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An investigation of JAK2 mutation in patients with ulcerative colitis with a history of thrombosis

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Ethics Committee Approval
This study was approved by the Clinical Research Ethics Committee of Manisa Celal Bayar University (approval no. E: 31.05.2018-E.49233). Consent was obtained from the participants in this study. This consent form permits to use of participant's data in the studies
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: JAK2 is a gene that provides instructions for making a protein called Janus kinase 2, which is involved in the signaling process that regulates the growth and division of cells. Variations in the JAK2 gene have been associated with several different diseases, including certain blood disorders like myeloproliferative neoplasms (MPNs) and ulcerative colitis (UC). The exact reason for ulcerative colitis is not fully understood. This study aimed to examine the possible role of JAK2 V617F mutation in the etiopathogenesis of ulcerative colitis.
Methods: The included patients were selected with UC and with signs of thrombosis. The DNA isolation was carried out from peripheral blood for all included patients. RT-qPCR methods were used to find JAK2 V617F mutations in UC patients with signs of thrombosis.
Results: 73.3% of the included patients in this study had bloody diarrhea and 80% had abdominal pain. Also, the JAK2 V617F mutation rate was detected in 6.6% of the patients included in the study.
Conclusion: In this study, it was found that the V617F mutation was relatively rare in ulcerative colitis patients and there was no correlation with the JAK2 V617F mutation in most of the ulcerative colitis cases with thrombotic symptoms.

Keywords: ulcerative colitis, JAK2, thrombosis, V617F mutation

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Introduction

Ulcerative colitis is an inflammatory disorder of the gastrointestinal tract that an abnormal mucosal immune response and has a chronic and relapsing course [1]. It is defined by ulcerations starting from the rectum and spreading to the proximal colon [2].

The majority of symptoms of ulcerative colitis are abdominal distress and diarrhea with blood or mucus [3]. While its annual incidence in Europe and North America is approximately 25/100000 and 20/100000, respectively, the annual incidence rate in Asia is lower (6.3/100000). This difference is thought to arise from the variation in the level of industrialization [2]. While ulcerative colitis can occur at any age, it has a bimodal distribution pattern, with a tendency to occur between 15-30 years and 50-70 years of age. Although the etiopathogenesis of ulcerative colitis has not yet been fully clarified, it is thought to develop as a result of an interaction between genetic, immunoregulatory, and environmental factors [4]. Demonstration of high concordance of the disease in monozygotic twin studies, a 10-fold increased risk for the disease in the presence of a positive family history, and co-occurrence with several genetic syndromes suggest that genetic factors are also related to the pathogenesis of these diseases [5].

Inflammatory bowel disease (IBD), is a gastrointestinal disorder thought to be triggered by environmental factors in genetically predisposed individuals. As with many other inflammatory diseases, the near relation between thrombosis and inflammation also influences the course and intensity of IBD. Thrombocytosis occurs as both a primary disease (primary myeloproliferative diseases) and a secondary disease (reactive bone marrow diseases) [1]. Infections, neoplasms, severe iron deficiency, bleeding, inflammatory conditions, hemolysis splenectomy, and some medications also lead to reactive thrombocytosis [6]. Chronic myeloproliferative disorders (CMDs) progress by proliferation, differentiation, and maturation in one or more hematopoietic cell populations. These include essential thrombocytosis (ET), polycythemia vera (PV), chronic myeloid leukemia (CML), and primary myelofibrosis (PMF) [7,8].

Thromboses are frequently seen in the lower extremity veins and respiratory system, and less commonly in the portal vein, cerebrovascular field, retinal veins, mesenteric vein, and hepatic veins. The incidence of thrombosis in people with IBD ranges from 1% to 7%. In autopsy studies, this rate can be as high as 39-41%. The rate of thrombosis is higher than that of well-known extraintestinal signs. Spondyloarthropathies, with prevalence rates ranging from 12% to 23%, are the only extraintestinal signs seen more frequently than thrombosis. Important findings were also obtained from the cohort study which was carried out by Horsted et al. [9]., including 13,756 IBD patients performed by Horsted et al While the general risk for Venous Thromboembolism (VTE) was found to increase 3.6-fold in IBD patients compared with controls, this risk can increase up to 8.4-fold during acute attacks. Although the risk of VTE decreases during remission periods, it was still found to be 2,1-fold higher than in the normal population.

In a previous study that evaluated patients hospitalized for VTE, patients with IBD had a 2,1-fold increased mortality risk and stayed at the hospital longer than patients without IBD [10]. A study investigating factors associated with the recurrence of VTE detected that patients with IBD had a 2.5-fold increased risk for the recurrence of VTE attacks. Also, in a study by Miehsler et al. [11] that investigated whether the increased risk of thromboembolism was specific to IBD, it was found that the risk of thrombosis was not increased in cases of rheumatoid arthritis (as the control for inflammatory disease) and in cases of celiac disease (as the control for intestinal disease), but was increased 3.6-fold in cases of IBD.

Hereditary factors that predispose to thrombosis in inflammatory bowel disease play an important role. Therefore, patients with IBD should be evaluated in terms of susceptibility to thrombosis, family history, and use of prothrombotic drugs. Also, JAK 2 gene mutation is among the potential hereditary risk factors that remain unconfirmed due to insufficient evidence, and 48 IBD patients with thrombosis in an exemplary previous study were tested for the gene, but all of them tested negative. This mutation in the JAK2 gene has been demonstrated in some cases of myeloproliferative neoplasms (MPN) [12] and most commonly in those with ET (23%-57%), PV (65%-97%), and PMF (35%-57%). Also, it is seldom available in cases of chronic myelomonocytic leukemia, and myelodysplastic syndrome [13]. In cases with BCR/ABL-MPN, the presence of this mutation can be used as a marker to differentiate PV, PMF, and ET from reactive hematopoietic diseases [14].

The JAK2 gene encodes cytoplasmic tyrosine kinase, a protein that mediates growth factor receptor signaling. G>T transversion occurring in the 2343 nucleotide region of the JAK2 gene results in the V617F variation. As a result of this change, the amino acid phenylalanine replaces valine (V617F) in the JAK2 protein [15]. The mutant protein has increased kinase activity. As a result of this mutation in the JAK2 gene, PV progenitor cells may become hypersensitive to growth factors and cytokines.

This study aimed to explore the association between JAK2 mutation and ulcerative colitis and to investigate whether this is a risk factor for the development of ulcerative colitis.

Materials and methods

Patients

The study was approved by the Clinical Research Ethics Committee of Manisa Celal Bayar University (Manisa, Turkey), and written informed consent was obtained from all subjects (approval no. E: 31.05.2018-E.49233). Patients diagnosed with ulcerative colitis, who had an accompanying history of thromboembolic events such as pulmonary embolism, deep vein thrombosis, coronary artery disease, hepatic vein or portal vein thrombosis at Manisa Celal Bayar University Gastroenterology Clinic were included in the study. The inclusion criteria were as follows: 1. Age above 18 years, 2. A diagnosis of ulcerative colitis, 3. History of a concomitant thromboembolic event(s).

The exclusion criteria were as follows: 1. Diagnosis of mental retardation, psychotic disorder, or substance abuse disorder, 2. History of polycythemia, essential thrombosis, or myelofibrosis.

A total of 15 patients who met the inclusion criteria were identified, and 3 ml peripheral venous blood samples were collected in sterile gel tubes from each patient. Also, 15 healthy candidates of peripheral venous blood samples were used as a control.

DNA Isolation

Peripheral blood samples were obtained from the patients and control, which were collected in EDTA tubes. According to the instructions of the manufacturer, genomic DNA was extracted from the samples using a DNA isolation kit (Qiagen GmbH, Hilden, Germany) and kept at −20 °C until an RT–qPCR test could be performed.

Determination of the Amount and Purity of DNA Samples

After the isolation procedure, the DNA samples obtained for performing RT–qPCR testing were analyzed with a NanoDrop (NanoDrop ND-100) spectrophotometer.

RT–qPCR Analysis

To determine the JAK2 V617F mutation the DNA samples were analyzed with ipsogen JAK2 MutaQuant Kit (Qiagen GmbH, Hilden, Germany) according to the instructions of the manufacturer by quantitative RT–qPCR method (Table 1).

Table 1: DNA amounts obtained from patient tissue samples.

Patient	DNA amount (ng/ul)
Patient 1	87.61
Patient 2	92.51
Patient 3	121.02
Patient 4	92.52
Patient 5	155.01
Patient 6	192.09
Patient 7	163.61
Patient 8	345.33
Patient 9	92.12
Patient 10	149.57
Patient 11	264.61
Patient 12	15.25
Patient 13	251.48
Patient 14	169.57
Patient 15	213.63

Statistical Analysis

The significant differences between the patients and controls were evaluated with the ANOVA test (SPSS 23.0 statistical program) using Tukey post-hoc analysis. A *P*-value of <0.05 was considered statistically significant.

Results

Demographic and medical data

A total of 4 (26.6%) of the patients were female, and 11 (73.4%) were male. Of the cases included in the study, 73.3% had bloody diarrhea, 20% had joint findings, 80% had abdominal pain, 53.3% had weight loss, 26.6% had skin findings, 40% had liver-gall bladder findings and 33.3% had ocular findings (Table 2).

Table 2: Demographic and medical data of the patients

	Yes	No
Bloody diarrhea	4	11
Joint signs	3	12
Abdominal pain	12	3
Weight loss	8	7
Skin signs	4	11
Liver-gall bladder signs	6	9
Ocular signs	5	10

JAK 2 Mutation Analysis in Patient Groups

Mutations were detected in one of the 15 patients (6.6%) included in the study (*P*=0.378). A statistically significant association between JAK2 mutation and ulcerative colitis in patients with thrombotic findings was not found in this study (Table 3).

Table 3: Primer sequencing data of transcripts of housekeeping and JAK2 genes.

JAK2	F:5'TTCCTTAGTCTTTCTTTGAAGC3'
	R:5'GTGATCCTGAAACTGAATTTTCT3'
IL17A	F:5'ACAATCCCACGAAATCCAGGA3'
	R: 5'AAGGTGAGGTGGATCGGTTG3'
HPRT1	F: 5'-CGTCTTGCTCGAGATGTGAT3'
	R:5'TTCAGTGCTTTGATGTAATCCAG3'
B2M	F:5' TCTCTCTTTCTGGCCTGGA3'
	R:5'TGTCGGATGGATGAAACCC3'

Discussion

The JAK2 V617F mutation is known to reason an overactive JAK-STAT signaling pathway, which can cause abnormal immune responses and inflammation in the gut. This can contribute to the development of UC. For this reason, some research has shown that this variation is present in a small subset of UC individuals, and it is relied on to play a role in the development and progression of the disease [16-18]. It was shown in a study performed on Korean patients by Yang et al. [19] that JAK2 variants could act a role in the etiopathogenesis of ulcerative colitis. Prager et al. [20] found that JAK2 could influence the etiopathogenesis of ulcerative colitis by disrupting the integrity of the intestinal barrier. Another hand, similar to our study, Karimi et al. [21] examined the role of JAK2 mutation in 48 patients with the inflammatory disease who had thrombotic complications but did not find JAK2 V617F mutation in any of the study participants.

It is known that there may be regional variations in the prevalence of JAK2 V617F mutations depending on the population [19-21]. In a study performed by Can et al. [22] in Turkey, JAK2 mutation was shown to be a factor in the cause of inflammatory bowel disease in the Turkish population.

Limitations

The limitations of this study were the small number of the included patients due to the difficulty to find UC with signs of thrombosis. For this reason, a statistically significant association between JAK2 mutation and ulcerative colitis in patients with thrombotic events was not demonstrated found in this study. However, it is important to mark that the V617F mutation is relatively rare in ulcerative colitis cases, and the majority of cases of ulcerative colitis are not directly caused by JAK2 mutations. More research is needed to fully understand its causes and potential treatments.

Conclusion

As a result of JAK2 mutation analyses applied in patients who met the inclusion criteria of this study, which analyzed the role of the JAK2 V617F mutation in the etiopathogenesis of ulcerative colitis an autoinflammatory disease with an etiopathogenesis that has not yet been fully elucidated. A statistically significant conclusion could not be reached regarding if the JAK2 V617F mutation is a risk factor for ulcerative colitis. This study provides data to the literature on this topic, albeit limited because of the small sample size.

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