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CLINICAL & BASIC RESEARCH

Differential Gene Expression Profiles in Inflammatory Bowel Disease Patients from Kurdistan, Iraq

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abstract: ***Objectives:*** Inflammatory bowel disease (IBD), generally comprising Crohn's disease (CD) and ulcerative colitis (UC), has become a significant global public health concern in the last decade. This study aimed to determine the alternations in the whole genomic expression profile of patients with IBD in this geographic location for the first time, as there are very few articles in the literature addressing this specific aspect of the field. ***Methods:*** The study was conducted in Erbil Governorate in the Kurdistan region of Iraq from July 2021 to July 2022. The genome expression profiles of 10 patients with IBD were compared to their matched controls. The sequences used in the design of the array were selected from GenBank®, dbEST and RefSeq. Whole blood RNA was extracted and hybridisation was conducted on the GeneChip® human genome U133A 2.0 array. The Scanner 3000 was used to scan high-resolution images and the General Comprehensive Operating System was used to read the results. ***Results:*** The upregulated genes shared between patients with UC and CD were *RIT2*, *BCL2L1*, *MDM2* and *FKBP8*, while the downregulated genes they shared were the *NFKBIB*, *DDX24* and *RASA3* genes. ***Conclusions:*** Upregulated and downregulated gene expression patterns were detected in individuals with IBD, offering diagnostic potential and opportunities for treatment by targeting the associated pathways.

Keywords: Crohn's Disease; Gene Expression; Genes; Colitis, Ulcerative; Iraq.

Advances in Knowledge

- *The study determined the alterations in the whole genomic expression profile of inflammatory bowel disease (IBD) patients.*
- *The RIT2, BCL2L1, MDM2 and FKBP8 genes were found to be upregulated in both patients with ulcerative colitis and Crohn's disease, while the NFKBIB, DDX24 and RASA3 genes were downregulated.*
- *The study highlights the importance of genomic alterations as potential targets for future treatments.*

Applications to Patient Care

- *The observed alterations in the gene expression of IBD patients have significant implications for diagnosis and treatment.*
- *The identified upregulated genes and downregulated genes can serve as potential biomarkers for IBD.*
- *Targeting the pathways associated with these differentially expressed genes may lead to the development of novel therapeutic interventions.*
- *The findings provide a basis for personalised medicine approaches in IBD, allowing for tailored treatments based on individual genomic profiles.*
- *Understanding the molecular changes in IBD enhances the potential for precision medicine and improved patient care.*

Inflammatory bowel disease (IBD) is a group of gastrointestinal disorders that clinically includes Crohn's disease (CD), ulcerative colitis (UC) and indeterminate colitis.[1] Over the past decade, IBD has emerged as a global public health challenge.[2] The incidence pattern of IBD has shifted during the past 20 years, with incidences increasing in previously low incidence regions such as Asia and the Middle East as well as continuing to rise in the West.[3] IBD is a complex and heterogeneous group of disorders that exhibits significant geographic and ethnic variations in both incidence and prevalence.[4] These differences underscore the importance of understanding the molecular underpinnings of IBD within specific populations.[5] While numerous studies have explored the clinical aspects of IBD, there is a growing need to investigate the underlying genetic and genomic factors contributing to the disease's pathogenesis.

The pathogenesis of IBD is not known; however, it is considered to be multifactorial, and a cure for IBD is yet to be discovered.[6] Recent experimental and clinical studies agree that genetics, environment, gut microbiota and immune response are responsible for the initiation and progression of IBD in susceptible hosts.[7] Additionally, it has been proposed that diet, lifestyle, pollutants and vitamin and mineral deficiencies contribute to the severity or development of IBD.[8,9] For example, a high prevalence of micronutrient deficiency has been reported among patients with IBD. These micronutrients include vitamins D, C and B12, folic acid and zinc.[10]

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Experimental studies revealed that the invasion of mucosal tissue with activated phagocytic immune cells that produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads a change towards prooxidants. This increases oxidative stress, which further disrupts cellular homeostasis by injuring important macromolecules, leads to cell damage, increases mucosal barrier permeability and increases locally existing inflammation.[11,12] Thus, oxidative stress has been mentioned as a potential contributor to the aetiology of IBD.[12] Oxidative stress levels can be evaluated indirectly by assessing the amount of DNA/RNA damage, lipid peroxidation and protein oxidation/nitration.[13] Lipids are the most involved class of macromolecules among the numerous biological targets of oxidative stress.[14] Malondialdehyde is one of the final products of polyunsaturated fatty acids peroxidation as well as a well-known oxidative stress biomarker; its overproduction is related to an increase of free radicals and a decrease in the levels of antioxidants.[15]

Methods

This study was conducted in Erbil Governorate, in the Kurdistan region of Iraq, from July 2021 to July 2022. The genome expression profiles of 10 patients (6 with UC and 4 with CD) were determined and compared to their matched controls (n = 5). The criteria for the diagnosis of IBD was a combination of clinical, radiographic, histological and endoscopic assessments and the collected data were reviewed by 3 independent physicians. The exclusion criteria were colitis from other causes and anyone who matched the following criteria: pregnant women, nursing mothers, people who had undergone a total colectomy in the past, people who were taking experimental medications, people who had cancer or any other concurrent end-stage organ disease and addicts. All participating patients who were not given an IBD diagnosis were considered part of the control population.

The collected samples were peripheral blood RNA extracted from blood samples and hybridised on the GeneChip® human genome U133A 2.0 array (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). This array is well designed to analyse the expression level of 18,400 transcripts and variants, including 14,500 well-characterised human genes. The sequences used in the design of this array were selected from GenBank®, dbEST and RefSeq. The sequence clusters were created from the UniGene database (Build 133, April 20, 2001), then refined by analysis and comparison with several other publicly available databases, including the Washington University EST trace repository and the University of California, Santa Cruz Golden-Path human genome database (released in April 2001). Oligonucleotide probes complementary to each corresponding sequence were synthesised *in situ* on the array. A total of 11 pairs of oligonucleotide probes were used to measure the level of transcription of each sequence represented on this array. The Scanner 3000 (Affymetrix, Santa Clara, California, USA) was used to scan high-resolution images and the General Comprehensive Operating System (GCOS), Version 1.3 (Affymetrix, USA) was used to read the results. Bioinformatic analysis started with preprocessing (normalisation and scatter plots), alignment was conducted to assemble transcripts, the analysis of variance (ANOVA) statistical tool was used to determine the top upregulated and downregulated genes, a heat map was created with TBtools, gene numbers were converted to gene IDs using g:Convert, the co-expression of related genes was identified with GeneMANIA's online tools and finally, the defected pathways related to the upregulated and downregulated genes were determined using the ShinyGO 0.76.3 tool.

All of the statistical analyses were performed in the Statistical Package for the Social Sciences (SPSS), Version 25.0 (IBM Corp., Armonk, New York, USA) and P values of <0.05 were considered statistically significance. The ANOVA was used to determine the fold changes in each gene.

Ethical approval for this study was obtained from the Salahaddin University College of Science (241.1B2.23). The study adhered to all ethical guidelines and regulations regarding the treatment of human subjects. Official permission to collect samples was obtained from the patients participating in the study. Prior to their participation, all the patients provided written informed consent, which detailed the purpose of the research, potential risks and benefits and their rights as participants. Participant confidentiality was strictly maintained throughout the study.

Results

Lists of the upregulated and downregulated genes in patients with UC and lists of the top upregulated and downregulated genes in patients with CD were created. The following genes were downregulated in patients with UC: *RNF19A*, *NFKBIB*, *EWSR1*, *DDX24*, *HES2*, *SART3*, *PPIG*, *TCAF1*, *DKC1*, *RASA3*, *CELF1*, *CCL23*, *SNRNP70*, *MXD4*, *CD6*, *HSP90AA1*, *PPIG* and *DNAJB4*. Conversely, the following genes were downregulated in patients with CD: *ZCCHC24*, *ILF3*, *RASA3*, *LAMB1*, *TRABD*, *TNFRSF25*, *BDH1*, *MAF*, *PIN1*, *GDPD5*, *PBXIP1*, *PRPF6*, *AP3D1*, *DDX24*,

DIO2, *GGA1*, *CORO1B* and *NFKBIB*. The significantly upregulated genes in patients with UC were *MDM2*, *SLC6A2*, *TRMT1*, *SNCA*, *CYP4B1*, *TNS1*, *RIT2*, *ZER1*, *SLC4A1*, *GNGT1*, *FOXH1*, *FKBP8*, *TNS1*, *CA1*, *TMOD1*, *SELENBP1*, *ALAS2* and *BCL2L1*. Conversely, the upregulated genes in patients with CD were *CXCL1*, *MDM2*, *GUCY1B3*, *DZIP1*, *RIT2*, *GYPA*, *FECH*, *PIGV*, *SHOX2*, *SARDH*, *DOHH*, *NR4A1*, *FKBP8*, *CXCL3*, *NFIX*, *MIA*, *ABLIM3* and *BCL2L1* [Figures 1–4].

Regarding the pathways of the upregulated (*RIT2*, *BCL2L1*, *MDM2* and *FKBP8*) and downregulated (*NFKBIB*, *DDX24* and *RASA3*) genes shared by patients with IBD, it was found that the *BCL2L1* and *MDM2* genes have roles in the p53 and NF-κB signalling pathways, respectively. The *NFKBIB* gene is related to the cytosolic DNA-sensing pathway and the adipocytokine, B-cell receptor and chemokine signalling pathways, while the *RASA3* gene is related to the Ras signalling pathway. Also, the *DDX24* gene was found to have a role in controlling p53 activities.

Discussion

To date, no DNA microarray-based studies in the Kurdistan region of Iraq have been published, and there are few such studies to investigate the alterations in gene expression in patients with IBD in the Middle East. Therefore, the present study investigated such gene expression via DNA microarray. Blood mRNA was collected from 10 patients with IBD (6 with UC and 4 with CD) and 5 controls, all of whom were from the Kurdistan region of Iraq. The results were explained using online and offline bioinformatic tools, such as TBtools for creating the heat maps, GeneMANIA for determining the co-expression of the genes, g:GOSt for converting the gene names to ensemble IDs and STRING for finding gene interactions and pathways. The top upregulated and downregulated genes in both patients with UC and patients with CD were determined based on the control group.

The lists of the upregulated and downregulated genes can be used for the diagnosis of IBD and its types in the Kurdish population. However, the co-expression reports of the downregulated and upregulated genes and their interactions were used to identify the candidate genes associated with the onset of IBD in terms of their pathways [Supplementary Figures 1–6]. For this purpose, the upregulated (*RIT2*, *BCL2L1*, *MDM2* and *FKBP8*) and downregulated (*NFKBIB*, *DDX24*, and *RASA3*) genes shared by the patients with UC and the patients with CD were used for further analysis through the KEGG database. The genes *BCL2L1* and *MDM2* were found to have roles in the p53 and NF-κB signalling pathways, respectively, while the *RIT2* and *FKBP8* genes' biological roles were not found on the KEGG database; therefore, their biological functions were determined based on previous studies. The *NFKBIB* gene was found to be related to the cytosolic DNA-sensing pathway and

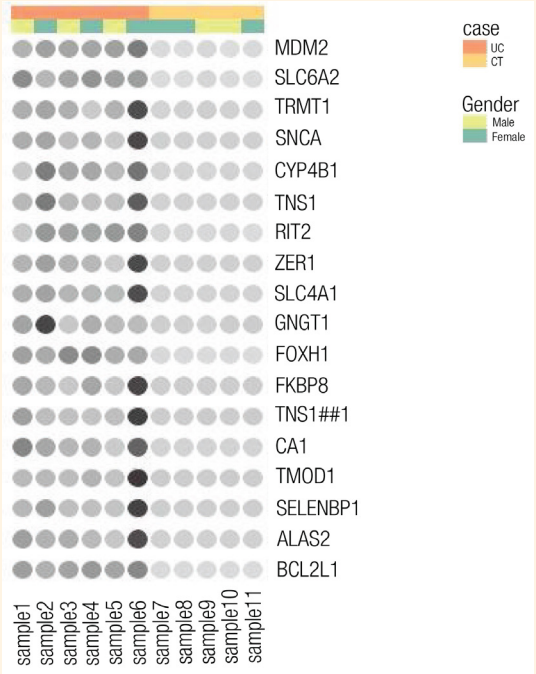

Figure 1: Heat map of upregulated genes in patients with ulcerative colitis.
UC = ulcerative colitis; CT = control
Dark color = higher expression.

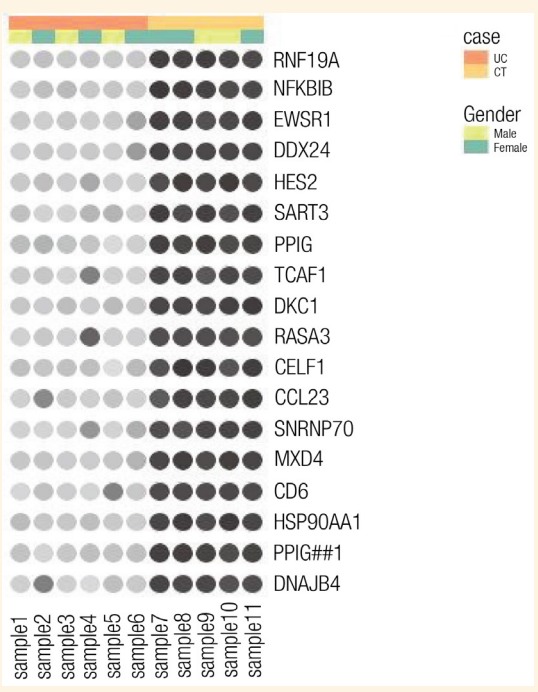

Figure 2: Heat map of downregulated genes in patients with ulcerative colitis.
UC = ulcerative colitis; CT = control
Light color = lower expression.

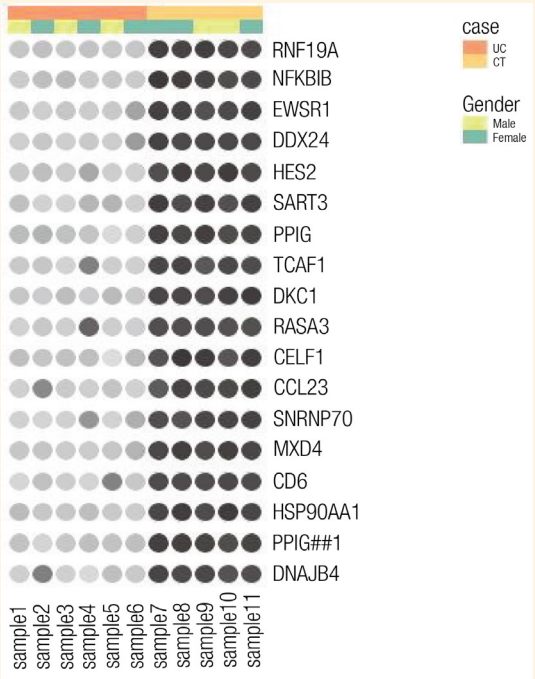

Figure 3: Heat map of upregulated genes in patients with Crohn's disease.
CD = Crohn's disease; CT = control
Dark color = higher expression.

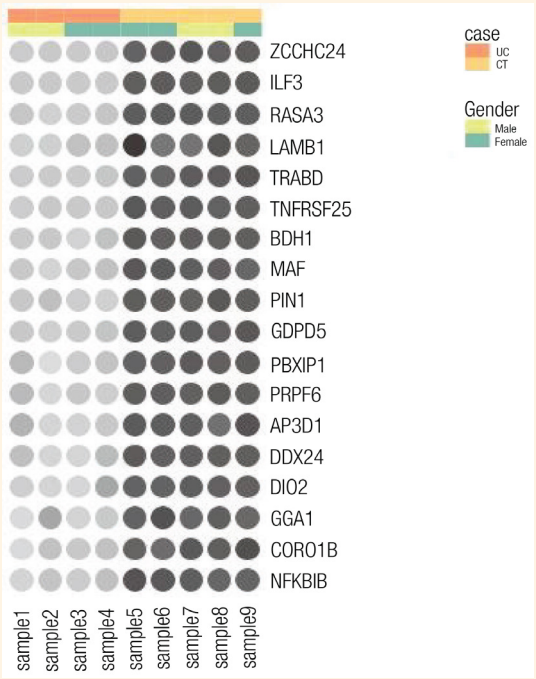

Figure 4: Heat map of downregulated genes in patients with Crohn's disease.
CD = Crohn's disease; CT = control
Light color = lower expression.

the adipocytokine, B-cell receptor and chemokine signalling pathways, while the *RASA3* gene was found to be related to the Ras signalling pathway. The pathway related to the *DDX24* gene was determined from previous studies. A previous study evaluated the expression of mucosal genes in ulcerative colitis patients, which reported the downregulation of the *NFKBIB* gene in infected tissues.[16] According to another study, the *DDX24* gene negatively regulates cytosolic RNA-mediated innate immune signalling.[17] Yet another study revealed the *DDX24* gene as an important regulator of p300 and suggests that the modulation of the p53-p300 interplay by the *DDX24* gene is critical in controlling p53 activities in human cancer cells.[18] The *RASA3* gene was not previously associated with IBD until studies determined that the differential methylation of the *RASA3* gene could potentially alter endothelial-leukocyte adhesions, known to be of major importance for the gut homing of inflammatory cells in IBD and which are targeted by drugs such as vedolizumab.[19]

A previous study measured *BCL2L1* gene expression levels in 116 paired colorectal cancer (CRC) and normal tissues and CRC cell lines by qRT-PCR and found that *BCL2L1* gene expression levels were significantly upregulated in the CRC tumour tissues and cell lines compared with the adjacent non-tumour tissues.[20] Another experimental study on mice confirmed that the upregulation of the *BCL2L1* gene is related to the onset of IBD.[21] The *MDM2* gene is a phospho-protein and a ubiquitin ligase for p53 that is responsible for inhibiting p53 activity and promoting its destruction.[22] Mutations in the *P53* gene have been identified in most human chronic diseases as well as in the gene's downstream signalling pathways, which are mediated by the *MDM2* gene; therefore, proper functioning of both these genes is important for the normal functioning of cells.[23] Consequently, when mutations in any of these genes disrupt critical signalling pathways, they can result in chronic diseases, including cancer.[24,25]

The variations in the *RIT2* gene have been shown to be associated with a number of neurological disorders, such as Parkinson's disease and autism.[26,27] However, a 2019 study on immune signalling revealed that non-immune genes, such as the *RIT2* gene, can impact immune function through the alteration of their expression.[28] The *FKBP8* protein is located on the outer membrane and has an anti-apoptotic role by interacting with B-cell lymphoma 2.[29,30] A study concluded that the *FKBP8* gene plays an essential role in mitochondrial fragmentation through LIRL (LIR motif-like sequence) during mitophagy and that this activity of the *FKBP8* gene, together with LIR, is required for mitophagy under stress conditions.[31] Consequently, disruption of mitochondrial function and increased expression of genes or proteins indicative of mitochondrial fragmentation have been observed in neurological diseases and in models of diabetes, intestinal inflammation, infection and sepsis.[32]

It is essential to note that while the findings of this study provide valuable insights into the genomic landscape of IBD, this study has its limitations as well, such as the relatively small sample size and the preliminary nature of the investigation. Therefore, caution should be exercised when interpreting and applying these results. Further research with larger and more diverse cohorts is warranted to corroborate the findings and enhance the understanding of the molecular mechanisms underpinning IBD.

Conclusions

This study identified a list of genes exhibiting both upregulation and downregulation in IBD patients, which can serve as a valuable tool for the diagnosis of IBD. Additionally, the associated pathways related to these gene alterations represent promising targets for potential treatments.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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No funding was received for this study.

AUTHORS' CONTRIBUTION

BIM was primarily responsible for sample collection, laboratory investigation and biological analysis of the data. BKA contributed to the project by working on the research's template (referring to a predefined structure or format that the researchers used to organize and present their research findings) and conducting the statistical analysis. BKA interpreted the research findings and provided guidance and supervision throughout the research process. Both BIM and BKM collaborated in the preparation and writing of this research paper. Both authors approved the final version of the manuscript.

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