Current Research in Microbiology and Biotechnology
Vol. 6, No. 1 (2018): 1400-1404
Research Article
Open Access

ISSN: 2320-2246

Interferon Gamma: A Profile of Gene Polymorphism and Serum Level in HBV and HCV Iraqi Patients

Osama B. Al-Saffar* and Ali H. Ad'hiah

[1] *Department of Biology, Madenat Al-elem University College.*
[2] *Tropical-Biological Research Unit, College of Science, University of Baghdad, Iraq.*

* Corresponding author: Osama B. Al-Saffar; e-mail: osamaalsaffar@gmail.com

Received: 26 November 2017 Accepted: 15 December 2017 Online: 01 January 2018

ABSTRACT

Serum level and gene polymorphism of Interferon gamma (IFN-γ) were investigated in 76 Iraqi Arab hepatitis patients; 38 for each of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, as well as 24 ethnicity, age and gender-matched controls. HCV patients showed a significant decreased level of IFN-γ compared to controls (38.69 ± 13.25 *vs.* 48.41 ± 8.09 pg/ml), and a decreased level was also observed in HBV patients, but the difference was not significant. Distributing patients and controls according to age and gender revealed that there was no significant variation between the age groups < 40 and ≥ 40 years or males and females. For *IFNG* gene polymorphism (*IFNG*$_{+874}$), the distribution of allele and genotypes in patients and controls showed no significant differences, with the excpetion of a significant decreased frequency of TT genotype in HCV patients compared to controls (18.4 *vs.* 45.8%; $p = 0.04$). Finally, no clear impact of *IFNG*$_{+874}$ genotypes on serum level of IFN-γ was observed in patients and controls, but TT genotype of HCV patients reported a significant decreased level of IFN-γ (33.0 ± 9.3 pg/ml) compared to almost all other genotypes in patients or controls.

Keywords: Hepatitis B virus, Hepatitis C virus, IFN-γ, *IFNG*$_{+874}$, Gene Polymorphism.

1. INTRODUCTION

Hepatitis is an inflammation of the liver that is most commonly caused by one of the five types of hepatitis viruses; A, B, C, D and E. These types are of a greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread worldwide; in particular, types B (HBV) and C (HCV) [1].

The balance between virus and host defense defines the course of viral infection and pathogenesis, and persistent viruses such as HBV and HCV are generally not directly cytopathic and have developed immune evasion mechanisms to survive without destroying the host [2]. For the host, the goal is to prevent, eliminate, or at least control viral infection while limiting undue collateral damage. These interactions are influenced by various host genetic, immunological and viral factors [3].

Interferon-γ (IFN-γ), or type II interferon, is a cytokine that is critical for innate and for innate and adaptive immunity against viral, some bacterial and protozoa infections [4]. IFN-γ gene is located on chromosome 12 at position 12q14, and the IFN-γ monomer consists of a core of six α-helices and an extended unfolded sequence in the C-terminal region [5].

When infecting the liver parenchyma, hepatotropic viruses such as HBV or HCV continuously release viral particles into the blood stream [6,7]. The first line of defenses that encounter viruses includes natural killer (NK) cells and natural killer T (NKT) cells, which are abound in the liver. These cells are activated by type-I IFNs (α and β) released by infected liver cells. NK and NKT cells, both can eliminate infected cells, but also constitute a relevant source of IFN-γ and tumor necrosis factor (TNF) alpha [8,9].

From the genetic point of view, the existence together of many forms of DNA sequences (polymorphism) at a locus within a population, or a discontinuous genetic variation may results in different forms or types of individuals among the members of a single species that differ in their immune resonse [10]. In this regard, many studies have examined the relationship between certain cytokine gene polymorphisms (single nucleotide polymorphisms; SNPs), cytokine gene expression, and susceptibility to and clinical severity of diseases [11]. One of these SNPs is *IFNG*$_{+874}$, and its alleles or genotypes have been suggested to effect susceptibility to several human diseases [12,13]. Therefore, the present study was planned to determine the role of IFN-γ in etiopathogenesis of HBV and HCV in terms of serum level and gene polymorphism.

2. MATERIALS AND METHODS

2.1 Subjects

After ethical clearance, the study was carried out at the Gastroenterology and Hepatology Teaching Hospital/Baghdad. The study was carried out on 100 subjects; 76 of them were suffering from viral hepatitis, and were divided into two clinical groups. The first group consisted of 38 HBV patients (20 males and 18 females), and their age ranged between 13–57 years (Mean ± SD: 43.36 ± 10.68) years. The second groups involved 38 HCV patients (13 males and 23 females), and their age ranged between 17-73 years (Mean ± SD: 33.63 ± 15.35 years). A control sample of 24 individuals (6 males and 18 females) was also included in the study, and their age ranged between 17-60 years (Mean ± SD: (39.20 ± 11.32) years. The controls were blood donors and their laboratory profile in the Central Blood Bank (Baghdad) revealed that they were negative for HBV and HCV infections.

From each participating subject, 5 ml were drawn and distributed into plain tube (3 ml) and EDTA tube (2 ml). After isolation of serum, it was tested by ELISA method to detect anti-viral (HBV and HCV) antibodies (Biomerieux HBs Ag HBV kit; France), and if it was positive, the diagnosis was confirmed further by real-time PCR analysis to detect the viral genetic material (COBAS® AmpliPrep/COBAS® TaqMan® HBV and COBAS® AmpliPrep/COBAS® TaqMan® HCV kits; USA). All patients were firstly diagnosed and none of them was under therapy.

2.2 Assessment of IFN-γ serum level

Sera of hepatitis patients and controls were assessed for the level of IFN-γ using a commercially available kit (PeproTech; UK), and the instructions of manufacturer were followed.

2.3 Detection of *IFNG* gene polymorphism

Genomic DNA was extracted from EDTA blood using AccuPrep® Genomic DNA Extraction Kit (Bioneer Corporation, Korea). The polymorphism was detected at one position of the promoter region (*IFNG*$_{+874}$) by polymerase chain reaction-specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% agarose-gel, by using CTS-PCR-SSP Tray Kit (Heidelberg, Germany). The thermocycling conditions were: initial denaturation at 94˚C for 2 minutes, followed by denaturation at 94˚C for 15 seconds, and then 10 cycles of annealing and extension at 65˚C for 60 seconds. This was followed by denaturation at 94˚C for 15 seconds, and then 20 cycles of annealing 61˚C at 50 seconds and extension at 72˚C for 30 seconds.

2.4 Statistical Analysis

Serum level of IFN-γ was given as mean ± SD, and significant differences between means were assessed by ANOVA (Analysis of Variance) followed by either LSD (Least Significant Test) or Duncan using the computer software SPSS (Statistical Package for Social Sciences) version 13.

Genotypes of IFN-γ were presented as percentage frequencies, and significant differences between their distributions in hepatitis patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, the relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between a genotype with the disease. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com

3. RESULTS AND DISCUSSION

3.1 Serum Level of IFN-γ

HCV patients showed a significant decreased level of IFN-γ compared to controls (38.69 ± 13.25 *vs*. 48.41 ± 8.09 pg/ml), and a decreased level was also observed in HBV patients (43.96 ± 9.64 *vs*. 48.41 ± 8.09 pg/ml), but the difference was not significant. pg/ml).Distributing patients and controls into two age groups (< 40 and ≥ 40 years) revealed that serum level of IFN-γ showed showed no significant difference between these age groups in patients or controls. Similarly, there was no significant difference between males and females in each group of patients or controls. However, male HCV patients showed a significant decreased level of IFN-γ (34.87 ± 10.90 pg/ml) compared to male HBV patients (45.82 ± 10.90 pg/ml) or controls (45.42 ± 7.02 pg/ml) (Table 1).

The present results demonstrate that IFN-γ serum level was down-regulated in viral hepatitis infection, especially HCV, and such finding confirms the protective role of IFN-γ against viral hepatitis. This is reasoned by the fact that IFN-γ is an important pro-nflammatory cytokine that has antiviral activity, and an experimental evidence declared that such cytokine can inhibit HCV infection [14]. Further data suggest a strong IFN-γ-mediated antiviral natural killer cell response is associated with a self-limited course of acute HCV in human immunodeficiency virus-positive patients [15]. However, the present study results demonstrated that neither age nor gender was of a significance in establishing the role of IFN-γ in pathogenesis of HBV and HCV. Both subjects (age and

gender) have been a matter of a controversy; therefore, it might be too-early to reach a final conclusion about this subject, and the differences between studies might be related to sample size, race and disease heterogeneity [16, 17,18, 19, 20,21,22].

Table 1: Serum level of IFN-γ and in hepatitis B and C patients and controls distributed by age group and gender.

Groups	IFN-γ Serum Mean Level ± SD (pg/ml)		
	Patients		Controls (No.= 24)
	Hepatitis B (No.= 38)	Hepatitis C (No.= 38)	
Total	43.96 ± 9.64^{AB}	38.69 ± 13.25^{B}	48.41 ± 8.09^{A}
< 40 years	43.68 ± 7.63^{AB}	38.55 ± 12.64^{B}	49.00 ± 7.30^{A}
≥ 40 years	44.38 ± 12.71^{AB}	38.76 ± 13.81^{B}	47.82 ± 9.10^{A}
p	N.S.	N.S.	N.S.
Males	45.82 ± 10.90^{A}	34.87 ± 10.90^{B}	45.42 ± 7.02^{A}
Females	41.85 ± 7.80^{A}	41.18 ± 13.72^{A}	49.41 ± 8.36^{A}
p	N.S.	N.S.	N.S.

Different superscript letters: Significant difference (P ≤ 0.05) between means of rows.
p: Probability of difference between males and females of each group.
N.S.Not significant ($p > 0.05$)

3.2 Genetic Polymorphism of *IFNG* Gene

The genetic polymorphism of *IFNG* gene was determined at position $IFNG_{+874}$, which was presented with three genotypes (AA, AT and TT) in HBV and HCV patients and controls. Analysis of Haedy-Weinberg equilibrium (HWE) revealed that HBV and HCV patients, as well as controls were in a good agreement with HWE, and no significant variation between the observed and expected genotype frequencies was observed. Also, comparing HBV to controls revealed that none of the genotypes or alleles showed a significant difference between patients and controls, but HCV patients contradicted such theme. They were observed to have a significantly decreased frequency of TT genotype (18.4 vs. 45.8%; $p = 0.04$), with a PF value of 0.33 (Tables 2, 3, 4 and 5).

The present analysis of $IFNG_{+874}$ genotypes in HBV and HCV patients revealed no association with HBV infection, while in HCV infection, a protective effect might be suggested by the TT genotype. These findings are not consistent with previous studies that considered $IFNG_{+874}$ polymorphism as marker for HBV infection [23]. However, in agreement with the present study, Cheong *et al*. (2006) found no significant association between $IFNG_{+874}$ genotypes or alleles and HBV infection. Such differences could be predominantly attributed to different population investigated and gene loci analyzed.

However, Gao *et al*. (2010) mentioned that the polymorphism in $IFNG_{+874}$ if not associated with either infection, it may influence the chronicity and outcome of HCV and/or HBV infection, but in an Iranian study, Sarvari *et al*. (2014) suggested that *IFNG* gene polymorphism at $IFNG_{+874}$ and perhaps $IFNG_{-183}$ loci do not seem to have any effect on the outcome of therapy in patients with HCV infection in general population. However, in a further recent study, it has been found that $IFNG_{+874}$ TT genotype and *T* allele was associated with a reduced risk of HBV infection in an Asian population [27].

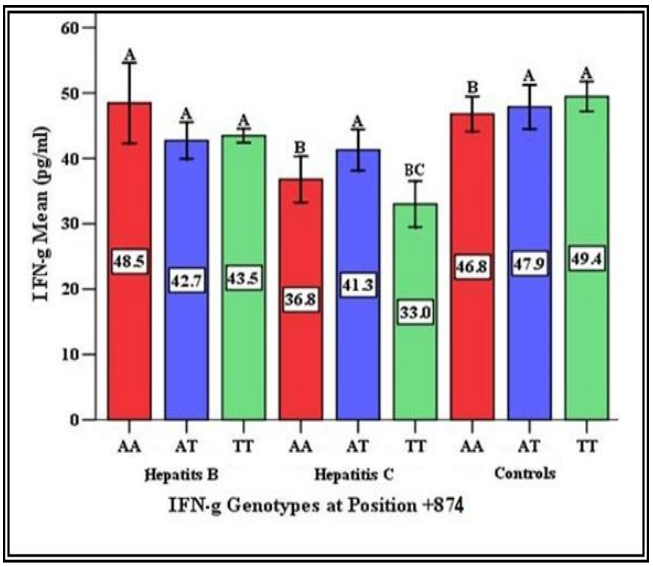

Figure 1: Serum level of IFN-γ in hepatitis B and C patients and controls distributed by $IFNG_{+874}$ genotypes. (Different superscript capital letters: Significant difference ($p \leq 0.05$) between means of bars).

Table 2: Observed numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of *IFNG*$_{+874}$ genotypes and alleles in hepatitis B patients and controls.

Groups			*IFNG*$_{+874}$ Genotype or Allele					HWE
			AA	AT	TT	*A*	*T*	*p* ≤
Hepatitis B (No. = 38)	Observed	No.	6	16	16	28	48	Not significance
		%	15.7	42.1	42.1	36.8	63.1	
	Expected	No.	5.1	17.6	39.8	Not Estimated		
		%	13.5	46.5	39.8			
Controls (No. = 24)	Observed	No.	4	9	11	17	31	Not significance
		%	16.6	37.5	45.8	35.4	64.5	
	Expected	No.	3.0	10.9	10.0	Not Estimated		
		%	12.5	45.7	41.7			

Table 3: Statistical evaluations of associations between *IFNG*$_{+874}$ genotypes or alleles and hepatitis B infection.

IFNG$_{+874}$ Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
AA	0.94	0.001	Not significance	0.24-3.65
AT	1.33	0.11	Not significance	0.47-3.76
TT	0.86	0.06	Not significance	0.31-2.36
A	1.06	0.02	Not significance	0.50-2.24
T	0.94	0.04	Not significance	0.45-1.98

Table 4: Observed numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of *IFNG*$_{+874}$ genotypes and alleles in hepatitis C patients and controls.

Groups			*IFNG*$_{+874}$ Genotype or Allele					HWE
			AA	AT	TT	*A*	*T*	*p* ≤
Hepatitis C (No. = 38)	Observed	No.	9	22	7	40	36	Not significance
		%	23.6	57.8	18.4	52.6	47.3	
	Expected	No.	10.5	18.9	8.5	Not Estimated		
		%	27.7	49.8	22.4			
Controls (No. = 24)	Observed	No.	4	9	11	17	31	Not significance
		%	16.6	37.5	45.8	35.4	64.5	
	Expected	No.	3.0	10.9	10.0	Not Estimated		
		%	12.5	45.7	41.7			

Table 5: Statistical evaluations of associations between *IFNG*$_{+874}$ genotypes or alleles and hepatitis C infection.

IFNG$_{+874}$ Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
AA	1.55	0.08	Not significance	0.43-5.60
AT	2.29	0.32	Not significance	0.82-6.41
TT	0.27	0.33	0.04	0.09-0.82
A	2.03	0.26	Not significance	0.97-4.23
T	0.49	0.32	Not significance	0.24-1.03

3.3 Genotype Impact on IFN-γ Level

No clear impact of *IFNG*$_{+874}$ genotypes on serum level of IFN-γ in HBV and HCV patients or controls was observed, but the TT genotype of HCV patients reported a significant decreased level of IFN-γ (33.0 ± 9.3 pg/ml) compared to almost all other genotypes in patients or controls (Figure 1).

In disagreement with the present results, Pravica *et al*. (2000) reported that the *IFNG*$_{+874}$ TT genotype was often associated with high IFN-γ production, while *IFNG*$_{+874}$ AA genotype was regarded as low producer in normal subjects. However, Ben-Ari *et al*. (2003) reported that AA genotype of *IFNG*$_{+874}$ was associated with low IFN-γ level in patients with chronic HBV infection compared to controls; an observation that was also shared by the present study, in which patients with the AA genotype were observed with a low level of IFN-γ compared to the corresponding controls (36.8 *vs*. 46.8 pg/ml). However, Conde *et al*. (2013) did not find a correlation between *IFNG*$_{+874}$ genotypes and IFN-γ level in chronic HBV patients. These differences could

be related the virus strain, as well as racial variation may also have its impact as these polymorphisms show different frequencies in different ethnic communities [31].

4. CONCLUSION

The serum level of IFN-γ was downregulated in hepatitis C patients. However, gene polymorphism at position might have no effect on susceptibility of HBV and HCV infections.

Acknowledgments

We thank all our friends for the critical support of the manuscript.

5. REFERENCES

1. WHO, (2016). Hepatitis. Retrieved 2016-6-20.
2. Bertoletti, A. and Gehring, A. (2006). The immune response during hepatitis B virus infection. J Gen Virol 87:1439-1449.
3. Saxena, R., Chawla, Y.K., Verma, I. and Kaur, J. (2013). IFN-γ (+874) and not TNF-α (-308) is associated with HBV-HCC risk in India. Mol. Cell Biochem 385:297-307.
4. Apte, S., Baz, A., Kelso, A. and Kienzle, N. (2008). Interferon-gamma and interleukin-4 reciprocally regulate CD8 expression in CD8+ T cells. Proc. Natl. Acad. Sci., 105(45):17475–17480.
5. Schoenborn, J. and Wilson, C. (2007). Regulation of interferon-gamma during innate and adaptive immune responses. Adv. Immunol., 96:41–101.
6. Su, A., Pezacki, J., Wodicka, L., Brideau, A., Supekova, L. and Thimme, R. (2002). Genomic analysis of the host response to hepatitis C virus infection. Proc. Natl. Acad. Sci. 99:15669-15674.
7. Chisari, F. V. and Ferrari, C. (2005). Hepatitis B virus immunopathogenesis. Ann. Rev. Immunol., 13:29-31
8. Guidotti, L. and Chisari, F. (2001). Noncytolitic control of viral infections by the innate and adaptative immune response. Annu. Rev. Immunol., 19:65-91.
9. Yang, Y., Qiu, Q., Yu, H., Zeng, Y. and Bei, H. (2012). TNF-α-863 polymorphisms and the risk of hepatocellular carcinoma. Exp. Ther. Med., 3(3):513-518.
10. Dai, C.Y., Chuang, W.L. and Lee, L.P. (2008). Association between transforming growth factor–beta 1 polymorphism and virologic characteristics of chronic hepatitis C. Translation Res., 152(4):151-156
11. Ulger, M., Emekdas, G., Aslan, G., et al. (2014). Determination of the cytokine gene polymorphism and genetic susceptibility in tuberculosis patients. Microbiol. bul., 47(2):250-264.
12. Bouzgarrou, N., Hassen, E. and Farhat, K., (2009). Combined analysis of interferon-γ and interleukin-10 gene polymorphisms and chronic hepatitis C severity. Hum. Immunol., 70:230-236
13. Wang, D., Zhong. X., Huang, D., Chen, R., Bai, G., (2014) Functional Polymorphisms of Interferon-gamma Affect Pneumonia-Induced Sepsis. PLoS ONE 9(1): e87049
14. Wei, X., Jia, Z., Lian, J., Zhang, Y., Li, J., Ma, L., Ye, L., Wang, J., Pan, L., Wang, P. and Bai, X. (2009). Inhibition of hepatitis C virus infection by interferon-gamma through downregulating claudin-1. J Interferon Cytokine 29(3):171-178.
15. Kokordelis, P., Krämer, B., Körner, C., Boesecke, C., et al. (2014). An effective interferon-gamma-mediated inhibition of hepatitis C virus replication by natural killer cells is associated with spontaneous clearance of acute hepatitis C in human immunodeficiency virus-positive patients. Hepatology. 59(3):814-827.
16. Gregg, R., Smith, C., Clark, F., et al. (2005). The number of human peripheral blood CD4+ CD25 high regulatory T cells increases with age. Clin Exp Immunol. 140:540-546.
17. Pawelec G. (2007). Immunosenescence comes of age. Symposium on Aging Research in Immunology: The Impact of Genomics. EMBO Rep. 8:220-223
18. Dur, M., Günter, b., Michaela, A., Stoffer e., V. Fialka-Moser, f., Alexandra, Kautzky-Willer, g., Clemens, D., Cem Ekmekcioglui, Birgit, j., Alexa, B., Josef, Smolenb, k., and Tanja, S. (2016). Initial evidence for the link between activities and health: Associations between a balance of activities, functioning and serum levels of cytokines and C-reactive protein. J. psyneuen. 65:138-148
19. Goetzl, E., Huang, M., Kon, J., Patel, K., Schwartz, J., Fast, K., Ferrucci, L., Madara, K., Taub, D. and Longo, D. (2010). Gender specificity of altered human immune cytokine profiles in aging. FASEB J., 24:3580-3589.
20. Poveshchenko, A., Orlov, N., Kazakov, O., Poveshchenko, O. Kim, I., Bondarenko, N., Miller, T., Strunkin, D., Kabakov, A., Reiter, T. and Konenkov, V. (2014). Age and Gender Differences in Cytokine Profile of Lymph and Blood Serum. Adv Aging Res, 3:216-221.
21. Kim, O., Kim, H., Youn, J., Shin, E. and Park, S. (2011). Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. J Transl Med., 9:113
22. Kleiner, G., Marcuzzi, A., Zanin, V., Monasta, L. and Zauli, G. (2013). Cytokine Levels in the Serum of Healthy Subjects. Mediators Inflamm., 2013:20-26
23. Zhou, J., Chen, D.Q., Poon, V.K., Zeng, Y., Ng, F. and Lu, L. (2006). A regulatory polymorphism in interferon-gamma receptor 1 promoter is associated with the susceptibility to chronic hepatitis B virus infection. Immunogenetics. 61:423-430
24. Cheong, J., Cho, S., Chung, S., Lee, J., Yeo, M., and Wang, H. (2006). Genetic polymorphism of interferon-gamma, interferon-gamma receptor, and interferon regulatory factor-1 genes in patients with hepatitis B virus infection. Biochem Genet., 44:246-255.
25. Gao, Q., Liu, D., Zhang, S., Jia, M. and Wu, L. (2010). Association between IFN-gamma+874 polymorphisms and the clinical outcomes of hepatitis B and/or hepatitis C virus infection. Zhonghua Liu Xing Bing Xue Za Zhi. 31(3):324-328
26. Sarvari, J., Norozian, H., Fattahi, M., Pirbonyeh, N. and Moattari, A. (2014). The Role of Interferon Gamma Gene Polymorphism (+874A/T, +2109A/G, and -183G/T) in Response to Treatment Among Hepatitis C Infected Patients in Fars Province, Southern. Iran Hepat. Mon., 14(1):16-20
27. Sun, X., Wu, J. and Tang, K. (2014). The interferon-gamma (IFN-γ) +874T allele reduces the risk of hepatitis B infection in an Asian population. J Viral Hepat., 4:281-287.
28. Pravica, V., Perrey, C., Stevens, A., Lee, J. and Hutchinson, I. (2000). A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. Hum. Immunol., 61:863-866.
29. Ben-Ari, Z., Mor, E., Papo, O., Kfir, B., Sulkes, J. and Tambur, A. (2003). Cytokine gene polymorphisms in patients infected with hepatitis B virus. Am J Gastroenterol., 1:144-150.
30. Conde, S. R., Feitosa, R. N., Freitas, F. B., Hermes, R. B., Demachki, S., Araujo, M. T., Soares, M. C., Ishak, R. and Vallinoto, A. C. (2013). Association of cytokine gene polymorphisms and serum concentrations with the outcome of chronic hepatitis B. Cytokine, 61:940-944.
31. Tunçbilek, S. (2014). Relationship between cytokine gene polymorphisms and chronic hepatitis B virus infection. World J Gastroenterol., 20:6226-6235.

*****