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Investigation of serum macrophage migration inhibitor factor and monocyte chemotactic protein-1 levels in irritable bowel syndrome

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Aim: Irritable bowel syndrome (IBS), a functional disorder of the bowel, has been thought to result from immune activation. The aim of this study was to evaluate macrophage migration inhibitory factor (MMIF) and monocyte chemotactic protein-1 (MCP-1) levels in IBS patients.

Materials and methods: We enrolled 30 IBS patients and 30 healthy controls. The MMIF and MCP-1 levels of all patients and controls were detected using commercial enzyme-linked immunosorbent assay kits.

Results: Serum MMIF and MCP-1 levels were markedly higher in IBS patients than in controls. White blood cell, neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts did not differ significantly between groups.

Conclusion: These results show that alterations in MMIF and MCP-1 affect the proinflammatory process. They also suggest that MMIF and MCP-1 may play a substantial role in IBS.

Key words: Irritable bowel syndrome, macrophage migration inhibitory factor, monocyte chemotactic protein, inflammation

1. Introduction

Irritable bowel syndrome (IBS) is one of the most prevalent gastrointestinal sicknesses and causes considerable morbidity amongst its sufferers (1). It is a functional bowel disease that usually occurs, or is exacerbated, during periods of emotional stress, and it progresses mainly with stomach aches or changes in defecation habits, like diarrhea or constipation. It is not linked to any known organic cause. Despite many studies, the pathophysiology of IBS is still uncertain. Hereditary and environmental factors affect the occurrence of IBS (2). Initially infections were blamed, but solid evidence could not be put forward to support this. Studies on visceral hypersensitivity and abnormal gastrointestinal motility as the primary sources of symptoms mainly focused on infections, inflammations, changes in microflora, excessive bacterial increase, genetics, and dysregulation of serotonin and the central nervous system. Both histological specimens obtained from endoscopy and serological cytokine studies have demonstrated low-grade inflammation in IBS (3).

Macrophage migration inhibitory factor (MMIF), which was first ascertained as the product of T cells, is described as a factor that inhibits desultory immigration of macrophages. Previous studies showed that MMIF could be produced as an immunoregulatory protein by several cells other than T cells (4). The immunological and hormonal effects of MMIF have been defined (5). Some findings refer to the fact that MMIF also promotes leukocyte accumulation during pathological inflammatory responses (6). As a proinflammatory cytokine, MMIF induces the secretion of some cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), thus taking a role in the pathogenesis of some inflammatory diseases and cancers (7).

Monocyte chemotactic protein-1 (MCP-1) is a member of the CC chemokine subfamily and is coded by the CCL2 gene (8). It is produced by monocytes, lymphocytes, vascular smooth muscle cells, tubular epithelial cells, and endothelial cells as a response to inflammatory stimuli including TNF-α, interferon-α, lipopolysaccharide, IL-1, and oxide low-density lipoprotein. It also acts as an intracellular messenger that controls leukocyte activation and migration linked to inflammatory reaction and immunity (9). It has been determined that in vitro MCP-

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1 could induce monocyte chemotaxis, which could carry a subgroup of T cells and IL-2-activated natural killer cells to the locality. Due to its target cell specificity, MCP-1 has a pathological role in several diseases described by mononuclear cell infiltration, including atherosclerosis, neoplasm, inflammatory diseases, and HIV (9).

It should be clear that both MMIF and MCP-1 can play a critical role in the pathogenesis of inflammatory diseases. We aimed to research whether there is a relationship between serum levels of these proinflammatory agents and the low-grade inflammation that has a role in the pathogenesis of IBS.

2. Materials and methods

2.1. Patients and controls

The study included 30 patients (20 females, 10 males; mean age: 45 ± 10 years) who had applied to Namık Kemal University Faculty of Medicine, Department of Gastroenterology, and were diagnosed with IBS according to the Rome III criteria and who had no alarm symptoms, had no chronic disease history, and had macroscopically normal colonoscopic findings (10). There were 30 control patients (17 females, 13 males; mean age: 49 ± 11 years) who had no history of chronic disease or drug use, who were undergoing colonoscopy for reasons not related to IBS, and who had macroscopically normal colonic mucosa. Exclusion criteria were alcohol and substance abuse or dependence, presence of severe organic disorders, use of any antioxidants, presence of gastroenterological disorder, presence of infectious or viral disease, and excessive obesity. The vital signs of all patients were recorded just before the colonoscopy by the anesthesiologist. All equipment needed for cardiopulmonary resuscitation was ready for use in the endoscopy unit. All drugs used during colonoscopy were administered by an anesthesiologist.

2.2. Biochemical analysis

Blood samples were obtained to determine MMIF and MCP-1 levels after overnight fasting. The serum samples obtained after centrifugation were then immediately frozen at –80 °C until further analysis of MMIF and MCP-1. The serum levels of MMIF (Quantikine Human MIF ELISA; R&D Systems) and MCP-1 (Quantikine Human CCL2/MCP-1 ELISA; R&D Systems) were specified with commercial enzyme-linked immunosorbent assay (ELISA) kits. MMIF and MCP-1 were measured in a sandwich-assay format using 2 specific high-affinity antibodies, streptavidin peroxidase conjugate and a chromogenic substrate. The minimum detectable level of MMIF and MCP-1 is 0.016 ng/mL and 1.7 pg/mL respectively. Complete blood cell count was determined by using a Roche Sysmex XT-2000i autoanalyzer and the same brand of commercial kits.

2.3. Statistical analysis

PASW 18 Statistics for Windows was used to record and analyze data on a computer. All results were expressed as mean ± SD. The Kolmogorov–Smirnov test was used to test the normality assumption for each variable. The group means were compared by using the independent sample t-test. The Pearson correlation coefficient was used to determine the relation between continuous variables. The results were considered to be statistically significant at $P < 0.05$.

2.4. Ethical considerations

The study was carried out in accordance with the Declaration of Helsinki II with approval of the ethics committee of the Namık Kemal University Faculty of Medicine. All participants were informed about the study protocol and written consent was obtained from each of them.

3. Results

Demographic data, white blood cell count, and subgroup cell counts are given in the Table. White blood cell, neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts did not differ significantly between groups (Table). Serum MMIF levels were significantly lower ($P = 0.013$) in the IBS group (37.43 ± 8.42 ng/mL) compared with the control group (32.66 ± 5.79 ng/mL) (Figure 1). MCP-1 levels were significantly lower ($P = 0.00$) in the IBS group (337.93 ± 58.77 pg/mL) compared with the control group (287.15 ± 41.76 pg/mL) (Figure 2). There was no correlation between MMIF and MCP-1 levels in IBS patients ($r = 0.019$, $P = 0.920$) (not shown).

Table. Demographic parameters, white blood cell count, and subgroup counts for the groups (mean ± SD).

	Control (mean ± SD)	IBS (mean ± SD)
Sex (M / F)	13 / 17	10 / 20
Age (years)	49 ± 11	45 ± 10
BMI (kg/m^2)	27.7 ± 8.3	30 ± 18
WBC (10^9/L)	7.6 ± 2.4	6.8 ± 1.6
NE (10^9/L)	4.4 ± 1.8	3.7 ± 1.3
LY (10^9/L)	2.4 ± 0.8	2.3 ± 0.5
MO (10^9/L)	0.65 ± 0.24	0.59 ± 0.18
EO (10^9/L)	0.17 ± 0.13	0.12±0.08
BA (10^9/L)	0.15 ± 0.07	0.15 ± 0.05

BMI: Body mass index, WBC: white blood cell count, NE: neutrophil count, LY: lymphocyte count, MO: monocyte count, EO: eosinophil count, BA: basophil count.

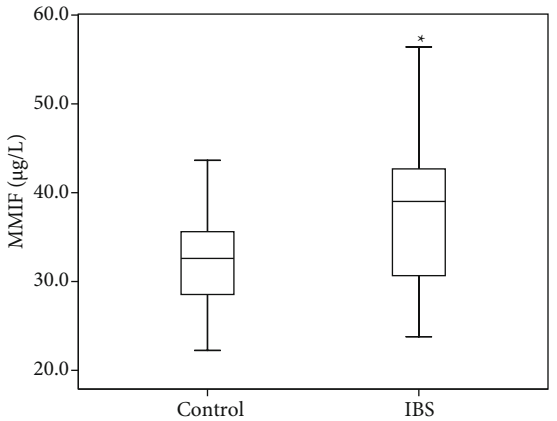

Figure 1. MMIF serum levels of the patients with IBS and controls (*: P = 0.013).

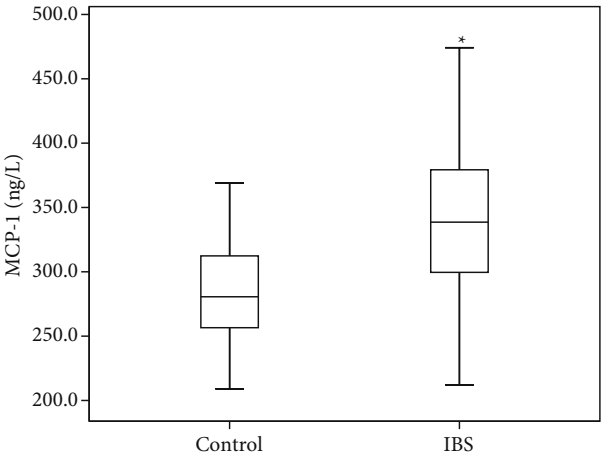

Figure 2. MCP-1 serum levels of the patients with IBS and controls (*: P = 0.000).

4. Discussion

In our study, we did not observe any significant change in the counts of white blood cells and subgroups. Nonsignificant decreases in counts of white blood cell and subgroups show that cellular immunity has a role in IBS development. Schoepfer et al. did not find significant changes in blood leukocyte levels of patients with IBS compared to healthy volunteers (11). In other research, Arévalo et al. suggested that there was no significant difference in mast cells, enterochromaffin cells, or eosinophils in the colonic mucosa of patients with IBS (12). Lee et al. supposed that there was no significant difference in lamina propria T lymphocyte counts between IBS patients and controls (13). Our findings match the results of these studies, which indicates that counts of white blood cells and subgroups cannot be a marker of IBS.

In our study, we found that serum MMIF levels of patients with IBS increased significantly compared with controls, which supports the development of inflammation in IBS. MMIF, which is a proinflammatory cytokine, is considered to have strong antiglucocorticoid effects and is linked to a variety of inflammatory diseases. MMIF inhibits MKP1 induction and thus inhibits the antiinflammatory effects of glucocorticoids, which induce MMIF (14). MMIF was shown to specifically counteract the glucocorticoid-induced suppression of inflammatory cytokine (TNF and interleukins) secretion in activated macrophages (15). Ishiguro et al. found that MMIF expression was enhanced in colonic mononuclear cells of patients with glucocorticoid-resistant ulcerative colitis and they stated that the antiinflammatory response to glucocorticoids in these cells was restored by anti-MMIF antibody (16). In their experimental study, Ogawa et al. stated that tumorigenesis due to an increase in angiogenesis resulting from the treatment of murine colon adenocarcinoma cells with anti-MMIF antibodies was suppressed, which suggests that MMIF could be associated with the development of tumor cell development (17). Huang et al. observed a significant upregulation in MMIF and MMIF mRNA and suggested that MMIF was mainly produced by macrophages, not neutrophils, in murine acute gastric ulcers. This was linked to the significant inhibition of macrophage and neutrophil accumulation and activation, which reduced ulcer sizes and attenuated ulceration (18). It has been suggested that MMIF protein was significantly increased in Crohn's disease and ulcerative colitis (UC). It was also suggested that MMIF significantly increased the production of IL-8 in UC patients compared with non-UC patients (19). Immune deficiency and inflammation are considered to be significant factors in the pathogenesis of IBS. Cytokines can affect bowel motility, permeability, and secretion, since they can affect epithelial cells, smooth muscles, and the enteric nervous system. Thus, it is suggested that cytokines could lead to IBS symptoms (20). Long et al. reported that immune cells were increased and mucosal immune systems were activated in the biopsy samples of patients with IBS (21). Studies on patients with IBS reported that proinflammatory cytokine levels were increased while antiinflammatory cytokine levels were decreased. In their study, Hua et al. reported that levels of IL-10, which is an antiinflammatory cytokine, were decreased in patients with IBS, whereas levels of proinflammatory cytokines such as IL-6 and TNF-α were increased. In the same study, it was claimed that the increase in the expression of IL-6, IL-8, and TNF-α, together with a decrease in the expression of IL-10 and IL-12, could be a risk factor in the development of IBS symptoms (22). Olivio-Diaz et al. stated that the inducement of IL-8 production and a decrease or deficiency in IL-10 production could trigger

the inflammatory process in patients with IBS (23). Dinan et al. stated a relation between IL-6 levels and IBS pathophysiology (24). These studies indicate a possible relation between the pathogenesis of IBS and an increase in proinflammatory cytokines caused by MMIF and a decrease in antiinflammatory cytokines.

Our findings suggest that MCP-1 levels increased significantly in patients with IBS, which matches the findings of previous studies. Studies have also found that, as well as proinflammatory cytokines, chemokine levels, and especially levels of MCP-1, IL-8, IL-1, and TNF, were increased in inflammatory bowel disease and showed a possible relation between MMIF and MCP-1 (25). The presence of MCP-1 is considered to be responsible for the massive invasion of blood monocytes and granulocytes to the inflamed tissue. Herrero et al. showed that MCP-1 levels were significantly reduced in rats with MMIF deficiency (26). Exogenous MMIF is considered to promote monocyte recruitment in vivo, partly through inducing endothelial release of CCL2/MCP-1. Our findings suggest that endogenous MMIF promotes TNF-induced p38 activation, which supports increased expression of adhesion molecules and chemokines (27). MMIF deficiency also has some inhibitory effects on CCL2-induced leukocyte adhesion and migration in vivo, migration in vitro, and cellular responses such as mitogen-activated protein kinase activation and actin polymerization. Experiments in CCL2-/- mice showed that MMIF-stimulated leukocyte adhesion and out-migration were both significantly decreased compared with MMIF-treated wild-type mice. It was also found that exposure to MMIF significantly increased CCL2 release. Specificity of MMIF for monocyte clustering resulted from a pathway involving the monocyte-attracting chemokine CCL2 and its monocyte-expressed receptor, CCR2. Blockade of CCL2 or CCR2 significantly reduces leukocyte adhesion and transmigration. Release of CCL2 from endothelial cells is induced by MMIF, and thus CCL2 promotes the adhesion and recruitment of monocytes. Macrophage-mediated inflammatory responses were also promoted by MMIF, through inducing the recruitment of monocytes into affected areas. MMIF could also have a role in the initiation and perpetuation of inflammation-associated diseases through attracting and activating monocytes or macrophages (28).

In conclusion, high levels of such proinflammatory mediators as MMIF and MCP-1 in patients with IBS were observed in the present study, which supports the claim that inflammation could be an effective factor in the pathogenesis of IBS and clarifies the increased production of proinflammatory cytokines. We consider that development of mechanisms or molecules inhibiting the activation of MMIF and MCP-1 could promote the treatment of IBS.

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