Slides were incubated in 200 µl of substrate working solution for 10 min, washed with deionized water for 5 minutes, washed with wash buffer for 5 minutes, and washed with deionized water for 5 minutes. Dehydration of the slides by series alcohol concentrations as below: 70%, 80%, 90%, then 100% for 1 minute each. Clearing by xylene for 1 minute and covered by a cover slide, slipping with DPX[15].

Reading slide and scoring method

Pathologist examine each slide using the double-blind procedure. The pathologist was instructed to recount any counts where there was a variance of more than 10%. After DAB formation, the presence of brown-yellow pigmentation or brown-yellow granules in the cell was interpreted as an indication that IL-6 and IL-10 were present. Utilizing the semi-quantitative integration method [16]. The results were calculated. In particular, three randomly selected locations were examined using a light microscope with a magnification of x200, and the percentage of cells that stained positively and the staining intensity were used to calculate scores. The immunohistochemical staining results were quantified using a standardized semi-quantitative scoring system that evaluated both the percentage of positively stained cells and the staining intensity, which directly correlates with target protein expression levels. For the percentage of stained cells, 1 point was assigned when less than one-third (1/3) of cells were positive, 2 points for one-third to two-thirds (1/3-2/3) positive cells, and 3 points when more than two-thirds (>2/3) of cells showed positive staining. Staining intensity was graded on a 4-point scale: 0 indicated no detectable staining (colorless), 1 represented weak expression (light yellow), 2 denoted moderate expression (brownish yellow), and 3 signified

strong expression (brown). The brown color intensity progression from light yellow to dark brown reflects increasing amounts of target protein. These two scores for percentage of positive cells and staining intensity were combined for each tissue segment to generate a comprehensive evaluation of protein expression. This dual-parameter scoring approach has been widely adopted in inflammatory bowel disease research as it provides both quantitative and qualitative assessment of protein expression patterns in tissue samples. Two experienced pathologists independently evaluated all slides to ensure scoring consistency, with any discrepancies resolved through a consensus review[17].

3. Results and Discussion

This study evaluated the expression levels of IL-6. Positive IL-6 expression was observed in all UC tissues, as illustrated in Figure 1A. In contrast, Figure 1B demonstrates that normal tissues exhibit negligible IL-6 expression.

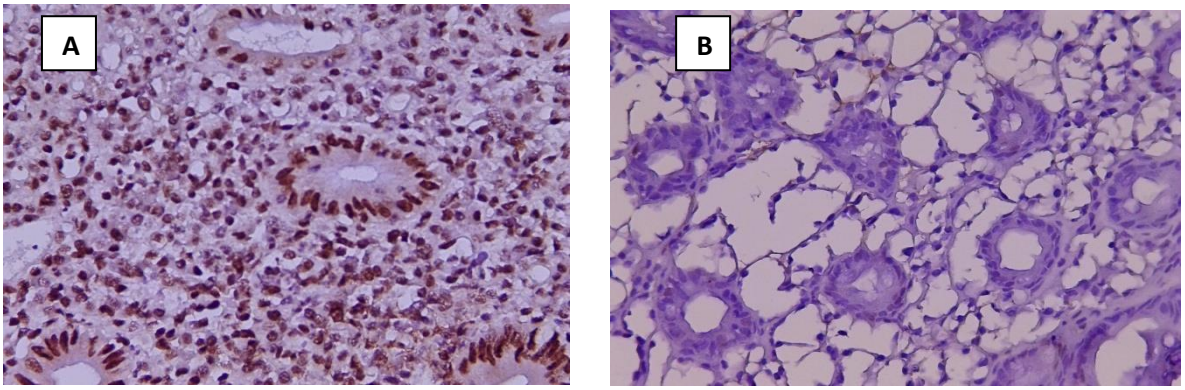

Figure 1: Expression of IL-6 in tissues with UC (A) and normal colonic tissues (B). (immunohistochemical staining at x40 magnification).

Figures 1A and 1B demonstrate the dual role of IL-6 in inflammatory signaling and the impairment of the epithelial barrier. The findings are consistent with previous research on the molecular mechanisms and clinical significance of IL-6 in inflammatory bowel disease (IBD)[18].

The low expression of IL-6 in normal colonic tissue indicates that it acts as an inducible cytokine during inflammatory stress rather than as a constitutively active molecule in homeostatic settings. IL-6 is significantly overexpressed in the inflamed mucosa of patients with UC, while its levels are modest in healthy colonic tissue. This differential expression pattern supports studies indicating a substantial increase in IL-6 levels in mucosal biopsies, which correlates with endoscopic disease severity and reinforces the link between IL-6 expression and active inflammation in UC patients [19].

Table 1 confirms that IL-6 expression was detected in all UC tissues, with positive staining primarily localized to lymphocytes and the epithelial cell membrane in a scattered distribution pattern.

Table 1: Summary of IL-6 score on normal and UC tissues.

Tissue samples	No score	+	++	+++	Total
IL-6 score in UC tissues	3	8	14	10	35
IL-6 score in normal tissues	14	1			15

This result aligns with a previous study analyzing cytokine levels in UC patients, which revealed significantly higher IL-6 mRNA expression in inflamed colonic mucosa compared to non-inflamed sections, demonstrating localized proinflammatory signaling during active disease. [20].

The increase was related to the movement of more immune cells and changes in the cells that can lead to long-term inflammation and scarring. Colonic epithelial cells, activated immune cells (such as macrophages, lymphocytes, and dendritic cells), and UC all trigger the production of interleukin-6 in response to inflammation[21]. The cytokine maintains continual inflammation by activating other signaling pathways such as JAK/STAT3, Mitogen-Activated Protein Kinase (MAPK), and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) through the IL-6 trans-signaling pathway, which comprises the soluble IL-6 receptor (sIL-6R) and gp130 [22].
Furthermore, IL-10 expression was markedly elevated in all UC tissues, as depicted in Figure 2. Specifically, Figure 2A illustrates IL-10 expression in UC tissues, while Figure 2B presents IL-10 expression in normal tissues.

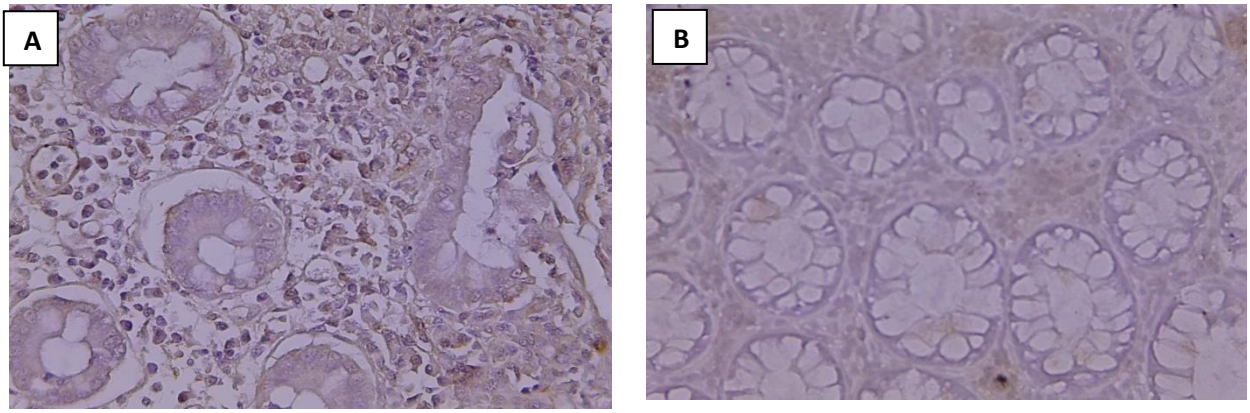

Figure 2: Expression of IL-10 in tissues with UC (A) and normal colonic tissues (B). (immunohistochemical staining at x40 magnification).

This is consistent with data in Figure 2, which shows that an elevated level of IL-10 expression in UC tissues may play a significant role in chronic inflammation and the maintenance of immune system homeostasis in the gut mucosa. IL-10 is a key immunosuppressive cytokine responsible for the negative regulation of proinflammatory cytokines, including TNF-α, IL-6, and IL-1β, in macrophages and dendritic cells within the inflamed tissues of UC. The overexpression of IL-10 appears to serve a compensatory role in mitigating the overactivity of the immune system and the damage to the epithelial barrier [23].

Table 2: Summary of IL-10 score on normal and ulcerative colitis tissues.

Tissue samples	No score	+	++	+++	Total
IL-10 score in UC tissues	7	18	9	1	35
IL-10 score in normal tissues	13	2			15

The finding that all UC tissues showed positive IL-10 expression, primarily in lymphocytes and epithelial cells, albeit at low levels, is consistent with current literature regarding the intricate role of IL-10 in the pathogenesis of UC. IL-10 is an essential anti-inflammatory cytokine synthesized by diverse cell types, such as T lymphocytes and epithelial cells, to modulate immune responses and preserve intestinal homeostasis. Studies have indicated that UC patients exhibit elevated IL-10 expression in mucosal tissues, implying an upregulation of IL-10 production in response to inflammation [24].

Descriptive Analysis of Cytokine Expression in UC Patients

Immunohistochemical evaluation revealed significant changes in mucosal cytokine expression patterns in colonic tissue specimens from UC. With a mean score of 2.1 ± 0.7, the expression of the proinflammatory cytokine IL-6 was much higher; the mean score of the anti-inflammatory cytokine IL-10 was 1.5 ± 0.6. The average score of 1.6 ± 0.5 indicates a significant shift towards a proinflammatory environment in tissues affected by UC, due to a significant change in the IL-6/IL-10 expression ratio.

Figure 3 graphically shows this information using a bar chart showing the mean ± standard deviation of IL-6 and IL-10 expression levels together with the computed IL-6/IL-10 ratio. The data underlines unequivocally the preponderance of IL-6 expression in comparison to IL-10, therefore supporting the idea of a distorted cytokine milieu favoring inflammation in the UC group

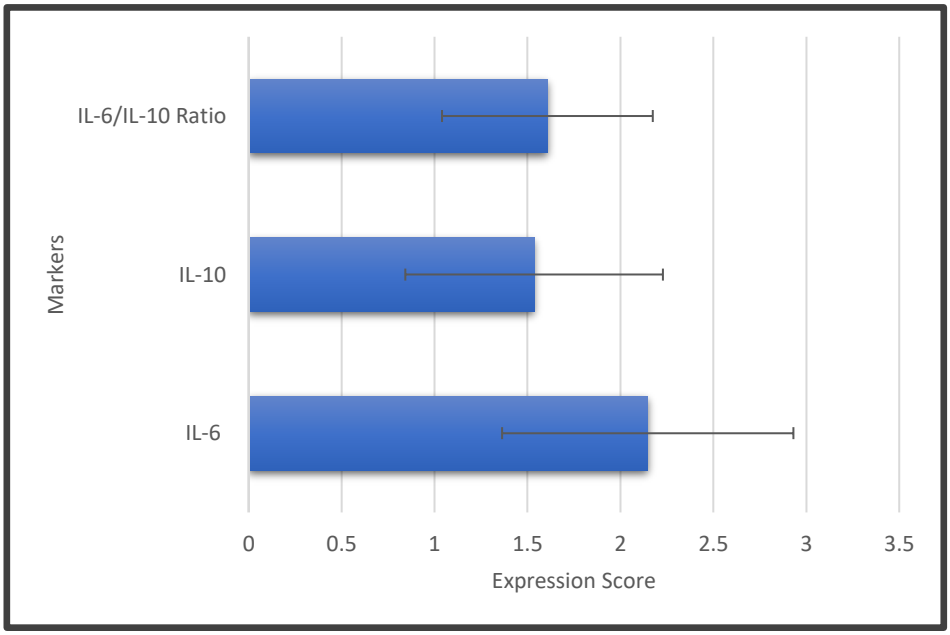

Figure 3: Bar chart illustrating mean (± SD) expression scores of IL-6, IL-10, and IL-6/IL-10 ratio in UC patient tissues. Elevated IL-6 expression and a higher IL-6/IL-10 ratio indicate a proinflammatory cytokine bias in the UC mucosa.

Together with its relatively low expression levels in this study, the observed rise of IL-10 points to an inadequate anti-inflammatory response that can aggravate the chronic inflammation unique to UC. This shortfall could result from either the influence of elevated proinflammatory cytokines that limit IL-10 function or from compromised IL-10 signaling pathways [25]. Particularly in innate immune cells, deficiencies in IL-10 receptor signaling have been associated with increased intestinal inflammation, thereby highlighting the crucial importance of operational IL-10 pathways in controlling mucosal immune responses [26].
Moreover, the observed cytokine imbalance confirms results from the literature that pinpoint IL-6 as a major component of chronic intestinal inflammation. Both of which are known to worsen tissue damage in ulcerative colitis, IL-6 causes sustained activation of the JAK/STAT3 signaling pathway and promotes Th17 cell proliferation [27]. On the other hand, IL-10 is crucial for preserving mucosal homeostasis and immunological tolerance; its reduced expression could point to an impaired anti-inflammatory counter-regulation, a clear trait of UC pathogenesis[28].
Finding IL-10 in epithelial cells points to a localized attempt to control inflammation and protect the mucosal barrier. It has been shown that epithelial cell generated IL-10 improves mucosal immune responses and increases barrier integrity [29]. The endogenous synthesis of IL-10 is insufficient in ulcerative colitis to counteract the predominance of inflammation [30].
Ulcerative colitis's dysregulated immune response, which is characterized by higher IL-6 and lower IL-10 activity, greatly adds to ongoing intestinal inflammation [31]. This finding highlights the therapeutic potential of targeting cytokine pathways to restore immunological balance. Targeting IL-6 is a possible approach shown by the use of poly lactic-co-glycolic acid (PLGA). nanoparticles loaded with berberine that especially block the IL-6/IL-6R axis, therefore reducing inflammation and promoting mucosal healing [32]. Presenting an original approach to treating UC, the nanoparticle-based delivery system enhances the bioavailability and therapeutic efficacy of berberine [33].
Similarly, increasing IL-10 activity has become a practical course of action. To significantly reduce inflammation in UC models, a polyphenol-mediated IL-10 mRNA delivery mechanism has been developed to increase IL-10 expression in colonic tissues [34]. This method presents a promising treatment technique for reducing inflammation related to UC by using the anti-inflammatory features of IL-10 [35].
Moreover, phytochemicals have been investigated for their control of cytokine patterns. While boosting anti-inflammatory cytokines like IL-10, some bioactive compounds have shown the capacity to block proinflammatory cytokines such as IL-6, offering an alternative approach to restore immunological balance in ulcerative colitis [36, 37]. Furthermore, showing promise in preclinical conditions is the development of innovative fusion proteins designed to enhance IL-10 distribution to intestinal areas. These proteins are designed to reduce mucosal inflammation in UC and increase the anti-inflammatory properties of IL-10 [38].

Therapeutic approaches targeted at the IL-6/IL-10 axis have a great possibility to restore immunological homeostasis in UC. Correcting cytokine imbalances may improve clinical outcomes and open up new therapeutic options for UC treatment.

4. Conclusion

Levels of IL-6 and IL-10, key molecules involved in inflammation and the immune system, were massively higher in tissue samples from UC compared to samples from healthy colon tissue. Whereas IL-10 is involved in the de-escalation of inflammation and tissue protection, IL-6 induces pathways that activate inflammation, such as JAK/STAT3, therefore, chronic inflammation continues. Unlike IL-10, which attenuates inflammation and

protects the tissue from damage, IL-6 activates proinflammatory pathways, including JAK/STAT3, resulting in chronic inflammation. But IL-10 does not work well as a therapy, either because IL-6 generates a strong inflammatory environment or because IL-10 receptors falter. Other treatments, such as monoclonal antibodies and JAK inhibitors, have also shown some potential success in reducing inflammation by halting IL-6. Yet approaches to soluble Interleukin-10 Receptor (IL10R) activating IL-10, for therapy to IL-10 could be used to modulate the immune response and support the intestine while helping tissue repair. Conversely, it may be possible to put a leash on the immune system and rescue the gut with treatments that stimulate IL-10 production, including receptor agonists or recombinant IL-10 therapy. Due to their opposite roles in UC treatment, the combination of IL-6 and IL-10 may help reduce inflammation and promote anti-inflammatory effects.

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Ethical responsibilities of authors

The local ethics council at the University of Baghdad approved this study.

Statements on compliance with ethical standards and standards of research involving animals

"This article does not contain any studies involving animals performed by any of the authors."

Disclosure and conflict of interest

"The authors declare that they have no conflicts of interest."

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