The TNFA -308G>A and IL10 -1082A>G Polymorphisms as Predictive Biomarkers of Chronic HCV Infection

Angélica Menezes Santiago

Universidade Federal do Para

Ednelza da Silva Graça Amoras

Universidade Federal do Para

Maria Alice Freitas Queiroz

Universidade Federal do Para

Simone Regina Souza da Silva Conde

Universidade Federal do Para

Izaura Maria Vieira Cayres-Vallinoto

Universidade Federal do Para

Ricardo Ishak

Universidade Federal do Para

Antonio C. R. Vallinoto (✉ vallinoto@ufpa.br)

Universidade Federal do Pará <https://orcid.org/0000-0003-1135-6507>

Research

Keywords: HCV, TNF- α , IL-10, polymorphism

Posted Date: August 31st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-64090/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Genetic changes may induce dysregulated cytokine production and affect the progression of the chronic disease caused by the hepatitis C virus (HCV) because the balance of pro- and anti-inflammatory cytokines determines the outcome of infection. This study evaluated the *TNFA*-308G>A and *IL 10*-1082A>G polymorphisms in the susceptibility and progress of chronic hepatitis C

Methods: The study included 101 samples from patients with chronic hepatitis C and 300 samples from healthy donors. Polymorphisms were typed by real-time PCR and were analyzed for associations with histopathological parameters (according to METAVIR classification) and HCV viral load.

Results: The polymorphic genotype for the *TNFA*-308G>A variant was not present in the group of patients with chronic hepatitis C and was associated with protection against HCV infection ($p = 0.0477$). Patients with the polymorphic genotype of the *IL 10*-1082A>G polymorphism had higher HCV viral load than wild-type patients ($p = 0.0428$). Neither polymorphism was associated with different levels of necroinflammatory activity or fibrosis scores.

Conclusion: The polymorphic genotype at *TNFA*-308G>A protected against chronic HCV infection, and the polymorphic genotype at the *IL 10*-1082A>G variant was associated with viral persistence.

Background

Hepatitis C virus (HCV) is the causative agent of hepatitis C and is considered the main cause of liver cancer. It is estimated that over 150 million people are chronically infected by the virus, among whom the 20-year risk of cirrhosis is 15–30% [1]. Persistent HCV infection leads to chronic hepatitis, which is mainly due to the inability of the immune system to eliminate the virus [2]. Dysregulated cytokine production is related to the chronicity of hepatitis C; however, no profile of cytokines involved in the development of liver injury has been identified [3].

Tumor necrosis factor (TNF- α) is a pro-inflammatory cytokine that acts both as a mediator of innate immunity and in the cellular immune response. Abnormal TNF- α levels have been associated with chronic HCV infection [4]. Some polymorphisms in the *TNFA* gene are associated with the regulation of cytokine production and coincide with the binding regions of transcription factors [5]. The *TNFA* - 308G > A polymorphism has higher transcriptional activity than the wild-type allele [6] and has been associated with different infectious diseases [7–9].

Interleukin (IL)-10 is a potent suppressor of the effector function of T cells, natural killer (NK) cells and, mainly, activated macrophages [10]. Several functional polymorphisms have been described in the promoter region of the *IL 10* gene [11], among which the *IL 10*-1082A > G polymorphism promotes changes in cytokine levels, with the A allele being related to lower levels and the G allele with higher levels of IL-10 [12]. This polymorphism has been associated with chronic and infectious diseases [13, 14].

Because the liver is a highly immunotolerant organ, an imbalance of the components related to its suppressor and effector functions may contribute to the persistence of HCV and the progression of chronic cases of hepatitis C. On this background, the present study investigated the influence of the *TNFA* - 308G > A and *IL 10*-1082A > G polymorphisms on the susceptibility to chronic HCV infection, the progression to different disease stages, and viral persistence.

Material And Methods

Study population

The study included patients treated at the liver disease outpatient clinics of the Santa Casa de Misericórdia do Pará Foundation and the João de Barros Barreto University Hospital. Consecutive patients with chronic HCV were included. The HCV group consisted of 101 patients with chronic hepatitis C, characterized by clinical changes, abnormal liver tests and HCV RNA positivity.

The inclusion criteria adopted for the individuals were as follows: age 18 or older, both sexes, and positivity for HBsAg for more than 6 months or positivity for HCV RNA. Individuals coinfecting with hepatitis B virus (HBV), hepatitis delta virus, or human immunodeficiency virus (HIV) were excluded from the study.

A control group was formed to compare the genotypic and allelic frequencies of the investigated polymorphisms, which included 300 blood samples from volunteer donors from the Foundation Center for Hemotherapy and Hematology of Pará (Fundação Centro de Hemoterapia e Hematologia do Pará). These volunteers were matched with the patient group for age and sex; were seronegative for HCV, HBV, HIV-1, human T-lymphotropic virus 1/2; and patients who used or were using specific antiviral therapy against HCV.

DNA extraction

DNA was extracted from peripheral-blood leukocytes using the Puregene kit (Gentra Systems, Minneapolis, Minnesota, USA) according to the manufacturer's protocol. The procedure included the steps of cell lysis, protein precipitation, DNA precipitation, and DNA hydration.

Genotyping

Polymorphisms were genotyped by quantitative real-time polymerase chain reaction in the StepOne PLUS Sequence Detector (Applied Biosystems, Foster City, CA, USA). The assay used for each polymorphism contained a pair of primers and a pair of VIC- and FAM-labeled probes for the respective alleles. For both polymorphisms, predesigned and customized TaqMan® SNP Genotyping Assays were used: C_7514879_10 for *TNFA*-308G>A and C_1747360_10 for *IL 10*-1082A>G (Thermo Fisher, Carlsbad, California, USA). For each reaction, 2X TaqMan® Universal PCR Master Mix, 1X TaqMan® Assay (diluted from 20X), and 20 ng of DNA was used in a final reaction volume of 10 µL. The following temperature

cycle was used for the amplification: 60 °C for 30 seconds, 95 °C for 10 minutes, and 50 cycles of 92 °C for 30 seconds and 60 °C for 1 minute and 30 seconds.

Complementary exams

All selected patients were clinically evaluated and subjected to a complimentary investigation consisting of hematological, biochemical, serological, virological, and ultrasound examinations. These data were transcribed from the medical records into a form developed specifically for this study.

Histopathological procedures

Liver biopsy specimens were obtained only from patients with medical indications for the investigation of liver parenchyma changes, in compliance with the clinical care protocol. The liver biopsies were performed by a medical professional from one of the study hospitals using a Tru-Cut needle under ultrasound guidance. The sample was sent to the Department of Pathological Anatomy of Federal University of Pará, where they were examined following the department's routines, which included hematoxylin–eosin (HE), chromotrope aniline blue (CAB), Gomori's reticulin, and Shikata's orcein staining.

The histopathological diagnosis followed the METAVIR classification [15], which classifies the activity of the portal and periportal inflammatory infiltrate from 0 to 3 (A0-A3), A0-A1 indicating absent to mild inflammation and A2-A3 indicating moderate to severe inflammation. The structural changes in the liver parenchyma (degree of fibrosis) were classified from 0 to 4 (F0-F4), F0-F1 indicating absent to mild liver fibrosis, F2 indicating moderate liver fibrosis, and F3-F4 indicating liver fibrosis that has progressed to cirrhosis. All data regarding the histopathological profile were obtained from the patients' medical records.

Statistical analysis

Hardy-Weinberg equilibrium analysis was performed in all samples through the chi-squared test. Comparative analyses of the allelic and genotypic frequencies were done using the G-test and chi-squared test. Comparisons of viral load levels (HCV RNA) were done using the Kruskal-Wallis test and the Mann-Whitney test. Statistical analyses were done with BioEstat software version 5.3 [16], adopting a significance level of $p < 0.05$.

Results

Most patients with chronic HCV were male ($n = 52$; 51%). The median alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase concentrations were 77.9 UI/L, 69.9 UI/L, and 99.6 UI/L, respectively. The mean viral load of 5.4 log (Table 1).

Table 1
Characterization of patients with chronic HCV.

Variable	HCV
Sex (F/M)	49/52
ALT (IU/L), median/IQR	77.9 ± 58.4
AST (IU/L), median/IQR	69.9 ± 48.6
GGT (IU/L), median/IQR	99.6 ± 95.2
Viral load (log ₁₀), median/IQR	5.4 ± 0.9
Fibrosis score	
0 to 2	67 (66.3)
3 to 4	34 (33.7)
Inflammatory activity	*
0 to 1	54 (60)
2 to 3	36 (40)
ALT: alanine aminotransferase (reference value: 16–40 IU/L); AST: aspartate aminotransferase (reference value: 8–54 IU/L); GGT: gamma-glutamyltransferase (reference value: 8–63 IU/L). *Inflammatory activity n = 90.	

According to the METAVIR classification (Table 1), most patients with HCV had absent to moderate fibrosis, F0-F2 (n = 67; 66.3%). Inflammatory activity was evaluated only in 90 patients because 11 were diagnosed with liver cirrhosis by imaging tests and therefore did not meet the medical indications for biopsy. The majority of the evaluated patients presented with absent or mild inflammatory activity, A0-A1 (n = 54; 60%).

The evaluation of the *TNFA* - 308G > A polymorphism showed that no patient with chronic HCV had the homozygous polymorphic genotype (AA). The comparison of genotypic frequencies showed a significant difference between the HCV group and control group (p = 0.0477). However, no significant differences were found in allelic frequencies (Table 2).

Table 2
Genotypic and allelic frequencies of the *TNFA* – 308G > A and *IL 10*-1082A > G polymorphisms in the study groups.

Allelic or Genotypic Profile	HCV n (%)	GC n (%)	<i>p</i>
TNFA - 308G > A			
GG	82 (81.2)	222 (74)	0.0477*
GA	19 (18.8)	69 (23)	
AA	0 (0)	9 (3)	
*G	0.91	0.86	0.3753**
*A	0.09	0.14	
IL10 -1082A > G			
AA	62 (61.4)	164 (54.7)	0.4847**
AG	32 (31.7)	109 (36.3)	
GG	7 (6.9)	27 (9)	
*A	0.77	0.73	0.6242**
*G	0.23	0.27	
HCV: patients with chronic hepatitis C; CG: control group; *G-test; **chi-squared test.			

At the *IL 10*-1082A > G polymorphism, the wild-type genotype and allele (AA and A, respectively) were the most frequent in the HCV and control groups, with no significant differences between the groups (Table 2).

The evaluation of the frequencies of genotypes and alleles of *TNFA* – 308G > A and *IL 10*-1082A > G in relation to histopathological markers of inflammatory activity and fibrosis score showed no significant differences (Table 3).

Table 3

Genotypic and allelic frequencies of the *TNFA* – 308G > A and *IL 10*-1082A > G polymorphisms in relation to the histopathological aspects of chronic HCV carriers.

Genetic profile	Inflammatory activity*		Fibrosis score*			
	0 to 1	2 to 3	p	0 to 2	3 to 4	p
	n (%)	n (%)		n (%)	n (%)	
TNFA – 308G > A						
GG	43 (79.6)	30 (83.3)	0.8688	54 (80.6)	28 (82.4)	0.9553
GG	11 (20.4)	6 (16.7)		13 (19.4)	6 (17.6)	
AA	0	0		0	0	
*G	0.90	0.92	0.8048	0.90	0.91	1.000
*A	0.10	0.08		0.10	0.09	
IL10 -1082A > G						
AA	31 (56.4)	22 (62.9)	0.7530	40 (59.7)	22	0.6839
AG	20 (36.4)	10 (28.6)		23 (34.3)	9	
GG	4 (7.2)	3 (8.5)		4 (6.0)	3	
*A	0.75	0.77	0.8685	0.77	0.78	1.000
*G	0.25	0.23		0.23	0.22	
*G-test; **chi-squared test.						

The analysis of viral load showed no significant difference between patients with different *TNFA* – 308G > A genotypes. However, patients with the polymorphic genotype of *IL 10*-1082A > G had higher HCV viral load than those with the wild-type genotype (p = 0.0428; Fig. 1).

Discussion

The course and outcome of HCV infection are determined by its virological characteristics and the immune responses triggered by the virus [17]. HCV is a hepatotropic virus that induces the development of acute and chronic necroinflammatory disease, escaping the immune system in up to 85% of cases [3].

Several cytokines play dual roles in viral infection and are responsible for viral clearance and tissue damage [18]. TNF- α is an important cytokine in the immune response, mediating the inflammatory process through innate immunity pathways and activation of the cellular response, which induces apoptosis or necrosis [19]. Thus, genetic variations in the *TNFA* gene that alter cytokine production levels may contribute to the progression of HCV infection.

In the present study, the polymorphic genotype for the *TNFA* – 308G > A variant was not present in the group of patients with HCV. This genotype is correlated with increased expression of the cytokine [6]. In this case, the presence of the homozygous allele may contribute to better immune control, preventing the progression of HCV infection. High levels of TNF- α increase the expression of vascular endothelial adhesion molecules and increase the stimulation of endothelial cells and macrophages, which may lead to better infection resolution [18].

The high frequency of the wild-type allele (G) in the group of patients with chronic HCV infection suggests that in addition to having a higher risk of developing the infection, these patients seem to have a greater chance of developing the chronic form of the disease. The inadequate production of TNF- α by dendritic cells favors the differentiation of CD4⁺ T cells into IL-10- and non-IFN- γ -producing cells [20]. As the IL-10 cytokine is not effective in resolving the infection, the infection progressed to the chronic form.

Studies on the *TNFA* – 308G > A polymorphism performed in other ethnic groups also observed different frequencies of the polymorphic genotype in patients with HCV than without, showing that although the presence of the homozygous polymorphism was not observed in patients from France [21], in India the prevalence of the polymorphism was higher in the group of patients with HCV [22]. As the population evaluated in this study is trihybrid, formed from the genetic contributions of whites, blacks, and indigenous people [23], the association of the polymorphism with the prevention or risk of HCV infection needs to be better investigated in other ethnic groups.

IL-10 is an anti-inflammatory cytokine produced by Th2 cells that inhibits the activity of Th1, NK, and macrophage cells, the main cells responsible for pathogen elimination. The cytokine acts by limiting the marked pro-inflammatory response and damage caused by inflammation [10]. In infectious processes, there is a direct correlation between lower IL-10 production and greater disease severity [24].

The *IL 10*-1082A > G polymorphism is associated with changes in IL-10 level, the wild-type genotype being associated with lower levels [12]. In the present study, no differences in genotype frequencies were found between the groups with and without HCV infection. The evaluation of this polymorphism in HCV infection by other studies has shown different results. Although the present results were similar to those of another study, which also did not find an association between the frequency of the *IL 10*-1082A > G polymorphism and susceptibility to HCV infection [25]. Ramos et al. (2012) observed that the GG (polymorphic) genotype was associated with increased chances of viral infection resolution [26]. The combined analysis of these results shows that the polymorphism does not influence the protection from

or susceptibility to HCV infection but can influence the disease resolution, reducing the chances of progression to the chronic form among those who develop hepatitis C.

The polymorphic genotype (GG) of the *IL 10*-1082A > G variant was associated with higher HCV viral load than the wild-type genotype. Most studies that investigated this polymorphism in HCV infection did not assess viral load levels. In the study by Abbas et al. (2005), no difference in viral load was observed between genotypes in patients from Pakistan [27]. Viral load has been associated with the frequency of the homozygous genotype (AA) and that of the wild-type (A) allele [28]. The divergence between the results of that study and the present study may be related to the type of analysis performed, Gao et al. (2009) evaluated the frequencies of genotypes in relation to the presence or absence of HCV RNA, while the present study evaluated the absolute plasma levels, which were converted to their base-10 logarithm [28].

The polymorphic genotype (GG) for *IL 10*-1082A > G is associated with higher IL-10 expression. This cytokine inhibits the activation of Th1, NK, and macrophage cells, which are the main cells responsible for the elimination of HCV; higher levels of IL-10 reduce inflammatory activity at the infection site, favoring the persistence of the virus in the tissue, the main characteristic of chronic infection [2] (Chigbu et al. 2019). In this sense, our findings suggest that the polymorphic genotype may favor the persistence of HCV in the liver tissue.

The *TNFA* – 308G > A and *IL 10*-1082A > G polymorphisms were not associated with different levels of necroinflammatory activity or with fibrosis score. Several studies have also found no relationship between these polymorphisms in the *TNF* and *IL 10* genes and different stages of the disease [21, 25, 29, 30]. Thus, these polymorphisms seem not to influence the progression of the histopathological processes of chronic HCV infection because in this disease, in addition to the host immunological factors, others factors inherent to the virus act directly on the inflammatory process, causing tissue damage.

Conclusions

In summary, the polymorphic genotype at *TNFA* – 308G > A was not present in the group of patients with chronic hepatitis C and conferred protection against HCV infection, while the polymorphic genotype for variant *IL 10*-1082A > G was associated with viral persistence.

Declarations

Authors' contributions

Concept and design: ACRV, IMVCV, SRSSC and RI. Financial support: ACRV; Provision of study materials or patients: SRSSC; Collection and assembly the data: SRSSC and AMS; Data analysis and interpretation: AMS, EGSA and MAFQ; Manuscript writing: AMS, MAFQ, EGSA, IMVC and ACRV; Final approval of the manuscript: ACRV, RI, AMS, MAFQ, EGSA, IMVCV and SRSC.

Funding

This study was funded by the National Council for Scientific and Technological Development (CNPq #480128/2013-8) and by the Federal University of Pará (PROPESP/PAPQ/2020).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present project was submitted to and approved by the research ethics committees of Santa Casa de Misericórdia do Pará (opinion 772,782/2014) and João de Barros Barreto University Hospital (opinions 962,537/2015 and 2,165,948/2017), in accordance with the principles of the Declaration of Helsinki.

Informed consent

All participants were informed about the objectives of the study, and those who agreed to participate signed an informed consent form and answered an epidemiological questionnaire.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. WHO. World Health Organization
WHO. World Health Organization. Hepatitis B. 2014. Disponível em:
<http://who.int/mediacentre/factsheets/fs204_Jul2014/en/>. Accessed in 03 March 2020.
2. Chigbu DI, Loonawat R, Sehgal M, Patel D, Jain P. Hepatitis C Virus Infection: Host-Virus Interaction and Mechanisms of Viral Persistence. *Cells*. 2019;8(4):376. doi:10.3390/cells8040376.
3. Fallahi P, Ferri C, Ferrari SM, Corrado A, Sansonno D, Antonelli A. Cytokines and HCV-related disorders. *Clin Dev Immunol*. 2012;2012:468107. doi:10.1155/2012/468107.
4. Kusumoto, Kusumoto K, Uto H, Hayashi K, Takahama Y, Nakao H, Suruki R, et al. Interleukin-10 or tumor necrosis factor-alpha polymorphisms and the natural course of hepatitis C virus infection in a hyperendemic area of Japan. *Cytokine*. 2006;34(1–2):24–31.
5. Abraham LJ, Kroeger KM. Impact of the – 308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J Leukoc Biol*. 1999;66(4):562–6.

6. Kroeger KM, Carville KS, Abraham LJ. The – 308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol Immunol*. 1997;34(5):391–9.
7. Cabrera M, Shaw MA, Sharples C, Williams H, Castes M, Convit J, Blackwell JM. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. *J Exp Med*. 1995;182(5):1259–64.
8. Pabalan N, Chaisri S, Tabunhan S, Tarasuk M, Jarjanazi H, Steiner T. Associations of tumor necrosis factor- α -308 polymorphism with dengue infection: A systematic review and meta-analysis. *Acta Trop*. 2017;173(2017):17–22.
9. Eskandari-Nasab E, Moghadampour M. The relationship between IFN- γ and TNF- α gene polymorphisms and brucellosis: A meta-analysis. *Adv Clin Exp Med*. 2018;27(12):1701–9.
10. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol*. 2008;180(9):5771–7.
11. Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci U S A*. 1998;95(16):9465–70.
12. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet*. 1997;24(1):1–8.
13. Lesiak A, Zakrzewski M, Przybyłowska K, Rogowski-Tylman M, Wozniacka A, Narbutt J. Atopic dermatitis patients carrying G allele in -1082 G/A IL-10 polymorphism are predisposed to higher serum concentration of IL-10. *Arch Med Sci*. 2014;10(6):1239–43.
14. Meenakshi P, Ramya S, Shruthi T, Lavanya J, Mohammed HH, Mohammed SA, et al. Association of IL-1 β + 3954 C/T and IL-10-1082 G/A cytokine gene polymorphisms with susceptibility to tuberculosis. *Scand J Immunol*. 2013;78(1):92 – 7..
15. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology*. 1996;24(2):289 – 93..
16. Ayres M, Ayres JR, Ayres DL, Santos AS. *BioEstat 5.3: aplicações estatísticas nas áreas das Ciências Biológicas e Médicas*. Pará: Mamirauá; 2011.
17. Shin EC, Sung PS, Park SH. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat Rev Immunol*. 2016;16(8):509 – 23..
18. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol*. 2001;19(2001):65–91.
19. Holbrook J, Lara-Reyna S, Jarosz-Griffiths H, McDermott M. Tumour necrosis factor signalling in health and disease. *F1000Res*. 2019;8:F1000 Faculty Rev-111. doi:10.12688/f1000research.17023.1.
20. Boks MA, Kager-Groenland JR, Mousset CM, van Ham SM, ten Brinke A. Inhibition of TNF receptor signaling by anti-TNF α biologicals primes naïve CD4(+) T cells towards IL-10(+) T cells with a regulatory phenotype and function. *Clin Immunol*. 2014;151(2):136 – 45.
21. Bouzgarrou N, Hassen E, Gabbouj S, Schvoerer E, Ben Mami N, Triki H, et al. Lack of effect of tumor necrosis factor-alpha – 308 G/A polymorphism on severity of liver fibrosis in Tunisian hepatitis C

- virus (HCV)-infected patients. *Gastroenterol Clin Biol*. 2010;34(4–5):297–304.
22. Dogra G, Chakravarti A, Kar P, Chawla YK. Polymorphism of tumor necrosis factor- α and interleukin-10 gene promoter region in chronic hepatitis C virus patients and their effect on pegylated interferon- α therapy response. *Hum Immunol*. 2011;72(10):935–9.
23. Santos NP, Ribeiro-Rodrigues EM, Ribeiro-Dos-Santos AK, Pereira R, Gusmão L, Amorim A, et al. Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. *Hum Mutat*. 2010;31(2):184–90.
24. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol*. 2011;29(2011):71-109.
25. Bouzgarrou N, Hassen E, Farhat K, Bahri O, Gabbouj S, Maamouri N, et al. Combined analysis of interferon-gamma and interleukin-10 gene polymorphisms and chronic hepatitis C severity. *Hum Immunol*. 2009;70(4):230–6.
26. Ramos JA, Silva R, Hoffmann L, Ramos AL, Cabello PH, Urményi TP, et al. Association of IL-10, IL-4, and IL-28B gene polymorphisms with spontaneous clearance of hepatitis C virus in a population from Rio de Janeiro. *BMC Res Notes*. 2012;5:508. doi:10.1186/1756-0500-5-508.
27. Abbas Z, Moatter T, Hussainy A, Jafri W. Effect of cytokine gene polymorphism on histological activity index, viral load and response to treatment in patients with chronic hepatitis C genotype 3. *World J Gastroenterol*. 2005;11(42):6656–61.
28. Gao QJ, Liu DW, Zhang SY, Jia M, Wang LM, Wu LH, et al. Polymorphisms of some cytokines and chronic hepatitis B and C virus infection. *World J Gastroenterol*. 2009;15(44):5610–9.
29. Talaat RM, Esmail AA, Elwakil R, Gurgis AA, Nasr MI. Tumor necrosis factor-alpha – 308G/A polymorphism and risk of hepatocellular carcinoma in hepatitis C virus-infected patients. *Chin J Cancer*. 2012;31(1):29–35.
30. Sheneef A, Esmat MM, Mohammad AN, Mahmoud AA, Moghazy HM, Noureldin AK. Interleukin-10 and Interferon Gamma Gene Polymorphisms and Hepatitis C Virus-Related Liver Cirrhosis Risk. *J Interferon Cytokine Res*. 2017;37(4):175 – 80..

Figures

Figure 1

Figure

Figure 2

HCV viral load according the genotype for each polymorphism: (A) *TNFA* -308G>A and (B) *IL10* -1082A>G.