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SNP in Tumor Necrosis Factor-Alpha (−308 A/G) Gene Association with HCV Infected Thalassemia Patients

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ABSTRACT

Background: Hemoglobin can develop abnormally as a result of hereditary blood illnesses called thalassemia that are inherited from a person's parents. **Objective:** The goal of this study was to identify the tumor necrosis factor alpha (TNF-α) A/G polymorphism and correlate TNF-α serum levels with illness progression.

Patients and methods: Eighty cases with beta-thalassemia who had been diagnosed at the thalassemia center at Al-Zahra Hospital in Al-Najaf province, Iraq participated in a case-control study with 40 healthy individuals serving as the control group. The 80 individuals were divided based on HCV status. From 50 seropositive hepatitis C virus (HCV) patients, 19 had IgG positivity, 15 had IgM positivity, and 16 had both IgG and IgM positivity. All patients and the control group had blood samples taken. DNA from blood was taken to be utilized in PCR to find the TNF-α A/G polymorphism. TNF-α levels were assessed using an ELISA test.

Results: The findings explained why HCV-infected individuals had high significant rates of thalassemia than non-infected ones. The findings showed that in thalassemia patients, AA genotype and A allele are risk factors for severity, whereas GG genotype and G allele are protective factors for severity.

Conclusions: The findings showed that having an AA genotype increases your chance of contracting HCV. This study also demonstrated a considerably higher level of TNF-α in thalassemia patients compared to controls, as well as a significantly higher level of TNF-α in thalassemia patients who also had HCV infection compared to controls and patients without HCV infection.

Keywords: Thalassemia, TNF-α, SNP, HCV, PCR.

INTRODUCTION

Genetic blood disorders known as thalassemia can cause faulty hemoglobin formation and are inherited from a person's parents [(1,2)]. Alpha and beta Thalassemia are the two primary kinds [(1)]. The Middle East region, Africa, the Indian subcontinent, and South-East Asia all have high rates of thalassemia [(3)]. Thalassemia affected 208 million individuals globally in 2013, with 4.7 million suffering from a severe version of the ailment [(4)]. Diagnosis is typically reached through the use of blood tests, a complete blood count, specific hemoglobin tests, and genetic investigations [(2)].

Hepatitis C virus (HCV) infection is one of the primary causes of chronic liver disease worldwide. This dangerous illness infects around 180 million individuals, with 20-40% of infected persons clearing the virus naturally and the remaining sustaining chronic Hepatitis C infection [(6)]. Transmission can occur by contaminated blood or blood products, infected syringes, or organ transplants [(6,7)]. HCV seroprevalence varies from 1 to 3% worldwide, but can reach 85% in high-risk populations such thalassemia, hemodialysis, and hemophilia [(8)].

One of the most prevalent transfusion-related infections among multi-transfused thalassemic (inherited hematological condition) patients who require multiple blood transfusions to survive is HCV infection. The pathophysiology of liver infection, as well as the intricate connection between HCV and its host, are now well recognized.Cytokines are low-molecular-weight proteins that regulate immunological responses such as proliferation, differentiation, lymphocyte activation, survival, and death. They are regulatory proteins produced by many different cell types, including monocytes, lymphocytes, antigen-presenting cells, fibroblasts, and endothelial cells [(9)]. Different cytokines' pro- and anti-inflammatory activities, which are encoded by single nucleotide polymorphisms (SNPs), may alter the outcome of HCV infection [(10,11)].

The host immune response to HCV infection is mainly reliant on tumor necrosis factor alpha (TNF-α), a strong antiviral cytokine located on chromosome 6 [(12,13)]. Hepatocytes infected with the virus stimulated its production [(5)]. Patients with chronic HCV infection exhibited increased liver TNF-α levels, demonstrating that viral hepatitis infection stimulates human hepatocytes to generate TNF-α [(12)].Interferon-gamma (IFN-γ) levels may influence antiviral medication outcomes in HCV patients. Furthermore, it was shown that IFN-γ modulates IFN-gamma treatment by interfering with Janus Kinase-Signal Transduction and Activator of Transcription (JAK-STAT) signaling pathways [(11)]. Immunogenetics has attributed a considerable influence on heredity in the host immune system's response to infections [(10)].

The TNF-α promoter single nucleotide polymorphisms (SNPs) located at locations -863, -308, and -238 are of particular interest among the many promoter SNPs examined in connection to HCV infection. The TNF-α 308 SNP was discovered to be intricately connected to HCV infection and its effects

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[11]H .igher TNF-α levels are associated to the severity of hepatic inflammation, fibrosis, and tissue injury [14]. Given that cytokines play an important part in the host's defense against viral infection, they may provide us with the knowledge we need to link viral clearance and treatment response in HCV-infected patients.

PATIENTS AND METHODS

Design study and laboratory methods:

Eighty thalassemic patients who visited Al-Zahra Hospital in the province of Al-Najaf from March 2019 to December 2019 were the subject of a case-control study. Along with 40 people who appeared to be in good health as the control group. The patients were split into two groups based on whether they had the human hepatitis virus or not. Each subject underwent a clinical examination, and the results were entered into a data sheet. Samples of blood obtained for following purposes: evaluation of the HCV infection (IgG and IgM by sight-USA kit), detection of serum TNF-α level (Elabscience, USA kit), sandwich ELISA system (Biotech, USA), Polymerase Chain Reaction amplification of TNF-α A/G polymorphism.

TNF-α (-308 G/A) gene polymorphism

Using a commercially available kit and the Genomic DNA kit system, genomic DNA was gained from peripheral blood white blood cells (Favorgen Biotech Corp., China). A PCR was used to produce amplified DNA. Three primers were used (position 144/164: 5′-TCTCGGTTTCTTCTCCATCG-3′), which is complement to the TNF-α A1 allele (position 328/308 G: 5′-ATAGGTTTTGAGGGGCATGG-3′), or TNF-α A2 allele (position 320/308A: 5′-ATAGGTTTTGAGGGGCATGA-3′), was used in combination with the allele-specific PCR primers.

The only difference between allele-specific primers was their 3' terminal sequences. Amplification not take place when the 3' ends does not match the origin DNA. Genomic DNA (250 ng), 200 mol/L dNTPs, 2 mMol/L $MgCl_2$, 1 L of 10× Taq DNA polymerase buffer, 1 unit of Taq DNA polymerase (Boehringer Mannheim, Mannheim, Germany), 10 pmol of each test primer, and 10 pmol of control primers included in the 10 Microliter PCR reaction mixture. The following reaction conditions were applied: 31 cycles of 95°C for 90 sec, 61°C for 150 sec, and 72°C for 60 sec, and a final extension step of 72°C for ten min, make up the 95°C for 5 min protocol. PCR products were then electrophoresed at 100 W for 45 min of 2.5% agarose gels contained 0.5 mg/mL ethidium bromide. Using a UV transilluminator imaging analyzer, the gels were seen.

Ethical statement: Patients and control Group: This study complied with the Declaration of Helsinki recommendations, which were approved by the Ethical Committees of the Al-Zahra Teaching Hospital and the Iraqi Ministry of Health. Verbal consent was also obtained from each participant prior to the collection of a sample.

Statistical analysis

Continuous variables were reported as mean ± standard deviation (SD) or median, whereas categorical data presented as frequencies. The chi-square test for linear trend was employed when appropriate to examine whether there were differences between groups, and odds ratios (ORs) with their 95% confidence intervals (95% CIs) were generated. $P \leq 0.05$ was used for defining the statistical significance. All statistical analyses conducted using the Statistical Package for Social Sciences (SPSS) software (version 16.0, SPSS Inc. Chicago IL, USA).

RESULTS

Family history on thalassemic patients:

The current study showed that the percentage of thalassemic individuals had a positive family history of thalassemia was 54% of patients (Figure 1a).

HCV in thalassemia patients

Out of 120 Case-Control of thalassemic patients there were 50 (62.5%) seropositive for anti-HCV antibodies remaining 30 (37.5%) seronegative (Figure 1b). From 50 HCV seropositive patients, 19 (38%) were positive toward IgG and 15 (30%) were positive for IgM and 16 (32%) were positive for both Ig and IgM.

Thalassemia patients' division according to gender and age:

The study showed that males were 45 (56.25%) and females were 35 (43.75%). Highest frequency age of patients was in 11-20 and lowest was 41-50, both male and female (Figure 2).

Molecular study Distribution of TNF-α (-308 A/G) Gene polymorphism in thalassemia patients and healthy control:

Results exhibited AA homozygous genotype 60% in the thalassemia patients and 15% in the controls that were statically significant whereas homozygous genotype GG represented 12.5% in thalassemics and 55% in controls as shown in (Figure 3). The results showed that A allele was prevalent in thalassemia (73.75%) compared to control (30%) (Figure 4).

Immunological study:

Concentration TNF-α in serum:

In current study, serum levels of TNF-α were found to be significantly ($P < 0.05$) higher in thalassemic patients (13.32 pg/mL) than that in controls (6.48 pg/mL) (Figure 5).

Correlation between TNF-α polymorphisms and its production in thalassemic patients:

The results showed that the mean TNF-α serum level in AA genotype thalassemia patients was about 12.3 pg/mL, which was significantly higher ($P < 0.05$) than the mean levels in GA (5 pg/mL) and GG (2.5 pg/mL) genotypes thalassemia patients (Figure 6).

TNF-α in serum levels in HCV infected and non-infected patients:

Both thalassemic patients with HCV and those without infection showed elevated levels of TNF-α compared to healthy controls (Figure 7).

Table (1): TNF-α (-308 A/G) genotype and allele frequency distribution in thalassemia patients and control

	Cases (n= 80)		Control (n= 40)				
Genotypes	**Number**	**%**	**Number**	**%**	**Odd ratio**	**95% CI**	***P*-value**
GG	12	15.0	21	52.5	0.117	0.04-0.36	<0.001***
GA	20	25.0	13	32.5	0.885	0.34-2.33	0.5000NS
AA	48	60.0	6.0	15.0	8.500	2.90-24.9	0.0001***
Allele							
G	32	40	55	68.75	0.153	0.08-0.30	<0.0001**
A	68	85	25	31.25	3.222	1.54-6.74	0.0015**

NS=nonsignificant

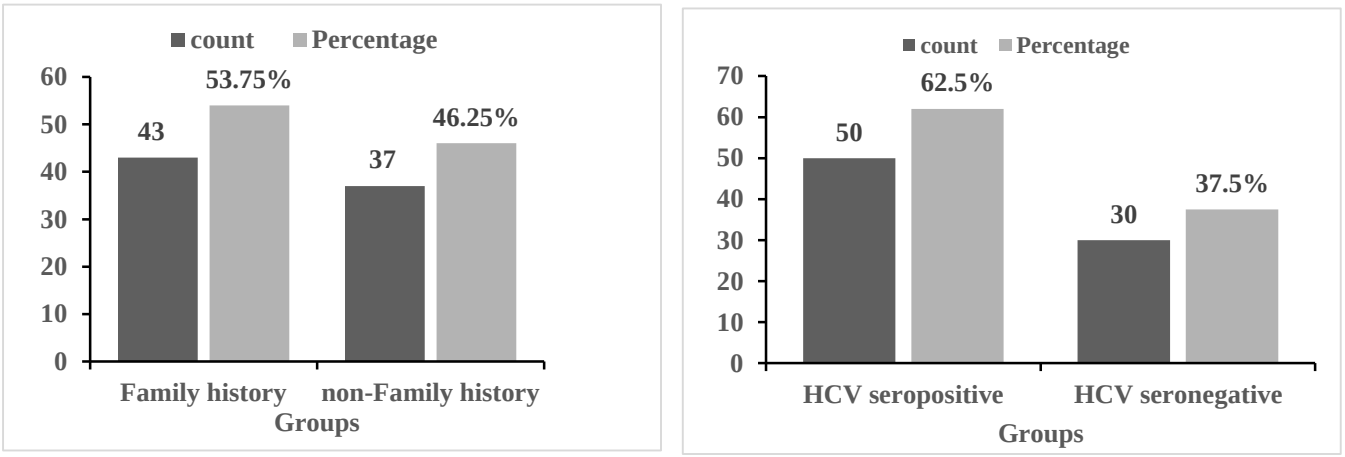

Fig. (1): Distribution of thalassemia patients according to family history (a) and HCV infection (b)

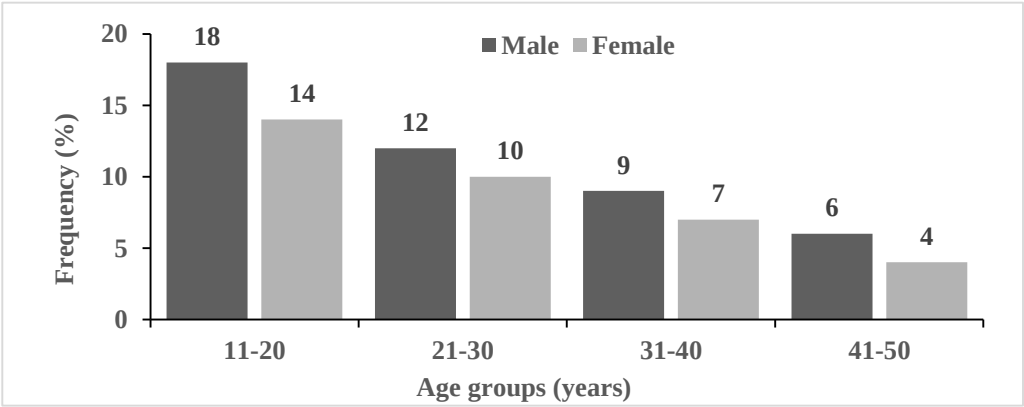

Fig. (2): Thalassemia patients' distribution according to age through gender**.**

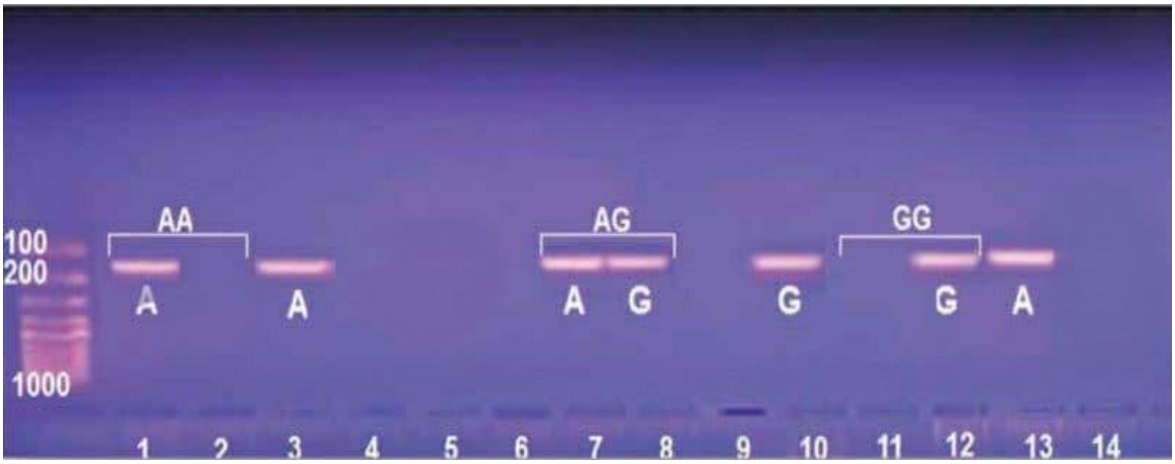

Fig. (3): PCR amplified 184 bp of TNF-α (-308 A/G gene) on an agarose gel stained with ethidium bromide. Display the DNA molecular size marker (100 bp Ladder), bands 1, 3, 5, 7, 9, 11, and 13 for A allele, while 2, 4, 6, 8, 10, and 12 for G allele, so bands 1, 2 represent AA genotype while 7, 8 for AG genotype, finally 9, 8 and 2 for GG genotype**.**

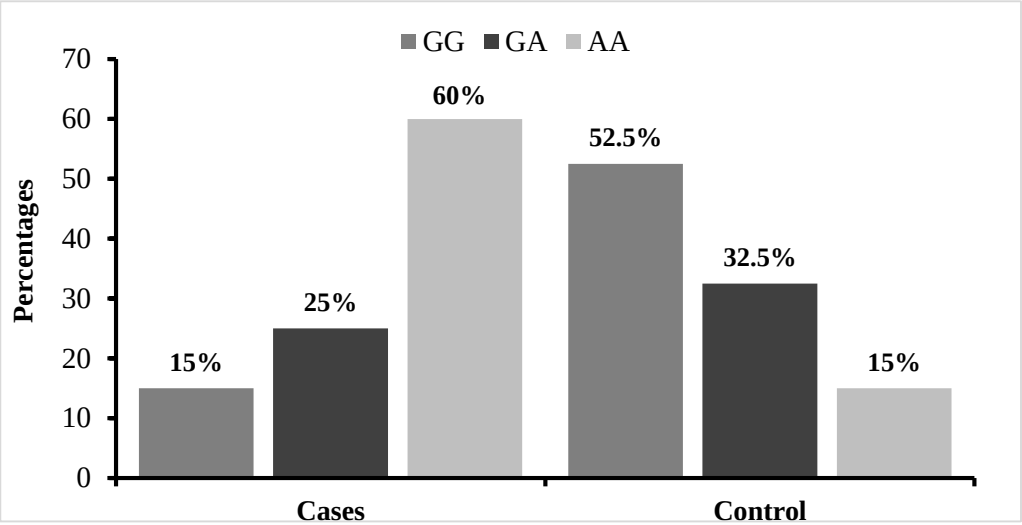

Fig. (4): Case-control comparison in relative frequency of three genotype TNF-α (-308 A/G gene(

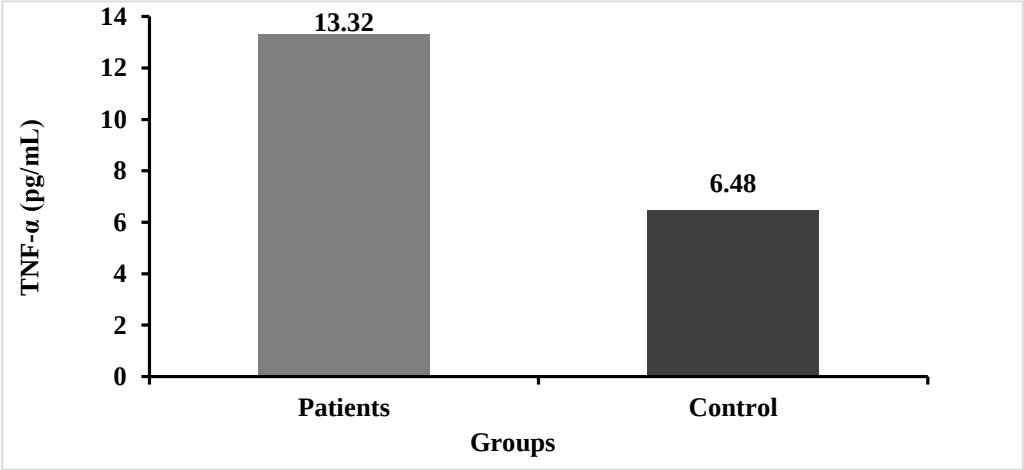

Fig. (5): Serum TNF-α concentration in patients and healthy

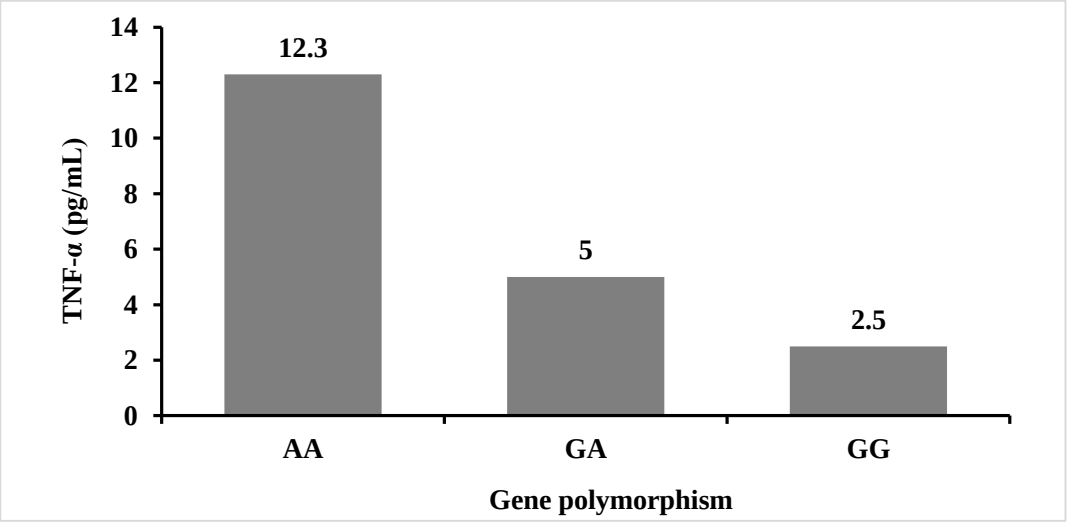

Fig. (6): TNF-α serum level in thalassemic patients according to gene polymorphism

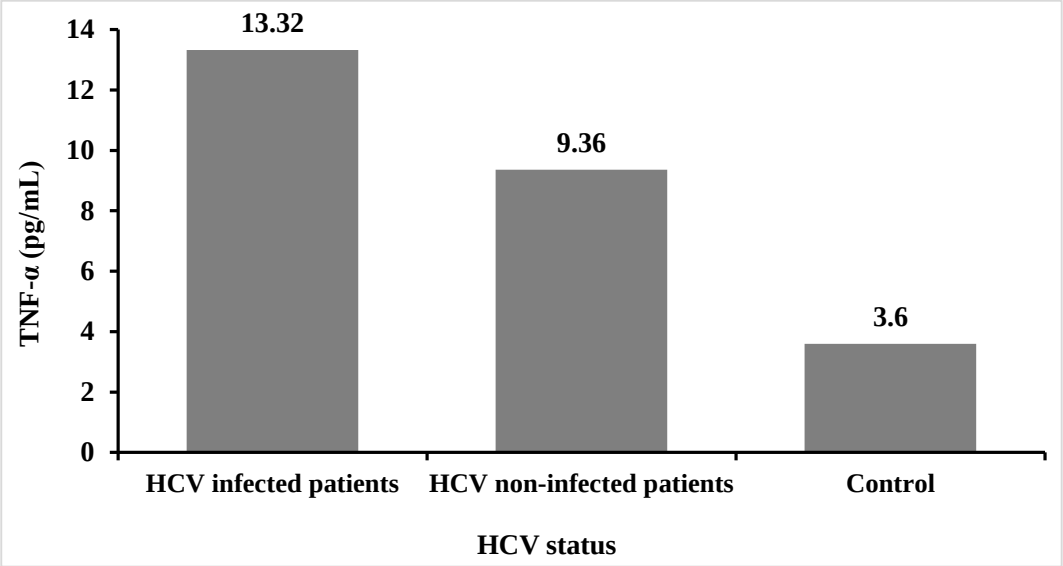

Fig. (7): TNF-α levels in thalassemic patients infected and non-infected with HCV were compared to controls

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DISCUSSION

Family history on thalassemic patients:

According to **Rivella** [15] and our study, the-thalassemia/Hb E syndrome is brought on by the co-inheritance of the hemoglobin E trait and thalassemia. From minimal to intermediate to significant, the disease's severity ranges greatly [15] .(Age, gender, alcohol use, the timing and length of the infection, the viral load, and the inheritance of the host immune response are all factors [8-16].

Thalassemia division according to gender and age:

The study shows a higher incidence in male than in female patients this may be caused by regional or folk practices [17] and it derives from the fact that immunity of males is greater than females, increasing the likelihood of his survival. This finding is consistent with **Khaled** [18] findings that there were 57% more men than women in Ninavha. Our research is consistent with that of **Bhavsar** [19] which found that 65% of the infected patients were men. The age range was 11–20in our data appears to be larger than other age ranges when results are grouped by age. This is because thalassemic individuals in their first or second decades of life lack hope for a future [20].

According to **Muhsin and Abdul-Husin** [21], 34.2% of thalassemic patients age range between 10 and 20. Additionally, according to **Falasca *et al.*** [22] findings there is no statistically significant difference between thalassemia patients who have HCV infection and those who are not HCV in Thalassemia.

Thalassemia patients are exposed to HCV infection, according to studies by **Muhsin and Abdul-Husin** [23] *in* **2013** as a result of blood transfusion.
Why people progress chronic infections so commonly is still a mystery. Gene variations, especially SNPs gene, expression that interacts with the host immunity scale is regulated by polymorphisms in the promoter region [24, 25]. Additionally, gene variations may influence how an illness manifests itself [25].

TNF-α is a major cytokine that maintains antigen-specific cell-mediated immunity [26-28]. The inflammatory response of the viral infection may induce liver damage in the host, resulting in a diverse cytokine response [29]. The persistence of the virus may be caused by the host's poor cytokine production and weak response to the virus [30].

Distribution of TNF-α G/A genotypes and alleles in patients and control groups:

Biswas *et al.* [23] investigated the connection between the host genotype single SNPs of TNF-α (-308) and HCV-infected thalassemic people in the Indian population [31]. Also, the G allele is the most prevalent genotype of TNF-α (308). The G allele associated with viral perseverance, whereas the A allele was much responsible for HCV infection. On the other hand, some researchers found no link between TNF-α (-308) SNPs and viral salvage [26, 32,33].

TNF-α serum concentrations in thalassemic patients infected and uninfected with HCV as compared to controls

Only a small percentage of the patients had high levels of TNF-alpha, as determined by the use of relatively sensitivity tests. IFN-g regulates the production of TNF-α by macrophages, which is consistent with the increased indecisiveness of patients with elevated TNF-α levels among those with unusually heightened IFN-g concentrations [34]. When using raised-sensitivity assays, 90% and 50% of the patients show noticeably elevated TNF-α and IL-1b values respectively. Serum TNF-α levels that are higher are a sign of immunological activation in thalassemia [17]. According to another study, around 50% of beta-

thalassemia patients who were transfusion dependent had elevated TNF-α levels that were higher than what was observed in our realization [17].

The TNF-α serum levels that are abnormally high have been associated with sickle cell disease and other hemolytic conditions [18]. Lymphohisitocytosis with hemophagocytosis [19]. There is also evidence that TNF-α stimulated the phagocytic activity of blood-borne macrophages [20]. TNF-α level following HCV infection increases the antiviral cytokine [21].

CONCLUSION

Polymorphism in TNF-α (-308AG) is associated with thalassemia advancement at the AA genotype. G allele act as protective factors for severity. AA genotype increases chance of contracting HCV, high level of TNF-α observed in thalassemia patients. Also, HCV-infected individuals had higher rates of thalassemia than non-infected ones.

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