Eurasian J Med 2014; 46: 176-81 The Eurasian Journal of Medicine **Original Article**

Association between Hepatocyte Growth Factor (HGF) Gene Polymorphisms and Serum HGF Levels in Patients with Rheumatoid Arthritis

Romatoid Artritli Hastalarda Serum Hepatosit Büyüme Faktörü (HGF) Düzeyleri ile HGF Gen Polimorfizmleri Arasındaki İlişki

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Abstract

Objective: Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by proliferation and insufficient apoptosis of synovial cell, inflammatory cell infiltration, angiogenesis, and destruction of joints. Hepatocyte growth factor (HGF) has many functions, such as regulation of inflammation, angiogenesis, and inhibition of apoptosis. The purpose of this study was to investigate the association between intron 13 C/A and intron 14 T/C HGF gene polymorphisms and serum HGF levels in patients with RA.

Materials and Methods: 100 patients with RA and 123 healthy controls were included in this study. Serum HGF concentrations were measured using ELISA kit. Gene polymorphisms were determined by allelic discrimination analysis using the real-time PCR method.

Results: HGF levels, frequency of AA genotype and A allele for intron 13 C/A polymorphism and frequency of CC genotype and C allele for intron 14 T/C polymorphism were increased in patients with RA compared to healthy controls. There was no overall associations between genotypes and serum HGF concentrations in both patient and control groups.

Conclusion: Our results indicate that HGF protein and gene may play an important role in the etiopathogenesis of RA. However, further studies are required for a better understanding of mechanisms related to the disease process.

Key Words: Hepatocyte growth factor, HGF intron 13 C/A, HGF intron 14 T/C, polymorphism, rheumatoid arthritis

Özet

Amaç: Romatoid artrit (RA), eklemlerde yıkım, anjiyogenezis, inflamatuar hücre infiltrasyonu, sinovyal hücre apoptozisinde yetersizlik ve proliferasyon ile karakterize kronik inflamatuar bir hastalıktır. Hepatosit büyüme faktörü (HGF), apoptozis inhibisyonu, anjiyogenezis ve inflamasyonun regülasyonu gibi bazı fonksiyonlara sahiptir. Bu çalışmanın amacı, RA'lı hastalarda serum HGF düzeyleri ile intron 13 C/A ve intron 14 T/C HGF gen polimorfizmleri arasındaki ilişkiyi araştırmaktı.

Gereç ve Yöntem: Bu çalışma 100 RA'lı hasta ve 123 sağlıklı kontrol üzerinde yürütüldü. Serum HGF konsantrasyonları, ELISA yöntemiyle; gen polimorfizmleri ise allelik diskriminasyon analiziyle real-time PCR yöntemiyle belirlendi.

Bulgular: RA'lı hastalarda, HGF düzeyleri, intron 13 C/A polimorfizmi için A alleli ve AA genotip frekansı, intron 14 T/C polimorfizmi için ise C alleli ve CC genotip frekansı kontrollere göre artmıştı. Hem hasta hem de kontrol gruplarında serum HGF konsantrasyonları ile HGF genotipleri arasında anlamlı bir ilişki yoktu.

Sonuç: HGF protein ve geni, RA etyopatogenezinde önemli role sahip olabilir. Bununla birlikte hastalık süreciyle ilişkili mekanizmaların anlaşılabilmesi için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Hepatosit büyüme faktörü, HGF intron 13 C/A, HGF intron 14 T/C, polimorfizm, romatoid artrit

Introduction

Rheumatoid arthritis (RA) is a chronic, progressive disease that affects the joints and leads to significant deformities that disrupt the quality of life. It is 3 times more prevalent in women than in men and it influences nearly 1% of the population. Although numerous studies on RA have been conducted over many years, its etiology still has not been clarified exactly [1, 2].

Studies on illuminating etiopathogenesis of RA gain attention for the equilibrium between pro- and anti-inflammatory cytokines that play role in initiation and continuation of chronic inflammatory process in synovial membrane

Received: September 22, 2013 / **Accepted:** March 15, 2014 / **Available Online Date:** August 26, 2014

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DOI:10.5152/eajm.2014.41

[3], angiogenesis [4, 5] and alterations in synovial cell apopitosis [6].

Hepatocyte growth factor (HGF) was originally identified in 1984 as a factor that stimulates hepatocyte proliferation and named according to this function [7, 8]. However, researches in the following years revealed that HGF also stimulates the proliferation of cells other than hepatocyte [9], and has angiogenic [10], anti-inflammatory [11], pro- and anti- apoptotic [12] properties.

Aim of our study is to investigate intron 13 C/A (rs2074725) and intron 14 T/C (rs2074724) HGF gene polymorphisms in patients with RA and evaluate serum HGF levels to compare patients with healthy control group.

Materials and Methods

This study was initiated after the approval of the Ethical Committee of Ataturk University Medical Faculty (date: 15.05.2009 and number: 137), and written informed consent was obtained from all patients. This study includes 100 patients with RA that diagnosed in the Department of Physical Medicine and Rehabilitation Medical Faculty of Ataturk University, and the control group consists of 123 healthy (having no systemic disease) participants whose mean age and gender percentage are similar to the patient group. The diagnosis of RA was made according to the criteria of the American College of Rheumatology [13]. None of the patients had clinically active disease, as defined by the presence of at least two of the following criteria: (1) morning stiffness duration >30 min, (2) six or more tender joints, (3) three or more swollen joints, and (4) erythrocyte sedimentation rate (ESR) >28 mm/h.

Venous blood samples were collected from each individual of patient and control group into serum-separator tubes for determining serum HGF levels and also into ethylene diamine tetra acetic acid (EDTA) tubes for DNA isolation. After waiting for coagulation of blood samples, it was centrifuged at 3000 rpm for 10 minutes for serum and serum aliquots stored at -80°C until the HGF measurement. DNA isolation was performed for the HGF polymorphism analysis from blood samples in EDTA tubes.

Serum HGF concentrations are evaluated by the solid-phase sandwich ELISA method using "Human Activated HGF Assay Kit" (IBL, Cat. No: RSC27401R) according to the instructions of the manufacturing company.

Venous blood samples in EDTA tubes are aliquoted for analysis of intron 13 C/A (rs2074725) and intron 14 T/C (rs2074724) HGF gene polymorphisms, and reserved at -80°C until use and DNAs are isolated using Invitrogen, PureLink Genomic DNA isolation kit (Cat. No: K1820-01). The analysis of intron 13 C/A (rs2074725) and intron 14 T/C (rs2074724) HGF gene polymorphisms was determined using the real-time PCR apparatus (Applied Biosystems 7300) by the TaqMan allelic discrimination method.

Allele specific TaqMan probes that are utilized in the analysis process were as follows: For intron 13 C/A of HGF gene 5'- FAM - GCACAAATTATAGTCCAGAGCTTACcGTCTGG CAAGCAGATGTGATCAGCT-Tamra-3' and 5'- VIC-G C A C A A A T T A T A G T C C A G A G C T T A C a G T CTGGCAAGCAGATGTGATCAGCT-Tamra-3'; forward primer 5'-CTACCTCTGGAGGCACAAA TTA-3' and reverse primer 5'-GGGTACAACCTTCAGGACCA-3'and for intron 14T/C 5'-FAM – CTACAGGAGAAAGAAGTAGTGAGGAtTGAAAAAAGCCTATTGA CAATTTAG-Tamra-3' and 5'-VIC-CTACAGGAGAAAGAAGTAGT GAGGAcTGAAAAAAGCCTATTGACAATTTAG-Tamra-3'.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SSPS) software package version 11.5 for Windows (SPSS Inc, Chicago, USA). The normal distribution of variables was evaluated using the Kolmogorov-Smirnov test. The Mann Whitney U and Kruskal Wallis tests were used to compare the analysis results which were not normally distributed. The Chi-square test was applied to evaluate the distribution of gene polymorphisms among the patient and control groups. For statistical significance, $p<0.05$ was accepted.

Results

While serum HGF concentration was found 1431±851 pg/mL in the patient group, it was 1135±647 pg/mL in the control group. Differences between the patient and control groups were statistically significant ($p<0.05$).

Genotypes for intron 13 C/A polymorphism were determined in 82 patients of which 57 were (69.5%) AA, 8 were (9.8%) CA and 17 were (20.7%) CC genotype. Also genotypes were determined in 106 controls of which 30 were (28.3%) AA, 27 were (25.5%) CA and 49 were (46.2%) CC genotype. The statistical significance test of difference in distribution of genotypes was carried out by the X^2 test and there were significant differences between groups ($x^2=31.661$, df=2; $p<0.001$) (Table 1). The allele frequencies of intron 13 C/A polymorphism analyses were performed on the patient and control groups were presented in Table 1. While 122 A alleles (74.4%) and 42 C alleles (25.6%) were determined in the patients, 87 A alleles (41%) and 125 C alleles (59%) were in healthy individuals. Statistically A allele frequency was significantly higher in patients ($p<0.001$).

Genotypes could be determined by the result of intron 14 T/C polymorphism analysis in 85 patients and 86 controls. CC, TC and TT genotypes were seen in 15 (17.7%), 38 (44.7%) and

Table 1. The results of intron 13 and intron 14 HGF gene polymorphism analysis

Polymorphism	Patient n (%)	Control n (%)	p value
Intron 13 genotype			
CC	17 (20.7%)	49 (46.2%)	x^2=31.66, df=2
AA	57 (69.5%)	30 (28.3%)	p<0.001
CA	8 (9.8%)	27 (25.5%)	
Allele frequency			
C	0.256	0.590	p<0.001
A	0.744	0.410	
Intron 14 genotype			
TT	32 (37.6%)	47 (54.6%)	x^2=9.33, df=2
TC	38 (44.7%)	35 (40.7%)	p<0.01
CC	15 (17.7%)	4 (4.7%)	
Allele frequency			
C	0.400	0.250	p<0.005
T	0.600	0.750	

Table 2. Effect of polymorphisms on serum HGF levels (mean±SD) in the patient group

Polymorphisms	HGF levels (pg/mL)	p value
Intron 13 genotype		
CC	1426±723	x^2=1.699, df=2
AA	1506±935	p>0.05
CA	1623±505	
Intron 14 genotype		
TT	1480±1022	x^2=0.094, df=2
TC	1429±815	p>0.05
CC	1507±893	

HGF: hepatocyte growth factor

32 (37.6%) of the patients, respectively. On the other hand; CC, TC and TT genotypes were found in 4 (4.7%), 35 (40.7%), 47 (54.6%) of controls, respectively. The statistical significance test of difference in distribution of genotypes was carried out by the x^2 test and there were significant differences between groups (x^2=9,334, df=2; p<0.01) (Table 1).

Sixty-eight (40%) C and 102 (60%) T alleles were detected in the patients performed intron 14 T/C polymorphism analysis. On the other hand, 43 (25%) C and 129 (75%) T alleles were in controls. Statistically C allele frequency was significantly higher in patients. (p<0.005) (Table 1).

However, a significant association was not observed between intron 13 and 14 HGF gene polymorphisms and serum HGF levels in both patients or controls (Tables 2 and 3).

Discussion

HGF is a multifunctional cytokine that is produced by various cells in response to tissue damage [14]. This situation gives rise to the thought whether HGF has a role in RA as a chronic inflammatory disease which leads to cartilage and subchondral bone destruction on occupied joints [15].

In the current study, we found higher serum HGF concentrations in patients with RA than in healthy controls. This result was consistent with the study results of Feuerherm et al. [16] and it was important in terms of showing that HGF may play a role in etiopathogenesis of RA. IL-1, IL-6 and TNFα are major cytokines that contribute fibroblast proliferation, tissue damage and progression of inflammation in RA. It is

Table 3. Effect of polymorphisms on serum HGF levels (mean±SD) in the healthy group

Polymorphisms	HGF levels (pg/mL)	p value
Intron 13 genotype		
CC	1035±378	x^2=1.624, df=2
AA	1186±673	p>0.05
CA	757±265	
Intron 14 genotype		
TT	1115±209	x^2=0.450, df=2
TC	1304±756	p>0.05
CC	1275±1129	

HGF: hepatocyte growth factor

known that HGF secretions from diverse tissues increase in response to these cytokines [17, 18]. Increase of serum HGF levels has been revealed in many inflammatory diseases such as inflammatory lung disease [19], hepatitis [20], inflammatory disease of the intestines [21], and then it has been proposed that HGF could be used as a prognostic tool in some of the diseases including myocardial infarction [14].

Studies have demonstrated that the synovial tissue is both target and source of HGF: It has been shown that c-Met were found in the synovial tissue of patients with RA and HGF was produced by synovial macrophages, fibroblasts and endothelial cells [8, 22, 23]. Yukioka et al. [15] found that the HGF concentrations in synovial fluid were higher in patients with RA than other arthritis such as osteoarthritis.

After HGF was discovered in 1984, rat and human HGF cDNAs were detected in a short period of time [8]. But, only a few studies have been carried out to date on determination of polymorphisms related to HGF gene and investigation of association between these polymorphisms and disease process [24, 25]. Existing studies were on the subject of coronary artery disease [26], hypertension and atherosclerosis [27, 28], myopia [29], autism [30], breast cancer [31], nephrolithiasis [32], and no article was written to investigate the HGF gene polymorphism in RA disease after scanning literature. Our study is the first study investigating HGF intron 13 C/A and intron 14 T/C polymorphisms in patients with RA. Thus, we expect that this research will make a substantial contribution to the literature.

Because there was no study evaluating the relationship between HGF polymorphisms and RA, it was difficult to compare our findings. Therefore, we might well consider other studies investigating the clinical relevance of HGF polymorphisms in different diseases for comparison. In previous studies, HGF intron 13 C/A and intron 14 T/C polymorphisms were examined in essential hypertension and nephrolithiasis cases [28, 32]. Although Motone et al. [28] did not found any association between essential hypertension and 14 T/C polymorphism, they observed an association between intron 13 C/A polymorphism and hypertension and they suggested that A allele could prevent hypertension through local HGF production.

In our study, we revealed that intron 13 AA genotype was more prevalent in patients with than in healthy controls, whereas CC genotype was rarer. Also, when allele frequency was examined, it was noted that A allele frequency was statistically significantly more common in patients. However, intron 13 C/A polymorphism did not affect the serum HGF concentrations in both patients and healthy controls. This result was consistent with the study of Ozturk et al [32].

In the current study, we found a statistically significant association between RA and intron 14 T/C polymorphism. CC genotype was more prevalent in patients than in control group, whereas TT genotype was rarer. For allele frequency, C allele was significantly more frequent in patients. However we saw that distinctiveness of genotype in the intron 14 T/C polymorphism did not have any statistically significant effect on HGF concentrations like intron 13 C/A polymorphism.

Although intron 13 A and intron 14 C allele frequencies were more common and serum HGF concentrations were higher in patients with RA than in healthy individuals, there was no association between HGF levels and genotype distinctiveness. This condition can be explained by many factors that involve HGF concentrations. Also it must not be forgotten that HGF in synovial fluid is more important than circulatory HGF in RA. Yukioka et al. [15] found that the levels of HGF in synovial fluid were higher than the serum ones that are collected from the same patients synchronously.

In conclusion, HGF reaches higher serum concentrations in patients with RA than in healthy controls, and although no relation was demonstrated between the genotype and HGF levels, a polymorphic distinctiveness was detected between patients and controls. We expect these will be able to con-

tribute to elucidation of etiopathogenesis of RA. However, further studies are needed to fully understand the role of HGF protein and gene.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Ataturk University Medical Faculty (15.05.2009 and number 137).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - F.K., A.Y.; Design - A.Y., S.K.; Supervision - E.B.; Funding - A.Y.; Materials - K.Y., S.K.; Data Collection and/or Processing - F.K.; Analysis and/or Interpretation - A.Y., F.K.; Literature Review - F.K.; Writing - M.G., F.K.; Critical Review - A.Y.; Other - F.K., M.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work was financially supported by the Ataturk University Research Council (Grant number: 2009/306).

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