RATE OF MICROSCOPIC COLITIS AND CYTOKINE LEVELS IN PATIENTS WITH IRRITABLE BOWEL SYNDROME

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ABSTRACT

***Aims**: The aim of this study is to determine the rate of microscopic colitis (MC) among patients diagnosed as Irritable Bowel Syndrome (IBS) in accordance with Roma III criteria and to investigate the correlation of cytokine levels with the type of IBS and presence of MC.*

***Methodology**: A total of Eighty-nine patients who were diagnosed with IBS based on Rome III diagnostic criteria were included in this study. Biopsies taken from caecum, ascending colon, transverse colon, descending colon and rectosigmoid region during colonoscopy were put in separate tubes and histopathologically examined. In addition, serum TNF-α, IL-1β and IL-6 levels were determined in peripheral blood samples of patients.*

***Results**: The frequency of MC was higher in the study groups compared to the general population (22.5% and 20.4% in IBS-D and IBS-C groups, respectively). Specimens taken from caecum, ascending colon, transverse colon and descending colon were more likely to lead to the diagnosis of MC compared to those taken from rectrosigmoid region ($p<0.001$). No significant difference was found between presence of MC and IL-1β, IL-6 and TNF-α levels. In addition, TNF-α levels were significantly higher in the IBS-C group ($p= 0.013$).*

***Conclusion**: Rate of MC in IBS patients is higher than the rate in normal population. In IBS patients, biopsy samples obtained solely from rectosigmoid area is not sufficient to preclude MC diagnosis. No difference exists between IBS patients with and without MC in terms of cytokine levels.*

***Key words**: Irritable Bowel Syndrome, Microscopic colitis, Cytokines.*

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Introduction

Irritable bowel syndrome (IBS) is a gastrointestinal syndrome characterized by chronic abdominal pain and change in bowel habits in the absence of any organic cause. It is the most commonly diagnosed gastrointestinal condition, accounting for about 30% of patient referred to gastroenterologists[(1)]. IBS prevalence is reported at 10-20% in the adult population worldwide[(2-4)].

Microscopic colitis (MC) is a term used to describe entities characterized by chronic aqueous diarrhea, normal radiological and colonoscopic appearance and microscopic anomalies in the colon. MC is the most common cause of chronic aqueous diarrhea particularly in the elderly population. MC has two forms: lymphocytic colitis (LC) and collagenous colitis (CC)[(5)]. MC diagnosis is made after histological examination of colon biopsy samples. The annual incidence was found to be 8,6/100.000 between 1985-2001 in the USA for MC, a marked increase was observed in incidence towards the end of the observation period[(6)].

No biological, anatomical and physiological markers are available to detect IBS[(1)]. Even if performed, no noteworthy findings could be made in colonoscopy and biopsy. Diagnosis is symptom-based, and dependent upon criteria set by experts (Maning, Roma criteria).

Due to symptomatic agreement in recent studies, there are studies showing an increased incidence of MC in IBS patients, particularly in the diarrhea-dominant form[7-9]. On the other hand, it has been reported that patients with MC corresponded to the IBS symptoms at a rate of about 50%[10-12]. Differentiating between these two clinical conditions is important for treatment approach.

MC is diagnosed according to histological criteria, and IBS is diagnosed according to symptomatic criteria (Maning, Roma criteria). MC and IBS have similar symptoms and normal mucosal appearing in colonoscopic examination. Both diseases have negative impact on the quality of life. The diagnostic agreement between IBS and MC is important due to the differing potential treatments of each disease[10].

In this study, we aimed to prospectively investigate MC incidence and cytokine levels in diarrhea and constipation-dominant IBS patients according to Roma III criteria. Our aim by incorporating cytokine levels in our study was to investigate whether or not MC patients who have been observed to have a higher incidence compared with the normal population in predicting IBS patients who would receive colonoscopic biopsy, an invasive intervention, as well as to establish whether there is any difference between IBS subtypes.

Materials and methods

Eighty-nine patients referring to our Gastroenterology outpatient clinic during September 2010 and March 2011 with a diagnosis of IBS were enrolled in this trial. Patients were classified into two groups as irritable bowel syndrome-diarrhea (IBS-D) (n=40) and irritable bowel syndrome-constipation (IBS-C) (n=49) in accordance with Roma III criteria and Bristol stool form scale. Detailed medical histories were taken and physical examinations performed in the polyclinic by gastroenterology experts. Routine examinations, parasite examination and occult blood in stool, microscopy and culture of stool, thyroid-stimulating hormone, thyroid antibodies and anti-endomysium antibodies of patients were evaluated and recorded.

Patients were included in the study if they had with a diagnosis of IBS in accordance with Roma III criteria. Exclusion criteria included age under 18 years; malignity in colon or in other body regions; severe respiratory and heart failure; current corticosteroid therapy; current anticoagulant therapy; active lower and upper gastrointestinal system bleeding; previous colon surgery; diagnosis of microscopic colitis.

The study was undertaken in compliance with the principles of the Helsinki declaration and was approved by the ethics committees of the Dicle University School of Medicine (Number; 27.08.10-114, Diyarbakir, Turkey). Patients were informed about the study, followed by verbal and written consents for colonoscopy and biopsy interventions.

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Following the sufficient cleaning of the colon with sodium phosphate solution, some gastroenterologists, who were not from the study team, performed colonoscopy in the patients using Olympus CF-Q260AL flexible video colonoscopy device (Olympus Optical Co., Tokyo, Japan) set. During colonoscopy, five biopsy samples were obtained from mucosa of caecum, ascending, transverse, descending and rectosigmoid colon to be placed in separate tubes and were evaluated. Samples were fixated for 24 hours in formaldehyde solution and following routine pathological tissue evaluation, 4 μm cross-sections were prepared by standard microtome with paraffin block. Cross-sections were stained with Hematoxylin-eosin and Masson-Trichrome and were examined under a Nikon Eclipse 80i light microscope (Nikon Corporation, Tokyo,Japan) with a magnification power of 200. The same pathologist performed all evaluations. LC in biopsy samples were diagnosed based on chronic inflammatory cell infiltration in lamina propria in addition to more than 20 lymphocytes in 100 epithelial cells counted during examination. For diagnosis of CC, subepithelial collagenous band ≥ 10 micron, determined by Masson-Trichrome staining was considered as a criterion.

Blood samples of patients were placed in plain biochemical tubes and centrifuged at 5000 cycles for 10 minutes at room temperature; following centrifuge process, serum samples were placed in Eppendorf tubes and stored in deep freezer at (-80) ºC until the date of examination for cytokines. Serum TNF-α level was determined by enzyme

linked immunoassay (ELISA) kits (Tanı Medikal Limited, Turkey). Serum IL-1β and IL-6 levels were measured by IMMULITE 1000 device and kits (EURO/DPC Limited, United Kingdom), using solid phase two-side chemiluminescence immunometric method.

Statistical analysis

Descriptive statistics related to continuous variables were indicated as mean and standard deviation (SD) values. Yates's correction and Pearson Chi-Square test were used in analysis of cross tables. Normal distribution assumption for study data was tested by Kolmogorov-Smirnov test. Student's t test was utilized in comparison of mean values of two groups. Hypothesis were two-sided and $p<0.05$ was accepted as a statistically significant result. The analysis of the data was performed by using the Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS Inc., Chicago, IL, USA) statistical software.

Results

Demographic data

Among 89 IBS patients enrolled in the study, 40 cases were diagnosed as IBS-D and 49 patients were IBS-C cases. Eighteen of IBS-D patients (45%) were females while 22 were males (55%); mean age was 35.60±12.97. In the group of IBS-C patients, 27 were females (55.1%) and 22 were males (44.9%) while the mean age was determined as 40.76±12.03. No significant difference was found in comparison of IBS-D and IBS-C groups in terms of age and gender (Table 1).

	IBS-D	IBS-C	P
Age (mean±SD)	35.60±12.97	40.76±12.03	0.058
Gender (M/F)	22/18	22/27	0.462

Table 1: Distribution of age and gender in patients with IBS-D and IBS-C.

Evaluation of patients in terms of diabetes mellitus, hypertension, surgical operations (cholecystectomy, appendectomy), drug use (Nonsteroidal anti-inflammatory drugs, proton-pump inhibitors, antihypertensive), addictions (alcohol, smoking), autoimmune thyroid disease revealed no correlation between IBS type and presence of MC.

Duration of IBS symptoms among patients with no detected MC was 50.67±50.72 months while the corresponding duration among cases with MC was shorter, as indicated by 28.89±14.80 months. Difference was statistically significant (p= 0.002).

Analysis of histopathological findings

In 19 of IBS patients (21.3%), LC was determined while no CC was seen in any of our patients. In subgroup evaluations; MC was determined in 9 of IBS-D patients (22.5%) and 10 of IBS-C cases (20.4%). No statistically significant difference was found between two groups in terms of age and gender.

Among patients with MC, rate of diagnosis in terms of biopsy localization were 36.8% for caecum, 31.6% for ascending colon, 52.6% for transverse colon and 21.1% for descending colon while biopsy results of all rectosigmoid samples were reported as normal (Figure 1). Probability of diagnosis in biopsy samples of caecum, ascending colon, transverse colon and descending colon was found to be statistically significant ($p< 0.001$).

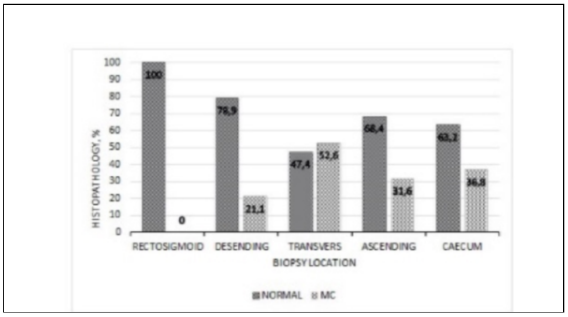

Fig. 1: Diagnostic rates among patients with MC in terms of biopsy localizations.
(Caecum, ascending colon, transverse colon and descending colon compared to rectrosigmoid region ($p<0.001$).

Cytokine levels

In our trial, no statistically significant correlation was found between levels of cytokines IL-1, IL-6 and TNF-α and presence of MC. In our trial, while no statistically significant correlation was found between IBS type and cytokine levels of IL-1 and IL-6, TNF-α levels was higher in IBS-C group with a statistically significant difference (p= 0.013) (Table 2).

	IBS-D	IBS-C	t	P
IL-1 (mean±SD)	5,01±0.09 pg/ml	5.18±0.83 pg/ml	-1.236	0.22
IL-6 (mean±SD)	3,03±3.00 pg/ml	3.93±9.36 pg/ml	-0.58	0.563
TNF-α (mean±SD)	23.18±7.29 pg/ml	49.25±64.91 pg/ml	-2.523	0.013

Table 2: Correlation of IBS type and cytokine levels.

Discussion

Irritable bowel syndrome is described as abdominal disturbance accompanied by alterations in bowel habits. Disease is diagnosed via symptoms, based on criteria developed by specialists (Manning, Roma criteria). MC is one of the most common causes of chronic diarrhea and it is generally accompanied by abdominal pain[13-15]. Colonoscopy reveals normal or near normal mucosa. In the presence of relevant clinical symptoms, MC diagnosis is based on histological examination results with an increase in intraepithelial lymphocyte (iEL) and presence of mixt type inflammatory changes in lamina propria[16]. MC is primarily analyzed in two subgroups, namely LC and CC. While both types have similar clinical and histological presentations, presence of subepithelial band is the distinguishing feature for CC. Diagnosis is based on histological criteria in MC while IBS is diagnosed by symptomatic criteria. MC and IBS present with similar symptoms and endoscopic findings.

Possible role of inflammation in pathogenesis of IBS is gradually increasing. Spiller et al. a mean of 2.5 iEL was detected in every 100 epithelial cells in postinfective irritable bowel syndrome (PI-IBS) group following Campylobacter enteritis while a mean of 0.5 iEL was determined in every 100 epithelial cells in the control group. In follow-up evaluations performed at 12th week, a reduction in increase in iEL count was observed (0.9 iEL in 100 epithelial cells)[17]. In the trial conducted by Dunlop et al.[18], change in iEL count among IBS patients (including PI-IBS cases and cases without PI-IBS) was found to be insignificant as compared to control group (mean iEL: 41.4 iEL/500 colon epithelial cells in PI-IBS patients, in the extra-PI-IBS IBS group, 32.8 iEL/500 colon epithelial cells, 43.1 iEL/500 colon epithelial cells in healthy control group). On the other hand, comparison of T lymphocyte count in lamina propria with the control group revealed a significant increase both in PI-IBS and in IBS cases without an infectious history (in each magnification area 101.6, 120.5 and 118.5 T lymphocytes, respectively).

Carmona-Sanchez et al. investigated the rate of MC in 300 patients diagnosed as IBS in accordance with Roma III criteria; while 155 of these cases were diagnosed as IBS-D subtype, 145 were IBS-C patients. MC rate was determined as 18% (n=28) in IBS-D and 0.7% (n;=1) in the IBS-C group (p= 0.0001)[7]. Among patients with MC, 15 had LC and 14 cases exhibited CC; endoscopic appearance was normal in twenty cases. In a trial conducted by Tuncer et al., rate of MC was investigated in 30 patients with IBS and in 20 control cases. MC was diagnosed in 23.3% of IBS and in 5% of control cases (p< 0.05)[9]. All patients presented with LC and no case of CC was detected.

In the trial performed by Chadwick et al. on 77 IBS patients in accordance with Roma II criteria (55% IBS-D, 14% IBS-C and 31% IBS-M) and on 28 asymptomatic control cases, rate of cases carrying histological criteria of LC in IBS group was determined as 10.3%. No case of LC was detected in the control group[8].

Madisch et al. evaluated 82 patients with MC and determined mean age as 57 and duration of symptoms prior to diagnosis as 28 months (mean). Among these cases, 28.2% was diagnosed as IBS-D in accordance with Roma II. Excluding the duration in calculations increased the rate to 65% (11). These results clearly indicate that a considerable amount of patients with MC have IBS-D symptoms. In the trial conducted by Barta et al., clinical presentations of 53 patients with MC were investigated. Authors determined constipation in 43.39% (n=23) and diarrhea in 56.61% of patients. They also indicated that MC leads to constipation symptoms in addition to diarrhea[19]. A patient with long term, painful, chronic constipation symptoms was operated and was diagnosed as CC based on evaluation of colectomy samples[20].

Erdem et al. determined MC rate as 11.5% (n=15) among 114 patients who were investigated for chronic diarrhea related to unknown causes. Among these cases, 12 patients were LC and 3 were diagnosed as CC[21].

Nooroudien et al. retrospectively re-evaluated all colonoscopic biopsy materials, excluding malignities (normal cases or patients diagnosed as chronic colitis, melanosis coli and MC). In addition to routine pathological evaluations, lymphocyte count was performed by using antibodies against CD3. More than 20 iEL were detected in 20 patients. Six patients (2%), who were previously reported as normal, were diagnosed as LC cases. Four of these cases were determined as being monitored for IBS-D[22].

In a large scale retrospective trial analyzing 547 patients (LC:376, MC:171) with MC[12] in the CC group, 30 patients (17.5%) were diagnosed as IBS prior to diagnosis of CC (p<0.001) and in the LC group, 43 patients (11.4%) were diagnosed as IBS prior to diagnosis of LC (p<0.001).

In our trial, 21.3% of our IBS cases were diagnosed as MC. All of these patients were LC cases. In analysis of IBS subtypes, rate of MC was determined as 22.5% in IBS-D patients while the rate was 20.4% in IBS-C group; the difference was statistically insignificant. Our results in IBS-D group displayed similar MC rates as compared to rates reported in literature (18-23%)[7, 9]. Nevertheless, these results are considerably high in comparison to the rate of 1.7% determined by Chey et al.[23] in IBS patients, excluding constipated cases. This discrepancy may be due to study design since Chey et al. performed biopsy in rectum and sigmoid regions. On the other hand, we performed biopsy in five different localizations (caecum, ascending, transverse, descending and rectosigmoid) and this may be one of the causes for the above mentioned discrepancy. Considering that rectosigmoidal biopsy samples of our cases were evaluated based on criteria of Chey et al. (> 15 iEL was used in diagnosis of LC), the corresponding rates would be calculated as 3.42% (3 patients) in all IBS patients and as 2.5% (1 patient) in IBS-D group, which would be partly similar to results of the above mentioned trial. Even though several articles exist indicating that MC may present with constipation[22-23], our MC rates in IBS-C group was found to be higher as compared to results in literature (0.7% & 20.4%)[7]. In our trial, biopsy samples from rectosigmoid region from 19 patients with LC were yielded normal findings and the most relevant results were achieved in biopsy samples from transverse colon (52% of MC cases). Our findings are in accordance with literature. Therefore, we may argue that rectal biopsy is not sufficient for precluding a diagnosis of MC.

Liebregts et al. (24) enrolled 55 IBS patients in their trial investigating the correlation of IBS and proinflammatory cytokine levels; patient groups were composed of 18 cases with mixt type IBS, 17 IBS-C and 20 IBS-D cases. Basal proinflammatory cytokine (IL-1β, IL-6, TNF-α) levels of IBS patients were found to be higher than the control group. In analysis of IBS subgroups, the highest proinflammatory cytokine level was detected in IBS-D group ($p< 0.05$). Dinan et al. (25) compared hypothalamo-pituitary-adrenal axis and plasma cytokine (IL-6, IL-6R, IL-8, IL-10, TNF-α) levels in a total of 76 IBS patients composed of 30 alternated type IBS, 36 IBS-D and 10 IBS-C cases and 75 control individuals. In conclusion, cortisol, IL-6, IL-6R and IL-8 were found to be statistically higher in all IBS groups as compared to controls ($p< 0.001$). Comparison of subgroups revealed that the most significant increase was in IBS-C group. No difference was determined in TNF-α and anti-inflammatory cytokine IL-10 levels.

In the current trial, we measured proinflammatory cytokine levels of IL-1β, IL-6 and TNF-α in peripheral blood. While levels of IL-1β, IL-6, and TNF-α of IBS patients were higher than MC cases, the difference was statistically insignificant. Comparison of cytokine levels and IBS subtypes revealed higher IL-1β, IL-6 and TNF-α levels in IBS-C patients as compared to IBS-D ones but the difference was statistically significant only for TNF-α values ($p= 0.013$). Increase in proinflammatory cytokines among IBS patients was shown in comparative trials against control groups[24-26]. On the other hand, it was suggested that cytokine mRNA levels at tissue level may indicate that plasma cytokine levels may not always reflect the expression and release of various cytokines in intestinal mucosa but the increase may be due to immunocytes in liver and spleen[27]. An example for this explanation may be that in inflammatory intestinal diseases, increase in intestinal mucosa cytokine expression does not always accompany the changes in plasma cytokine levels[28].

The limitations of our study are as follows; The most important limitation of this study was the relatively small number of patients. The study is a single-center study. In this study, we did not have the chance to study cytokine mRNA at tissue level. If we had studied this, we would have made additional comments on tissue inflammation and serum levels of cytokine.

In conclusion, rate of MC in IBS was found to be higher as compared to normal population. In addition, we showed that in IBS patients with suspected MC, performing rectosigmoidal biopsy alone does not always preclude the diagnosis of disease. Though significant inflammation findings at histopathological level were present in MC, we were unable to detect a significant difference in serum cytokine levels.

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