

Role of Tumor Necrosis Factor Alpha Promoter Polymorphisms in Interferon Related Side Effects in Chronic Hepatitis B Patients Under Interferon Alpha 2b Treatment

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ABSTRACT

Objective: Interferon alpha therapy is associated with series of adverse effects leading premature discontinuation of treatment. Here we aimed to determine the effects of Tumor necrosis alpha promoter polymorphisms on interferon related side effects during interferon alpha 2b treatment in chronic hepatitis B patients.

Material and Methods: This observational study enrolled 50 chronic hepatitis B patients, who were treated with interferon alpha 2b 10 tiw plus lamivudine 100mg/day at Selçuk University Meram Medical Faculty Department of Infectious Diseases and Clinical Bacteriology between 2006-2007. Patients were followed-up with complete blood count, transaminases and amylase monthly at out patient clinics of our department. All patients were asked to fill a questionnaire to evaluate the interferon related side effects at every visit. Tumor necrosis factor alpha -238 and -308 polymorphisms were investigated with PCR-Restriction fragment length polymorphisms.

Results: Arthralgia was present in 93.8% of patients. Exhaustion, loss of appetite, mouth dryness and fever are the leading complaints of the patients. Median complaint scores were 9, 9.5, 11 and 5 at 4th, 12th, 24th and 48th week visits, respectively. Thrombocytopenia and leucopenia were detected in 8 (16%) and 5 (10%) patients, respectively. One-third of the patients reported depressive mood however, depression was diagnosed in 5 (10%) patients. TNF alpha promoter 238GG and 308GG allele was present in 42/50 and 43/50 patients, respectively. No association was found between TNF promoter allele and certain patient-reported side effect, complaint score, hematological and psychological adverse effects.

Conclusion: TNF alpha promoter polymorphisms do not contribute to occurrence of IFN alpha treatment related side effects.

Keywords: TNF alpha promoter polymorphism, interferon alpha, interferon related side effect, chronic hepatitis B

Received: 10 March 2019, Accepted: 26 April 2019,
Published online: 30 June 2019

INTRODUCTION

There are more than 300 million people are chronically infected with hepatitis B virus (HBV) worldwide. Chronic viral hepatitis B (CHB) infection is associated with increased risk of liver-related morbidity, including cirrhosis, fulminant liver failure, liver

transplantation, hepatocellular carcinoma (HCC) and death [1,2]. In the Americas, HBV prevalence is relatively low, with hepatitis B surface antigen (HBsAg) positivity ranging from $\leq 2\%$ to 7% compared with

Asia, Africa and the Middle East, where HBV prevalence rates reach 5-20% of the general population [2,3]. Successful implementation of childhood HBV vaccination program in Turkey led to rapid decrease in HBsAg prevalence. Estimated number of Turkish CHB patients is 3.3 million with an overall prevalence of 4.7% [4].

Scaring outcomes of CHB infection necessitates an effective therapy. The main goal of CHB treatment is to suppress HBV replication and to induce remission of liver disease which in turn will prevent the development of cirrhosis or HCC. In 2006, interferon alpha (IFN α), lamivudine, entecavir, tenofovir and telbivudine were approved and available on market for CHB treatment.

Interferons are pleiotropic proteins, able to initiate and regulate a variety of responses, either directly or by stimulating the induction/activation of additional proteins. Different interferon subtypes intrinsically have the ability to stimulate different but overlapping sets of genes [5]. IFN α has wide variety of side effects on different organ systems which some times lead to premature discontinuations. Treatment with IFN α elicits an immune response leading to secretion of tumor necrosis factor alpha (TNF α), interleukin 1 (IL1) and interleukin 6 (IL6). These cytokines cause fever, chills and behavioral changes [6]. TNF α gene is mapped to the class III region of MHC between HLA-B and DR loci. Several single nucleotide polymorphisms, at TNF α promoter region, effecting TNF α 's transcription level were previously demonstrated [7].

Treatment of CHB with IFN α results in ever-complaining patients. Otherwise healthy patients become ill after IFN α administration. On the other hand, IFN α is the most effective agent in terms of HBsAg clearance which gives it a pivotal role in CHB treatment. This study was planned in order to determine the role of different TNF α promoter polymorphisms in IFN α treatment-related side effects in CHB patients.

MATERIALS and METHODS

This single center, prospective, observational study carried out at Selçuk University Meram Faculty of Medicine Department of Infectious Diseases and Clinical Bacteriology between 2006-2007. Ethics committee of Selçuk University Meram Faculty of Medicine reviewed and approved the study protocol (Decision: 12.02.2006, 2006/043). All patients provided written informed consent before

undergoing study procedures. The study was carried out in accordance with the Declaration of Helsinki and its amendments.

Patients

Fifty CHB patients followed-up at outpatient clinics of Selçuk University Infectious Disease Department were enrolled in this study. CHB diagnosed based on positive HBsAg over 6 months, elevated alanine aminotransferase and aspartate aminotransferase levels and positive antiHBc IgG type antibodies. All patients were screened for HBeAg antigen and antiHBe antibody. Hepatitis C, Hepatitis D, human immunodeficiency virus infection, autoimmune hepatitis, heavy alcohol consumption (over 40g/day) and cirrhosis were exclusion criteria. HBV DNA was measured with real time PCR. All patients underwent liver biopsy. Liver histology was evaluated according to modified Knodells' score. Thyroid function tests were performed before treatment. CHB patients were treated with interferon alpha 2b (Schering-Plough, USA) 10MU tiw plus lamivudine (Glaxo Smith Kline, USA) 100mg/day for 48 weeks.

Patient Follow-up

All patients were followed-up monthly in outpatient clinics of our department for treatment outcome and side effects. During the follow up visit patient was asked to report any adverse effect which he/she encountered since last visit. Furthermore, a questionnaire was applied to all patients to ease their adverse-effect reporting. Questionnaire was consisted of 28 questions interrogating subjective complaints of patients including chills, fever, myalgia, arthralgia, headache, exhaustion, lethargy, loss of appetite, loss of taste, weight-loss, nausea, vomiting, diarrhea, mouth dryness, forgetfulness, insomnia, convulsion, irritability, paresthesia, loss of hair, chest pain, cough, impotency, chest pain, herpes labialis, depressed mood, palpitation, dyspnea, itching. A complaint score was calculated as each patient-stated complaint scored 1 point. Complaint score calculated at 4th, 12th, 24th and 48th week visits. Median complaint scores were calculated and patients were reclassified with respect to this value as complainers and non-complainers. Difference between these two groups with respect to TNF α promoter polymorphisms was investigated. Complete blood count, transaminases, bilirubin, albumin, urea and creatinine values were evaluated at every visit. HBV DNA levels were measured four times a year (4, 12, 24 and 48 weeks). Thyroid

function tests were done when needed. Any complaint, which had any other possible explanation, was excluded. Leucopenia was defined as absolute neutrophil count below 1000/mm³ and thrombocytopenia was defined platelet count below 100000/mm³. Interferon induced thyroiditis was diagnosed with thyroid function tests, positive anti-thyroglobulin, anti-thyroid antibodies, thyroid ultrasonography and, when needed, thyroid scintigraphy. Psychiatric evaluation of patients was done when needed. Depressive mood was a patient defined phenomenon whereas, interferon induced depression was diagnosed and treated by an experienced psychiatrist with respect to DSM IV.

PCR-RFLP of TNF α Promoter

2ml of venous blood samples were drawn from all recruited subjects and stored at 4°C until DNA isolation. Genomic DNA was isolated from peripheral blood leucocytes using standard phenol-chloroform methods. Isolated DNA was kept at -80°C.

Table 1. Outline of TNF α promoter genotyping

Loci	Primer sequences (5' to 3')	PCR Product	Restriction enzyme	Genotype
- 238	F:AGAAGACCCCTCG GAAC R:ATCTGGAGGAAGCGG TAGTG	152bp	MspI	GG:132+20bp GA:152+132+20bp AA:152bp
- 308	F:AGGCAATAGGTTTTGAGGGCCAT R:TCCTCCCTGCTCCGATTCCG	107bp	NcoI	GG:87+20bp GA:107+87+20bp AA:107bp

Gene fragments containing the polymorphic sites were amplified by PCR. PCR was performed in a 20 μ L reaction mixture containing 0.4 μ L DNA Taq polymerase (Fermentas, England), 50 ng genomic DNA, 20 μ L 10 x buffer, and 1.5 mmol/L MgCl₂, 1.2 μ L primers and 10 pmol/L dNTPs. The PCR cycles were as follows: 94°C for 3 minutes, 30 cycles of denaturing at 94 °C for 15 seconds, annealing at 57°C for 15 seconds, extension at 72 °C for 15 seconds, and a single final extension at 72 °C for 3 minutes in Applied Biosystems GeneAmp PCR System model 2700 thermocycler. After amplification, products were identified under ultraviolet light after electrophoresis in 3% agarose gel stained by ethidium bromide (EB). Then PCR products were digested by allele-specific restriction enzymes (NcoI, Fermentas, England) (MspI Promega, USA) overnight at 37 °C. Cleaved DNA fragments were then identified by ultraviolet light after electrophoresis in 3% agarose gel stained by EB. pBR322 DNA/BsuRI (Fermentas, England) DNA ladder was used during analysis. TNF α promoter genotyping was reviewed in Table 1.

Statistical Analysis

Data were evaluated by χ^2 test using SPSS 10.0 programme with $p \leq 0.05$ as statistical significance.

RESULTS

Patient Characteristics

Total 50 patients (38 men, 12 women) were enrolled in this study. Mean age of the study groups was 36.7 \pm 11.8. Two patients were lost in follow-up that is why total 48 patients were included in statistical analyses.

Side Effects

Most common side effect was arthralgia observed

in 93.8% of patients. Exhaustion, loss of appetite, mouth dryness and fever were the leading complaints of the patients. Convulsion was not observed. Complaints of the patients were reviewed at Table 2. Median complaint score was 9, 9.5, 11 and 5 at 4th, 12th, 24th and 48th week visits respectively. TNF alpha promoter 238GG and 308GG allele was present in 42 (84%) and 43 (86%) patients, respectively. No statistically significant difference detected between certain TNF alpha promoter polymorphism and complainer (C) and non-complainer (NC) groups. Results of genotypic analyses are summarized in Table 3.

Table 2: Review of patient reported side-effects in CHB patients receiving interferon alpha 2b

Side Effect	Positive %	Side Effect	Positive %
Arthralgia	93.8	Itching	60.4
Exhaustion	89.6	Paresthesia	58.3
Loss of appetite	85.4	Herpes labialis	52.1
Mouth Dryness	85.4	Insomnia	52.1
Fever	83.3	Nausea	50
Myalgia	83.3	Cough	45.8
Headache	81.3	Chest pain	43.8
Loss of taste	77.1	Impotence	43.8
Weight-loss	75	Depressed mood	33.3
Lethargy	72.9	Dyspnea	29.2
Loss of hair	72.9	Palpitation	25
Chills	68.8	Vomiting	22.9
Irritability	66.7	Diarrhea	20.8
Forgetfulness	62.5	Convulsion	0

Table 3: Prevalence of TNF α promoter polymorphisms in complainer (C) and non-complainer (NC) groups.

TNF α promoter		Month 1		Month 3		Month 6		Month 12	
genotype		C	NC	C	NC	C	NC	C	NC
-238	GG	18	24	24	18	22	20	28	14
	GA	2	6	2	6	3	5	3	5
		p: 0.345		p: 0.095		p: 0.440		p: 0.119	
-308	GG	18	25	23	20	20	23	27	16
	GA	2	5	3	4	5	2	4	3
		p: 0.506		p: 0.602		p: 0.221		p: 0.775	

Hematological Side Effects

Thrombocytopenia and leucopenia diagnosed in 8 (16%) and 5 (10%) patients, respectively. No statistically significant association was present between a hematological side effect and TNF promoter polymorphisms (Table 4).

Table 4: Hematological side effects of interferon alpha 2b with respect to TNF promoter polymorphisms.

TNF α promoter					
	genotype	Thrombocytopenia	Normal	Leucopenia	Normal
-238	GG	7	35	3	39
	GA	1	7	2	6
		p: 0.768		p: 0.123	
-308	GG	7	36	4	39
	GA	1	6	1	6
		p: 0.894		p: 0.684	

Psychiatric Side Effects

One-third of the patients suffered depressive mood but depression was diagnosed in 5 (10%) patients. Patients complaining from depressive mood did not differ from others with respect to TNF α polymorphisms (Table 5).

Table 3. Comparison of risk factors of patients according to complication groups.

TNF α promoter					
	genotype	Depressive Mood	Normal	Depression	Normal
-238	GG	14	27	4	38
	GA	2	5	1	7
			p: 0.772		p: 0.797
-308	GG	13	28	4	39
	GA	3	4	1	6
			p: 0.563		p: 0.684

DISCUSSION

IFN- α , which has both antiviral and immunomodulatory activities, was the first agent approved for the treatment of both HBeAg-positive and HBeAg negative CHB [8]. The main advantage of IFN- α therapy is a finite-duration and sustained off-therapy responses in a proportion of patients with HBeAg-negative CHB [9]. Furthermore, serum half-life of IFN α was boosted as a result of pegylation technology and pegylated forms of IFN α has been widely implemented in CHB treatment [9]. Interferon and pegylated forms are associated with better virological outcomes when compared to nucleoside/nucleotide based antiviral treatment [9]. Conversely, poor tolerability due to frequent and potentially severe side effects is the main drawback of treatment [10]. Side effects of IFN α treatment may lead to dose reduction or premature withdrawals from treatment regime. Side effects of IFN α treatment changes from person to person, suggesting a genetic susceptibility to IFN α induced side effects.

TNF α is cytokine capable with versatile functions. Nevertheless, when TNF α is present in peripheral circulation, it always induces inflammatory phenotype. Despite inflammation is key to overcome infections, number of studies proved the role of TNF α in variety of conditions including autoimmunity [11,12].

Impact of interferon administration in addition to antiviral treatment attracted many scientists all around the world due to unmet needs in CHB treatment [13,14]. It has been shown that addition of IFN α to lamivudine lower the rates of emerging lamivudine resistance [14]. Treatment outcomes

can be affected by treatment duration, definition of treatment success, ethnic background, infection mode, patient demographics as well as virus itself [14]. Nevertheless, all trials have focused on the treatment success rather than focusing on patient reported outcomes and adverse events.

In this trial we focused on patient reported outcomes. To our knowledge this is the first study in literature exploring certain polymorphisms with regard to patient reported outcomes in CHB treatment. We observed arthralgia, exhaustion, loss of appetite and mount dryness in majority of IFN α treated patients. Patient-reported-fever was present in 83.3% of patients. Acute toxicity of IFN α treatment consists of fever, myalgia, headache and chills. Flu like symptoms associated with IFN α therapy is found to be associated with TNF α , IL1 and IL6 secretion [6]. In addition to cytokine activation, prostaglandin E2 induction usually occurs during interferon treatment [15]. Effective suppression of prostaglandin E2 by antipyretic agents may alleviate the symptoms triggered by IFN α administration. In this trial, patients were extensively informed on the possible side effects of IFN α and left free to take paracetamol when needed that is why fever was found lesser than other acute side effects. Fever was effectively controlled with paracetamol treatment, whilst arthralgia was not. In this study we evaluated the role of two different polymorphisms of TNF α gene promoter on IFN α induced side effects. We found that TNF α promoter polymorphisms did not contribute to development of acute interferon related toxicities in CHB patients treated with IFN α .

Our observations in complaint rates were in consistent with previous findings [6,14]. In addition, our results proved complaint-rate was highest at 24th week and lowest at the end of 48th week suggestive of getting-used-to treatment as time passes by. Our findings suggest that TNF α promoter polymorphisms was not associated with complaint-rate at any time point.

Hematological side effects under interferon treatment is common and dose limiting [6]. In patients treated with low dose IFN α grade 3-4 bone marrow toxicity was low, occurring in 10-15% of patients [15]. Thrombocytopenia and anemia gradually increases over time under IFN α treatment. Mechanisms underlying hematological side effects include IFN α induced bone marrow inhibition, maturation block, up-regulation of endothelial adhesion molecules which in turn leads to redistribution of blood cells to other compartments and induction of autoimmunity [6]. In this trial, thrombocytopenia and leucopenia was detected in 16% and 10% of patients, respectively. Hematological manifestations did not lead to premature discontinuations. We found that, TNF α promoter polymorphisms at position -238 and -308 did not contribute to leucopenia and thrombocytopenia. Thrombocytopenia and leucopenia could be resulted because of redistribution rather than TNF mediated myelosuppression under IFN α treatment.

Psychiatric symptoms are common among CHB patients [17] and studies with major depression revealed elevated TNF α levels in the sera of patients suggesting a mild inflammatory response [18]. Elevated serum TNF α levels were found in IFN α induced depression [18]. IFN α treatment may result in cognitive, behavioral and affective disturbances and lead to major depression, concentration difficulties and anxiety [17]. IFN α induced depression was more common among Caucasians when compared to Asian population [19]. Depression and premature discontinuations occur in 4-5% and 6-8% of CHB patients undergoing IFN α treatment, respectively [20]. In this study we found that 16 out of 48 patients complained from depressive mood whereas, only 5 patients (10.4%) were diagnosed to have depression. TNF α -238 and TNF α -308 genotypes did not confer to development of depressive mood

or depression. This finding could be explained by induction of other cytokines rather than TNF α played crucial role in IFN α induced depression.

TNF α levels are subject to control at transcriptional, translational and posttranslational levels. Transcriptional control of TNF α gene can be managed at promoter region of the gene [21]. Some studies showed variable TNF α production upon antigenic stimuli in different promoter genotypes. Polymorphisms at TNF α promoter region -238 and -308, may confer to differential TNF production in-vivo [22, 23]. Certain polymorphisms of TNF α promoter may contribute to viral clearance, response to HBV vaccination and disease severity [21, 24]. In this study we evaluated the role of TNF α promoter polymorphisms in IFN induced side effects. No association was found between TNF α genotype and leucopenia, thrombocytopenia, depression, depressive mood and median complaint values.

Our study has some limitations. First it is a single center study enrolled limited number of CHB patients. Thus, results of our study may not be applicable to diverse clinic settings. Furthermore, we might have overlooked the possible association with TNF α promoter polymorphisms due to limited sample size. As interferon is still the only definitive and finite treatment for CHB and may result in diverse side effects, treating physician desires to foresee the outcome of therapy: Who will respond? Who will fail in IFN treatment? These questions are still waiting their answers. Microbiological factors are known to influence the outcome of the infection but, host factors are still remains obscure. Many studies are needed to clarify the questions: Who will benefit from treatment and who will develop adverse reactions?

ACKNOWLEDGEMENT

Authors are pleased to extent their sincere gratitude to the volunteering patients who contributed to this study. Without their enthusiastic involvement this study was destined to fail.

CONFLICT OF INTEREST

Authors declare no conflict of interest regarding this manuscript



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