

ROLE OF INTERLEUKIN 10 GENE (-1082 G/A) AND INTERLEUKIN 12B GENE (-1188 A/C) POLYMORPHISMS IN SUSCEPTIBILITY TO HCV INFECTION AND ITS CLEARANCE AMONG EGYPTIAN HEMODIALYSIS PATIENTS

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Abstract:

Background: The natural outcome of hepatitis C infection varies dramatically among individuals. Host genetic factors may affect the clinical outcome of hepatitis C virus. Many studies have focused on the role of cytokine gene polymorphisms on natural outcome of HCV.

Aim of the work: The aim of the present study is to determine the genotypes and allele frequency of interleukin 10- 1082 G/A (rs 1800896) gene and interleukin 12 β -1188 A/C (rs 3212227) gene polymorphisms among Egyptian hemodialysis patients to assess their role on susceptibility and outcome of hepatitis C viral infection

Patients and methods: This cross-sectional study included one hundred patients on regular hemodialysis for more than six months. They consisted of two groups; HCV-Ab positive and negative patients. Positive patients were confirmed by HCV-RNA at dialysis initiation. Our study repeated HCV-RNA for HCV positive patients, further subdividing them into persistent infection and viral clearance groups, according to presence or absence of HCV-RNA. All groups were subjected to genotyping for polymorphisms in IL 10 -1082 A/G and IL12b -1188 A/C using PCR-RFLP method.

Results: Although the HCV antibody negative and spontaneous clearance groups had higher frequency of the wild genotype of IL-10 1082 (AA) and of the mutant genotypes of IL-12b 1188 (AC and CC), this was not statistically significant. There was no association between IL-10 1082 A/G and IL-12b 1188A/C gene polymorphisms and outcome of hepatitis C virus infection.

Conclusion: Genetic variations in IL-10 and IL-12 gene have no effect on susceptibility to HCV or its outcome.

Keywords: HCV, IL-10 gene, IL-12 gene, hemodialysis.

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INTRODUCTION

Hepatitis C virus infection is a major cause of liver disease in hemodialysis (HD) patients. Nosocomial transmission can explain the higher prevalence in these patients than in the general population [1]. Inappropriate handling of medical equipment or disinfection of surfaces in the hemodialysis unit can lead to the perpetuation of this problem [2]

The prevalence of HCV in hemodialysis patients in the DOPPS study from 2012-2015 (phase 5) was about 9.9%, ranging from 4.1% in Belgium to 20.1% across the Gulf Cooperation Council Countries. Factors associated with increased HCV prevalence included younger age, longer duration on dialysis, hepatitis B virus (HBV), HIV positivity, substance abuse and glomerulonephritis as a cause of end-stage renal disease (ESRD)[2].

The annual incidence of HCV infection in Egyptian dialysis patients decreased after implementation of infection control programs by the Egyptian Ministry of Health and Population in 2001 from 28% to 6% within 3 years [3]. In 2018, the first annual report of the Egyptian Renal Data System (ERDS) announced the HCV prevalence in end stage renal disease (ESRD) patients in Egypt to be around 35% [4]. This estimate is expected to be significantly reduced in the anticipated new report of the Egyptian Registry, after the launch of the 100 Million Lives campaign in March 2019.

Factors related to development of persistent HCV infection, which is the natural course in most patients, or to spontaneous viral clearance, are not well defined. The individual susceptibility and the outcome of HCV infection have been associated with polymorphisms in cytokine genes.

Cytokines are small proteins secreted by a variety of T cells in response to pathogens and mediate the immune response to infectious agents such as HCV. They carry out this function by inducing inflammation to clear the infection. However, excessive inflammation can lead to unwanted tissue damage. Therefore, anti-inflammatory cytokines serve a much-desired goal of limiting this damage [5]. In some cases, however, this anti-inflammatory action leads to viral persistence.

Thus, several studies have examined polymorphisms in a variety of cytokine genes to determine their role in persistence or clearance of HCV [6]. This has been the case with IL-10. It is a cytokine with multiple pleiotropic effects in immunoregulation. IL-10 is also known as human cytokine synthesis inhibitory factor (CSIF) and has been implicated in maintaining persistence and pathogenicity of some viral infections as HCV. This is mediated by direct targeting of immune effector cell types or by preventing maturation of macrophage and dendritic cells indirectly,

with limitation of antigen presentation and chemokine secretion capacity of the host [5]. IL-10 downregulates the Th1 immune response and suppresses pro-inflammatory cytokine secretion, such as TNF- α and IFN- γ [7].

Single nucleotide polymorphisms (SNPs) at positions - 1082 G> A, - 819 C > T, and - 592 C >A have been identified in the IL-10 gene promoter region [8]. These polymorphisms are involved in controlling IL-10 transcription rate, with subsequent Th1 response inhibition and viral persistence, if IL-10 was upregulated [9].

IL-12 is an immunomodulatory cytokine that induces the development of Th1 cells with down-regulation of Th2 cells. This results in stimulation of pro-inflammatory cytokine secretion, such as TNF- α and IFN- γ . Therefore, IL-12 plays an important role in cell mediated immunity, clearance of viral infections and response to antiviral therapy [10]. It is a heterodimer composed of a light chain (p35) and a heavy chain (p40). The gene encoding IL-12 p40 (IL12B) is polymorphic and is located on 5q31–33. A single nucleotide polymorphism (A/C) in the 3' untranslated region at position 1188 of this gene has been identified. This polymorphism influences IL-12 synthesis and may be functionally relevant in diseases with an altered Th1/Th2 balance [11].

SUBJECTS AND METHODS

This cross-sectional study included one hundred ESRD patients who have been on dialysis for more than 6 months in the hemodialysis unit of Theodor Bilharz Research Institute (TBRI). Their ages ranged between 19 and 76 years. They were 28 (28%) females and 72 (72%) males.

Patients

They consisted of two groups; HCV-Ab positive and HCV-Ab negative patients. HCV-Ab negative patients would serve as a reference group for the frequency distribution of tested polymorphic variants. HCV infection had been confirmed in HCV-Ab positive patients by HCV-RNA PCR at dialysis initiation. We repeated HCV RNA reverse transcription polymerase chain reaction (RT/PCR) for HCV positive

patients, further subdividing them into persistent infection and viral clearance groups. The persistent infection group were positive for HCV-RNA at the initiation of renal replacement therapy and at the time of the study. Viral clearance group were positive for HCV-RNA at the initiation of renal replacement therapy but negative for HCV-RNA at the time of the study and remained negative thereafter. None of the patients studied received anti-viral therapy.

Laboratory investigations

All patients were subjected to the following investigations: CBC using automated hematology analyzer (DXH500, Beckman Coulter, USA), films were spread and stained for determination of differential leucocytic count. Prothrombin time (PT) and prothrombin concentration (PC) were performed using automated coagulation analyzer (Stago compact, France). Kidney function tests (serum creatinine and serum urea), Liver function tests (serum bilirubin, serum alanine transaminase (ALT), serum aspartate transaminase (AST) and serum albumin) were performed using AU480 chemistry analyzer, Beckman Coulter, USA.

Serology

The presence of HCV antibodies was determined using ADVIA, Centure HCV Abs chemiluminescent assay (Siemens, Germany).

Viral Load

The presence of HCV-RNA was determined using reverse transcriptase polymerase chain reaction (RT-PCR) light cyclor 480 class II (ROCH, Basel, Switzerland).

Genomic extraction and PCR amplification

Genotyping of IL10 -1082 G/A (rs 1800896) and IL12 β -1188A/C (rs 3212227) was performed using restriction fragment length polymorphism (RFLP). Genomic DNA was extracted from peripheral blood using Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Mini Kit (Cat. No. K0781, Thermo Scientific, Massachusetts, USA).

Table 1: Primers sequence for PCR

Gene	PCR	Annealing
IL-10 A\G	F1(5'-CTC GCT GCA ACC CAA CTG GC-3')	58°C
	F2 (5'-TCT TAC CTA TCC CTA CTT CC-3')	
IL-12b A\C	F3(5'- GATATCTTTGCTGTATTTGTATAGTT-3')	56°C
	F4 (5'- AATATTTAAATAGCATGAAGGC-3')	

Using Thermo scientific™ DreamTaq™Green PCR Master Mix and following cycle condition: An initial heating step at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, followed by 35 cycles of denaturing (at 94°C for 1 min), annealing (58°C for IL 10 -1082A\G and 56°C for IL12b -1188A\C for 1 min), and chain extension (72°C for 1 min), followed by a final cycle of 94°C for 1 min, 60°C for 1 min and 72°C for 5 min. The amplified DNA fragments were digested by specific restriction enzyme. For IL 10 1082A\G gene polymorphism the restriction enzyme used is FastDigest MNII, Cat.No.00455751 and the recognition sequence is (5'- CCTG-3') and (3'- GGAG-5'). For IL 12b -1188A\C gene polymorphism the restriction enzyme used is FastDigest Taq1 Cat.No.00405152 all from Thermo scientific, USA.

The digested products were electrophoresed on 2% agarose gel, Sigma, St. Louis, USA. DNA molecular marker Cat No. 00014637 Fermentas, USA 100 bp DNA ladder was used to assess the size of the PCR-RFLP products. The site of the

band in agarose gel was detected using ultra-violet trans-illumination by staining with ethidium bromide dye. DNA-ethidium bromide complexes absorb ultra-violet light at 260, 300 or 360 nm and emit at 590 nm in the red orange region of the visible spectrum.

IL 10 -1082A\G gene polymorphism shows that Wild A/A genotype gives a single band 231 bp, Heterozygous A/G genotype gives 3 bands 231, 121bp and 110 bp and Homozygous G/G genotype gives two bands of 121 bp and 110 bp Figure (1).

IL 12b -1188A\C gene polymorphism shows Wild A/A genotype gives two bands of 87 bp and 118 bp, Heterozygous A/C genotype gives 3 bands 118, 92 and 26 bp and Homozygous C/C genotype gives two band 92 bp and 26 bp Figure (2).

STATISTICAL METHODS

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Data were analyzed using SPSS statistical package version 17. Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square tests (Fisher's exact test or Pearson Chi-Square) were used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test. Calculated Odds ratio (OR) and 95% confidence intervals (CI) were done for

risk estimation. A p-value < 0.05 was considered significant.

RESULTS

Among 100 studied HD patients, 24 patients (24%) were HCV Ab +ve, while 76 patients (76%) were HCV Ab -ve patients. Among Ab +ve patients, 17 patients (71%) had persistent infection and 7 patients (29%) spontaneously cleared the virus.

Table (2): Comparison of demographic features between HCV Ab -ve and HCV Ab +ve patients

	HCV Ab -ve N=24(24%)	HCV Ab +ve N=76(76%)	P value
Age	53.7±11.07	52.9±11.78	0.7
Gender			
Male	16(66.7%)	56(73.7%)	0.5
Female	8(33.3%)	20(26.3%)	
Blood transfusion			
Yes	4(16.7%)	20(26.3%)	0.3
No	20(83.3%)	56(73.7%)	
Duration of dialysis (Years)	8.33±4.58	8.01±4.37	0.7

Regarding the genotyping of IL-10 1082, the distribution of wild genotype (AA) was higher in HCV Ab -ve patients (44.7%) compared to HCV Ab +ve patients (33.3%), while mutant genotypes (AG and GG) were higher in HCV Ab +ve patients (66.7%) compared to HCV Ab -ve patients (55.3%), although however no statistical significance was found between the two groups (Table 3).

Regarding the frequency of A and G alleles, we found the frequency of A alleles higher in anti-HCV -ve (60%) compared to anti-HCV +ve patients (50%), while frequency of G alleles was higher in anti-HCV +ve patients (50%) compared to anti-HCV -ve patients (40%).

Regarding the effect of IL-10 1082 A/G polymorphism on spontaneous clearance of the virus, statistical analysis of retrieved data showed that frequency of A alleles was higher in the spontaneous clearance group (70%) compared to persistent infection group (50%), while

frequency of G alleles was higher in persistent infection group (50%) compared to spontaneous clearance group (30%) (Table 4).

As regard results of genotype distribution of IL-12b 1188 A\G gene polymorphism; the wild type (AA) was higher in HCV Ab +ve patients (41.7 %) compared to HCV Ab -ve patients (35.5 %), while mutant genotypes (AC and CC) were higher in HCV Ab -ve patients (64.5%) compared to HCV Ab +ve patients (58.3%), although however no statistical significance was found between the two groups (Table 5).

The frequency of A alleles was higher in HCV Ab -ve patients (52%) compared to HCV Ab +ve patients (48%), while frequency of C allele was higher in HCV Ab +ve patients (52%) compared to HCV Ab -ve patients (48%). The A and C allele frequency distributions were near each other in patients reactive and non-reactive to HCV antibodies.

Table (3): Comparison of frequencies of genotypes and alleles of IL 10 -1082 A\G between HCV Ab -ve and HCV Ab +ve patients

Polymorphism IL10-1082 A\G	HCV Ab -ve N= 76 (76% of total patients)	HCV Ab +ve N=24 (24% of total patients)	AOR (95%CI)	P. value
AA Wild	34 (44.7% of anti-HCV -ve)	8 (33.3% of HCV +ve)	1 (Reference)	
AG Hetero	25 (32.9% of anti-HCV -ve)	10 (41.7% of HCV +ve)	0.59 (0.203-1.704)	0.3
GG Homo	17 (22.4% of anti-HCV -ve)	6 (25.0% of HCV +ve)	0.67(0.19-2.23)	0.5
AG+GG	42 (55.3% of anti-HCV -ve)	16 (66.7% of HCV +ve)	1.619(0.62 - 4.24)	0.3
Allele frequency				
A	93 (60%)	26 (50%)		0.6
G	59 (40%)	22 (50%)		0.5

AOR: Adjusted Odds Ratio; CI: 95%Confidence Interval.

Table (4): Comparison of frequencies of genotypes and alleles of IL 10 -1082 A\G between HCV spontaneous clearance and HCV persistent infection patients

Polymorphism A/G	IL10-1082	Spontaneous clearance N=7 /24 (29% of HCV +ve)	Persistent infection N=17/24 (71% of HCV +ve)	AOR (95%CI)	P. Value
AA		4 (57.1% of spontaneous)	4 (23.5% of persistent)	1 (Reference)	

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	clearance)	infection)		
AG	2 (28.6% of spontaneous clearance)	8 (47.1% of persistent infection)	0.25 (0.031 - 1.99)	0.1
GG	1 (14.3% of spontaneous clearance)	5 (29.4% of persistent infection)	0.2 (0.016 - 2.57)	0.2
AG+GG	3 (42.9% of spontaneous clearance)	13 (76.5% of persistent infection)	4.33(0.67-28.12)	0.1
Allele frequency				
A	10 (70%)	16 (50%)		0.3
G	4 (30%)	18 (50%)		0.2

AOR: Adjusted Odds Ratio; CI: 95%Confidence Interval.

Table (5): Comparison of frequencies of genotypes and alleles of IL12b - 1188 A\C between HCV Ab -ve and HCV Ab +ve patients

Polymorphism	HCV Ab -ve N= 76 (76% of total patients)	HCV Ab +ve N=24 (24% of total patients)	AOR (95%CI)	P. value
AA	27 (35.5% of anti-HCV -ve)	10 (41.7% of HCV +ve)	1 (Reference)	
AC	25 (32.9% of anti-HCV -ve)	3 (12.5% of HCV +ve)	3.086(0.761-12.519)	0.1
CC	24 (31.6% of anti-HCV -ve)	11 (45.8% of HCV +ve)	0.808(0.292-2.236)	0.6
AC+CC	49 (64.5% of anti-HCV -ve)	14 (58.3% of HCV +ve)	0.771(0.302-1.97)	0.5
Allele frequency				
A	79 (52%)	23 (48%)		0.7
C	73 (48%)	25 (52%)		0.7

AOR: Adjusted Odds Ratio; CI: 95%Confidence Interval.

Regarding the role of IL 12b -1188A\C gene polymorphism in hepatitis C viral clearance in HD patients, we found that the wild type (AA) was higher in the persistent infection group (47.1 %) compared to the spontaneous clearance group (28.6 %), mutant genotypes AC and CC were higher in the spontaneous clearance group (71.4 %) compared to the persistent infection group (52.9 %), the difference between both groups, however, was not statistically

significant (Table 6).

The A allele frequency was higher in the persistent infection group (60 %) compared to the spontaneous clearance group (30 %), while the C allele frequency was higher in the spontaneous clearance group (70 %) compared to the persistent infection group (40 %), again without statistical significance.

Table (6): Comparison of frequencies of genotypes and alleles of IL12b -1188A/C between HCV spontaneous clearance and HCV persistent infection patients

Polymorphism	Spontaneous clearance N=7/24 of HCV +ve (29%)	Persistent infection N=17/24 of HCV +ve (71%)	AOR (95%CI)	P. Value
AA	2 (28.6% of spontaneous clearance)	8 (47.1% of persistent infection)	1 (Reference)	
AC	0 (0.0% of spontaneous clearance)	3 (17.6% of persistent infection)	-	-
CC	5 (71.4% of spontaneous clearance)	6 (35.3% of persistent infection)	3.33(0.47-23.47)	0.2
AC+CC	5 (71.4% of spontaneous clearance)	9 (52.9% of persistent infection)	0.45(0.068-2.99)	0.4
Allele frequency				
A	4 (30%)	19 (60%)		0.2
C	10 (70%)	15 (40%)		0.2

AOR: Adjusted Odds Ratio; CI: 95%Confidence Interval

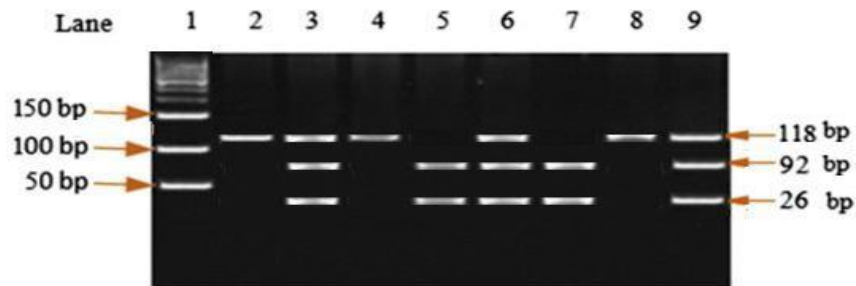


Figure (1): Results of IL10 - 1082A\G gene polymorphism on cases

Lane 1 showing: DNA ladder. Lane 2, 4 and 8 showing: cases with wild AA genotypes. Lane 3, 6 and 9 showing: cases with heterozygotes AG genotypes. Lane 5 and 7 showing: cases with homozygotes GG genotypes.

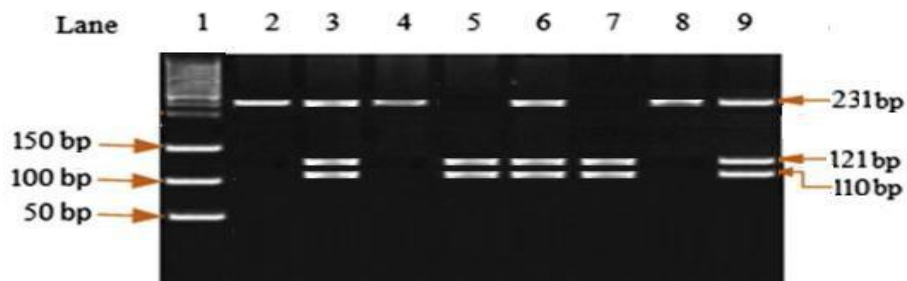


Figure (2): Results of IL 12b -1188A\C gene polymorphism on cases

Lane 1 showing: DNA ladder. Lane 2, 4 and 8 showing: cases with wild AA genotypes. Lane 3, 6 and 9 showing: cases with heterozygotes AC genotypes. Lane 5 and showing: cases with homozygotes CC genotypes.

DISCUSSION

Hepatitis C virus has been recognized as a major cause of acute and chronic hepatitis among hemodialysis patients. The elimination of HCV requires an efficient cell-mediated immune response, which largely depends on Th1 cells. IL-12 is the most important cytokine promoting this type of cells. On the other hand, a predominant Th2 response is associated with viral persistence. A Th2 immune response is mediated by the immunoregulatory cytokine IL-10 [9].

In the present study, we estimated the rate of spontaneous HCV clearance, which is defined by the presence of positive HCV Ab and undetectable HCV RNA by RT/PCR on follow up without antiviral therapy. The results showed that out of 24 patients 17 patients (71%) had persistent HCV infection, while 7 patients (29%) showed spontaneous HCV clearance.

The course of HCV infection in renal patients could be different from non-renal patients due to the effect of uremia in establishing an inflammatory milieu. This would increase inflammatory cytokines like TNF α and IL-12 but could also inhibit the cells of the immune system [3].

In the present study, gene polymorphism of IL10 -1082 A/G promoter (rs 1800896) was investigated. The frequency of G alleles and mutant genotypes (AG and GG) was higher in HCV Ab +ve patients, which suggests that presence of G allele may increase risk of HCV infection. However, there was no statistically significant difference. This is in agreement with a study by Dogra et al. [12] who showed that the GG genotype is more frequent in individuals infected with HCV than in healthy individuals although the difference was no significant. Similar to our observation, Sepahi et al. [14] reported higher frequencies of G allele and GG genotypes at position IL-10 1082 in HCV infected

patients compared to healthy controls, even after adjusting for confounders. Sheneef et al. [13] observed a higher frequency of GG genotype in chronic HCV infected Egyptian patients compared to controls.

Furthermore, Afzal et al. [15] demonstrated higher frequency of GG genotype in HCV infected patients more so than in healthy controls

Other authors have not observed a significant difference in the polymorphisms of IL10 -1082 A/G of the gene promoter between HCV infected and healthy patients [16, 17].

Regarding the effect of IL10 -1082 A/G polymorphism on spontaneous clearance of the virus, the distribution of wild homozygous genotypes (AA) was higher in patients with spontaneous clearance (57.1%) when compared to those with persistent infection (23.5%). The distribution of mutant genotypes (AG and GG) was higher in patients with persistent infection (76.5%) when compared to those with spontaneous clearance (42.9%). However, the difference between both groups was nonsignificant.

The frequency of G alleles and mutant genotypes (AG and GG) was higher in the persistent infection group, which suggests that presence of G alleles may play a role in persistent infection, although this was not statistically significant. This might be explained by the fact that GG genotype is responsible for high IL10 production, which in turn negatively affects the cellular immune response [18]. Similarly, Moudi et al. [20], found G alleles to be associated with high levels of IL10 in serum [19] which inhibits T cell proliferation and Th1 cytokine production

On the other hand, Rees et al. [21] reported that the IL10 -1082 A, not G, allele confers an increase in transcriptional activity of the IL10 promoter. While, Constantini et al. [22] reported no relationship between IL10 -1082 A\G polymorphism and outcome of HCV infection.

IL12b is one of the most important proinflammatory

cytokines, produced mainly by antigen presenting cells. It induces IFN γ stimulation and plays an important role in Th1 differentiation, favoring an anti-viral immune response [23]. In the present study, frequency of C allele was higher in HCV Ab +ve patients (52%) compared to HCV Ab -ve patients (48%). The A and C allele frequency distributions were near each other in patients reactive and non-reactive to HCV antibodies.

The mutant genotypes (AC and CC) of IL-12b 1188 A\C were higher in HCV Ab -ve patients (64.5%) compared to HCV Ab + ve patients (58.3%). This may suggest that the presence of the C allele may decrease the risk of HCV infection.

Similarly, **Hegazy et al.** [24] found a protective effect of the CC genotype and C allele of the SNP of IL12b -1188 in subjects exposed to HCV who remained uninfected compared to HCV infected patients. Moreover, **Zhu et al.** [9] found that, the C allele was associated with decreased susceptibility to HCV infection. Also, **Youssef et al.** [23] found that the C allele frequency was lower than the A allele frequency in HCV infected Egyptian patients in their study. This further reinforces the hypothesis that the C allele of IL12b -1188A\C polymorphism is associated with decreased susceptibility to persistent HCV infection.

Regarding the role of IL 12b -1188 A\C gene polymorphism in HCV clearance in HD patients, the higher frequency of C alleles and mutant genotypes (AC and CC) in HCV clearance patients suggests that presence of the C allele may play a role in spontaneous clearance of virus.

The study done by **Zhu et al.** [9] on genotyping of IL12b -1188A\C found no statistically significant difference in IL12-1188A\C gene polymorphism distribution between those with persistent infection and those with spontaneous clearance, although, as previously stated, regarding susceptibility, they found that the C allele was associated with a decreased risk of susceptibility to HCV infection.

Houldsworth et al. [25] however, did find an association between the A allele of the IL12b -1188 and between persistence of HCV infection in their study [25]. This has been linked to decreased IL-12 production by the A allele and increased production by the C allele.

Mueller et al. [26] revealed that the C allele of IL12b -1188 conferred better response to antiviral therapy, due to an enhanced Th1 immune response. However, no link could be found between this allele and spontaneous HCV clearance.

CONCLUSION

This study suggests that polymorphisms of IL10 -1082 A\C and IL12b -1188 A\C may contribute to susceptibility and spontaneous clearance of HCV infection in the HD population. However, since the link between these polymorphisms and susceptibility or outcome of HCV has not been clearly defined, we could not positively define specific alleles of IL10 -1082 A\C gene or IL12b -1188A\C gene as predictive or prognostic markers in HCV infection in HD patients.

Other studies however have reported such a genetic link. This may be explained by differences in sample size, structure of examined groups, ethnic and environmental factors. Further studies are needed to clarify the effect of genetic and immunological factors on HCV infection in HD patients.

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