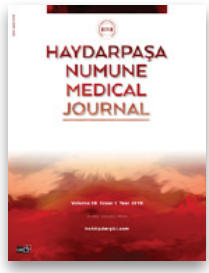
HAYDARPAŞA NUMUNE MEDICAL JOURNAL

DOI: 10.14744/hnhj.2019.06332
Haydarpasa Numune Med J 2020;60(2):182–187



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ORIGINAL ARTICLE

Association of Macrophage Migration Inhibitory Factor Gene - 173 G/C Polymorphism with Occurrence and Severity of Acute Pancreatitis

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Abstract

Introduction: Promoter polymorphism -173G/C of macrophage migration inhibitory factor (MIF) is related to higher MIF levels. In this study, we have investigated the effects of this polymorphism with the occurrence and severity of acute pancreatitis (AP).

Methods: Sixty-two AP patients and 83 healthy volunteers were included in our study. The demographical, clinical, laboratory and radiological findings were recorded, and peripheral blood samples were genetically analyzed.

Results: In this study, 35 female and 27 male patients were included. The mean age was 51. The AP severity was mild at 37 patients, moderate at 21 and severe at four patients. Genotype and allelic distribution at patient and control groups were statistically different (p<0.001; p=0.03). Although no significant difference between mild, moderate and severe AP groups detected, there was a tendency of CC genotype and C allele frequency being higher at moderate and severe AP and GG genotype being higher at mild AP. Eight of nine SIRS developed patients had CC genotype and C allele frequency was 88.9%. The leucocyte count at CC genotype and at C allele increased.

Discussion and Conclusion: According to our results, CC genotype and C allele frequency were related with AP occurrence. However, considering the severity of AP, the statistical significance could not be proven.

Keywords: Cytokine; genetic; macrophage migration inhibitory factor; pancreatitis; polymorphism.

Acute pancreatitis (AP) is an inflammatory disorder characterized with abdominal pain with increased pancreatic enzymes[1]. The clinical progression of the disease has a wide spectrum and many patients recover after a self-limiting mild disease with a couple of days of hospitalization. However, approximately 3.7-28.5 % of all AP cases were severe having 20-80% mortality risk[2–7]. Whatever the inducing factor, the same events occur after the first injury of pancreatic acinar cell. During the progression of the disease, there are three phases respectively that are local inflamation, systemic inflammation and multiple organ failure. In case of failure to restrict inflammatory reaction, these phases progress to severe AP, a clinical condition may result with death of the patient. The primary humoral mediator of this period is cytokines[8]. If the balance between pro-inflammatory cytokines [Macrophage Migration Inhibitory

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Submitted Date (Başvuru Tarihi): 21.07.2019 **Accepted Date (Kabul Tarihi):** 26.07.2019

Factor (MIF), Tumour Necrosis Factor, Interleukin 1.6 and 8] and anti-inflammatory cytokines [Interleukin 10, Interleukin 1 receptor antagonist] is disturbed favoring pro-inflammatory side progression to severe acute pancreatitis occurs and morbidity, mortality increases[9, 10].

MIF is first identified as an inhibiting factor of macrophage migration that is secreted from activated T lymphocytes at 1966[11]. Recent studies proved its proinflammatory role at a wide spectrum of inflammatory disease pathophysiology[12, 13]. Both in experimental and clinical studies, the finding of increased serum levels at AP disorder compared to healthy controls and at severe AP compared to mild AP disorder were shown[7, 10, 14, 15].

There are two important MIF promotor region functional polymorphisms identified[16, 17]. First polymorphism identified was located at MIF gene promoter region at -173 position with a G nucleotide change to C nucleotide. This single nucleotide polymorphism is found to be associated with systemic-onset juvenile idiopathic artritis. This relation is explained with the functional influence of promoter sequence change to form a new binding site for activator protein transcription factor 4[16].

Other polymorphism is located at -794 position where a tetra-nucleotide CATT5-8 repeat is formed. Five repeat 5-CATT polymorphic allele had the lowest in vitro promotor activity and found to be related with low level disease severity of romatoid artritis[17]. In the first research article conserning relationship of MIF gene polymorphism and AP by Makhija et al.[18] -794 CATT5-8 repeat was reported to be insignificant. In the same paper -173 G/C polymorphic genotype distribution and -173 C allele frequency were statistically important between AP patients and healthy control. However, there was no statistical important relation among the clinical severity groups[18]. Makhija et al.[19] classified the patients according to Atlanta classification (AC). However, in 2012, there were important revisions and new definitions added to this classification system[1]. In this study, we have studied the relationship between MIF gene promoter -173 G/C polymorphism and AP occurrence and the severity of disease which is classified according to Revized Atlanta Classification (RAC)[1].

Materials and Methods

Study Design and Setting

This study was planned as an observational analytic cohort study. Our protocol and informed consent form were approved by University of Health Sciences Haydarpasa Numune Research and Training Hospital Clinical Research Ethical Council (Date/Number: March 9, 2015/49). Patient admission and blood sampling were carried out at general surgery clinic at Haydarpaşa Numune Research and Training Hospital and MIF -173 G/C gene polymorphism analysis were carried out at Marmara University School of Medicine Department of Medical Genetics Laboratories.

Patients and Controls

In this prospective study, 62 patients older than 18 years of age with AP diagnosis who were hospitalized and treated between January-December 2016 at Haydarpasa Numune Research and Training Hospital were covered but chronic pancreatitis patients were excluded from this study. Diagnosis of AP was confirmed with the presence of at least two of the three signs of AP (abdominal pain, serum lipase or amilase levels higher than three folds and the detection of characteristic radiological finding). For disease severity detection, RAC was used and patients were categorized as mild (no organ failure and no local and systemic complications), moderate (transient organ failure recovered at 48 hours and/or patients with local or systemic complications) and severe AP (patients with persistent organ failure) into three groups[1]. For detection of organ failure, Modified Marshall Organ Dysfunction Scoring was used. All patients' demographical, clinical properties, laboratory values, radiological imaging findings, AP etiology and Systemic Inflammatory Response Syndrome (SIRS) scores (at admission, 24 hours, 48 hours) were recorded at case report forms. Patients were treated according to up to date standart medical knowledge and their clinical progression was recorded at this form.

Control group was 83 healthy volunteers working at Haydarpasa Numune Research and Training Hospital.The peripheral blood samples were collected to EDTA tubes and kept at -20 degree celcius refrigerator until they are analyzed for MIF -173 G/C polymorphism.

MIF -173G/C genotyping

Genomic DNA was isolated from peripheral blood with colon purification method and polymerase chain reaction was carried out as described by Donn et al.[16]. A 366 bp fragment of MIF promotor region was amplified using Polymerase chain reaction. Forward primer was 5'-ACT-AAGAAA-GAC CCG-AGG-C-3' and the reverse primer was 5'-GGG-GCA-CGT-TGG-TGTTTA-C-3'. The annealing temperature for the primers was 59°C. After Alu I restriction endonuclease (New England Biolabs, Ipswich, MA, USA) overnight incubation at 37°C, the resulting digested frag-

ments were electrophoresed at 2.5% agarose gel and visualized by staining with 10% ethidium bromide under UV light. The 366 bp MIF PCR product had a restriction site revealing a 98 bp and a 268 bp fragments. The GG genotype did not have a second cutting site for Alu I but the CC genotype had a second cutting site resulting in three fragments: 205 bp, 98 bp, 63 bp size after the Alu I digestion.

The heterozygous GC genotype was characterised by four bands: 268 bp, 205 bp, 98 bp, 63 bp at gel electrophoresis. Fifty percent of the samples were repeated to control the band quality and to avoid sample or reading errors.

Statistics

Descriptive statistics were used for description of continuous data (mean, standard deviation, minimum, median, maximum). Independent and normally distributed two variables were compared using Student t-test and those variables that were not suitable for independent and normal distribution were compared with Mann-Whitney U test. Chi square test (in some occasions Fisher Exact test was used) was used to analyze the relationship between cathegorical variables. Variables were analyzed using Kruskal Wallis test in more than two groups, not suitable for normal distribution. The level of statistical significance was detected as 0.05. Data were analyzed using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013) programme.

Results

Among the 62 AP patient group, 35 were females and 27 were males and the mean age was 51±15.1 years of age. Among 83 healthy volunteers of the control group, 55 were females and 28 were males and there were no statistical significance about gender difference between the two groups (p=0.302, χ^2 test).

The most frequent etiology of AP was gallstones (n=41, 66.1%). When the disease severity is classified according to RAC, distribution of the patients was 37 patients with mild (59.7%), 21 patients (33.9%) with moderate and four patients with severe AP (6.5%). One of the severe AP patients was dead due to multiple organ failure. Mortality rate for all patients were 1.6%; for severe AP patients it was 25%. The general characteristics of the patients are summarized in Table 1.

When MIF -173 G/C gene polymorphism was analyzed in AP patients and control group, there was a statistically significant difference both at genotype distribution and -173 C allele frequency (p<0.001; p=0.03, respectively). While

Table 1. General characteristics of the patients

Characteristics	n	%
Age		
Mean±SD	51±15.1	
Median (Range)	50.5 (21-76)	
Gender		
Female	35	56.4
Male	27	43.6
Etiology		
Gallstones	41	66.1
Alcohol	2	3.2
Drug	2	3.2
Hyperlipidemia	1	1.6
Idiopathic	16	25.8
Severity		
Mild	37	59.7
Moderate	21	33.9
Severe	4	6.5

SD: Standard deviation.

CC genotype was more frequent in AP patients (p=0.015), GC genotype was more frequent at the control group (p=0.033). There was a tendency of GG genotype to be less frequent at AP patients, but there was no statistical significance (p=0.597). C allele frequency was higher at AP patients compared to the control group (p=0.03). When AP patients were categorized according to disease severity as mild, moderate and severe AP although there was no statistical significance (p=0.629; p=0,138, respectively) between the groups at both genotype distribution and -173C allele frequency, there was a tendency of the CC genotype and -173 C allele frequency being higher at moderate and severe AP patients and the GG genotype being higher at mild AP patients. MIF -173 G/C genotype and allelic distribution were shown in Table 2.

Among AP patients, nine of them developed SIRS and eighth of them had CC genotype. There was an important statistical difference between the genotypes for SIRS development (p<0.001). Patients who developed SIRS had -173 C allele at a frequency of 88.9 % and the frequency of having SIRS was higher at C allele carrying patients than the patients that had G allele with a statistical significance (p<0.001). The highest leukocyte count was present at CC genotyped and C allele carrying patients, and there was a significant leucocyte count difference between the C and G allele groups (p=0.023; p <0.001, respectively). The development of SIRS and the leucocyte count distribution between MIF -173 G/C genotype and different allele groups are shown in Table 3.

Table 2. Distribution of the MIF -173 G/C genotypes and alleles

	Acute pancreatitis n=62 n (%)	Control group n=83 n (%)	p	The severity of acute pancreatitis			
				Mild n=37 n (%)	Moderate n=21 n (%)	Severe n=4 n (%)	p
Genotypes							
GG	33 (53.2)	49 (59.0)	0.597	22 (59.5)	9 (42.9)	2 (50)	0.472
GC	4 (6.5)	17 (20.5)	**0.033**	3 (8.1)	1 (4.8)	0 (0)	0.597
CC	25 (40.3)	17 (20.5)	**0.015**	12 (32.4)	11 (52.4)	2 (50)	0.304
Overall p		**<0.001**				0.629	
Alleles							
G	70 (56.4)	115 (69.3)		47 (63.5)	19 (45.2)	4 (50)	
C	54 (43.6)	51 (30.7)		27 (36.5)	23 (54.8)	4 (50)	
Overall p		**0.03**			0.138		

Groups are compared with Fisher's Exact test.

Table 3. Progression to SIRS and blood leukocyte counts at MIF -173 G/C genotype and allele groups

	SIRS (+) n=9 n (%)	SIRS (-) n=53 n (%)	p	Blood leucocyte count Mean (/mm^3)±SD
Genotypes				
GG	1 (11.1)	32 (60.4)	**0.017**	11258.9±4462.5
GC	0 (0)	4 (7.5)	-	13392±4590.7
CC	8 (88.9)	17 (32.1)	**<0.001**	15112.1±5872.6
Overall p	**<0.001[a]**			**0.023[b, c]**
Alleles				
G	2 (11.1)	68 (64.1)		11380.9±4431.3
C	16 (88.9)	38 (35.9)		14984.8±5712.6
Overall p	**<0.001[a]**			**<0.001[d]**

[a]Fisher's Exact test, [b]Kruskal-Wallis test, [d]Mann-Whitney U test, SD: standard deviation; [c]For GG-CC, GG-GC and CC-GC p values are as <0.001, 0.407 and 0.408 respectively at Post-Hoc two-sided comparison. For this analysis, Mann-Whitney U test is used and for interpretation with Bonferroni correction <0.016 is accepted as significant.

Discussion

Since 1896, when Chiari first proposed pancreatic autodigestion due to pancreatic enzymes, AP pathogenesis studies have concentrated on trypsin[20]. Recently, oxidative stress, endoplasmic stress, disturbed autophagia and mitochondrial dysfunction were described with new studies that could not overcome the enigma at AP pathogenesis [21–23]. The uncertainty of how the initializing first event causing acinar cell damage gives rise to local inflammation and escalation to systemic inflammation is still a debate. The variability of the immune response may be an important determinative of AP severity. With the progression of local inflammation to systemic inflammation, accompanying tissue damage and disturbance of organ function, increases AP severity and ends with severe AP disorder. Thus, immune system activation and inflammatory mediators are of great interest to figure out new targets for therapeutic action[23, 24].

Herein when MIF -173 G/C gene polymorphism was analyzed, both genotype distribution and -173 C allele frequency were significantly different between the AP patients and healthy controls. Our findings are similar to Makhija et al.'s study results and the results of both of these studies were compatible with results of a similar study of Donn et al. which were covering Juvenile Idiopathic Artritis patients[18, 25]. Donn et al.[25] additionally reported that patients carrying MIF-173 C allele also had significantly higher serum MIF levels than the patients having MIF-173 G allele. It is reported that MIF intracellularly induces Nuclear Factor Kappa B (NF-κB) activity[26] and activation of NF- κB is identified as an important inflammatory pathway at AP pathogenesis[23]. Recently, at the trypsinogen knockout mice model, it is reported that during AP, absent pathological trypsinogen activation has no effect on intra-acinar NF-κB activation[27]. Thus, this NF-κB induction might be an early independent event from trypsinogen activation and might be sufficient to induce AP. Thus, with this knowledge, we can propose that the significantly higher CC genotype and C allele frequency detected at AP patients might be effective at AP pathogenesis by the effect of increased MIF and induction of NF-κB activity. We thought that since GC genotype is more frequent at the control group, the pathogenic effect of the C allele might be considered only if CC homozygosity is present. Both these relationships and other effective mechanisms should be analyzed with further studies.

In the studies covering the relationship of AP severity and MIF, it is reported that at severe AP disorder, the serum MIF was higher than mild AP[7, 10, 14, 15]. Sakai et al. reported that MIF was a critical mediator at severe AP that at experimental severe AP model ascitic fluid, serum and lung MIF levels were markedly increased and by application of prophylactic anti-MIF antibody mortality was decreased. At the clinical counterpart of the same study covering AP patients, serum MIF levels were significantly higher at severe AP patients compared to mild AP and healthy controls[14]. According to AC disease severity classification in Makhija et al.'s[18] study, they reported that there was no significant difference between MIF -173 G/C gene polymorphism and disease severity detected. In our study, according to RAC, we have classified our patients as mild, moderate and severe and we could not detect statistical significance both between the genotype distribution and also -173 C allele frequency. However, CC genotype and C allele frequency at moderate and severe AP groups had a tendency to be higher than mild AP group. Also, eighth of nine SIRS patients had CC genotype as a C allele frequency of 88,9%. Higher blood leukocyte count associated with C allele frequency was compatible with this clinical condition. These findings, when analyzed with the results of Gando et al. study[28], where high MIF levels and worse SIRS prognosis were related, we suggest that 173 G/C gene polymorphism and disease severity should be analyzed at larger patient groups for further analysis.

Conclusion

Herein in our study, although there is no statistical significance between CC genotype and C allele frequency and disease severity, CC genotype and C allele frequency and AP occurrence seem to be interrelated. To our knowledge, this research is the first study to use RAC and the second study analyzing MIF-173 G/C gene polymorphism and acute pancreatitis relationship. There is a need to have comprehensive studies to elucidate this relationship.

Ethics Committee Approval: The Ethics Committee of Haydarpasa Numune Training and Research Hospital provided the ethics committee approval for this study (March 9, 2015/49).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: M.A.U., S.A.K., P.A., M.T., S.B.O., T.D.; Design: M.A.U., S.A.K., P.A., M.T., S.B.O., T.D.; Data Collection or Processing: M.A.U., S.A.K., P.A., S.B.O., T.D.; Analysis or Interpretation: M.A.U., S.A.K., P.A., M.T.; Literature Search: M.A.U., S.A.K., P.A.; Writing: M.A.U., S.A.K., P.A., M.T., S.B.O, T.D.

Conflict of Interest: None declared.

Financial Disclosure: This study was supported by University of Health Sciences, Haydarpasa Numune Research and Training Hospital Research Planning Grant Commission.

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