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Journal of Medical and Life Science



Serum TNF- α levels as a biomarker in some liver diseases of Egyptian patients

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DOI: [10.21608/jmals.2023.329303](https://doi.org/10.21608/jmals.2023.329303)

Abstract:

Background: Tumor necrosis factor alpha (TNF- α) is physiologically essential for a healthy immune response. Although TNF- α can help the immune system regulate, producing too much or inappropriate of it can be dangerous and can cause diseases. **Patients and methods:** this study was conducted on 90 patients with liver diseases and 25 healthy control G1, patients divided into 4 groups, (G2) 25 patients with HCV infection, (G3) 25 HCC+HCV infection, (G4) 25 patients with HBV infection, (G5) 15 patients with HCC + HBV. **Results:** There was a statistically significant difference between the five groups with p-value <0.001*, Compared to the control group the four groups HCV (G2), HCV+HCC (G3), HBV (G4), and HBV+HCC (G5) have the same statistically significant difference with the same (P1) p-value <0.001* which was significant. The serum TNF- α expression level of the HCV group (G2) was significantly elevated than HCV+HCC (G3) with a (P2) p-value of 0.010* significant. Still, the Serum TNF- α expression level of the HBV group (G4) was higher than HBV+HCC (G5) (61.05 \pm 199.1, 14.36 \pm 2.18 respectively) with (P7) p-value 0.960.

Conclusion: Serum TNF- α can be used as a biomarker to differentiate between healthy and infected patients with HCV or HBV and the development of HCC.

Keywords: Tumor necrosis factor alpha (TNF- α), HBV, HCV, HCC, Liver diseases.

1. Introduction:

Tumor necrosis factor alpha (TNF- α) is a cytokine that affects different types of cells in pleiotropic ways. It is known as a major regulator of inflammatory responses and is known to be involved in the pathogenesis of some inflammatory and autoimmune diseases (1). TNF- α is a homotrimer protein consisting of 157

amino acids, that is mostly produced by activated macrophages, T-lymphocytes, and natural killer cells (2). It is functionally known to initiate a cascade of inflammatory molecules, such as chemokines and additional cytokines. There are two forms of TNF- α : transmembrane and soluble. The transmembrane TNF- α (tmTNF- α) is the initially synthesized precursor

form. It is required to be processed by TNF- α -converting enzyme (TACE), a membrane-bound disintegrin metalloproteinase, to be released as the soluble TNF- α (sTNF- α) (3).

TNF- α is physiologically essential for a healthy immune response. Although TNF- α can help the immune system regulate, producing too much or inappropriate of it can be dangerous and can cause diseases. TNF- α can be categorized as a critical factor in the pathological development of rheumatoid arthritis (RA), inflammatory bowel disease (IBD) (4), psoriatic arthritis (PsA), psoriasis (PS), and noninfectious uveitis (NIU) are induced by the abnormal secretion of TNF- α ; thus, TNF- α can be classified as a key factor in the pathological development (5). TNF- α has been associated with a poor prognosis in patients with severe acute respiratory syndrome (SARS), but the serum TNF- α level is not a significant biomarker for diagnosis or prognosis of mild COVID-19 patients (6).

TNF- α inhibitors have been created and used successfully in the treatment of autoimmune illnesses including Crohn's disease (CD) and RA since TNF- α is involved in the pathophysiology of these diseases (7).

TNF- α was originally identified as a circulating factor. It is a crucial mediator of many physiological states and has been linked to some liver disorders. TNF- α is expressed by both hepatocytes and infiltrating inflammatory cells in chronic liver lesions, and it has been proposed to play a significant role during tissue damage (8).

A major inflammatory cytokine in developing liver disease is tumor necrosis factor- α (TNF- α). This cytokine has the potential to induce hepatic injury, cirrhosis and eventually promote hepatocellular carcinoma (9).

Previous research revealed that patients with HCC had higher levels of circulating TNF- α . One might

reasonably conjecture that the elevated levels of TNF- α in the bloodstream seen in HCC patients could be ascribed to its SNPs. Furthermore, TNF- α may stimulate the secretion of additional inflammatory cytokines and induce the release of other fibrogenic factors, such as interleukin-1, interleukin-6, and tumor growth factor- β which can cause or aggravate liver damage (10). Elevated TNF production is linked to increased secretion of pro-inflammatory cytokines, activation of proto-oncogenes, and several genes linked to cell invasion, proliferation, and metastasis of cancer cells (11; 12).

This work aims to study TNF- α levels in Egyptian patients with liver diseases as hepatitis C infection HCV, hepatitis B infection HBV, and hepatocellular carcinoma HCC. to detect the role of TNF- α in the progression of liver disease and its ability to serve as a biomarker for early diagnosis of HCC.

2. SUBJECTS AND METHODS

2.1. Subjects

This study was carried out on 90 Egyptian hepatic patients and twenty-five healthy subjects. They matched in gender, social standard, and residency, all participants were selected from outpatient clinics, the National Liver Institute, Menoufia University at the time from October 2019 to December 2021 (During the COVID-19 pandemic), The study was conducted according to the ethical approval of the Research Ethics Committee, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt

All subjects were classified into five groups as follows:

Group (1): This group consisted of 25 healthy subjects which served as a control group.

Group (2): This group consisted of 25 patients with HCV infection.

Group (3): This group consisted of 25 patients with hepatocellular carcinoma (HCC) associated with HCV infection.

Group (4): This group consisted of 25 patients with HBV infection.

Group (5): This group consisted of 15 patients with hepatocellular carcinoma (HCC) associated with HBV infection.

2.2. Methods

2.2.1. Blood sample collection and preparation

3 milliliters of venous blood were taken from each patient in all five groups using a vacutainer system and septic venipuncture. Allow samples to coagulate for 30 minutes in a serum separator tube (SST) before centrifuging for 15 minutes at 1000 g. Removed serum was stored at -20°C or -80°C to the analytical time.

2.2.2. Quantification determination of TNF- α by Enzyme-Linked Immunosorbent Assay (ELISA)

TNF- α was determined using (Wuhan EIAab Science Co., Ltd, China)

Catalog No: E0133h, according to the manufacturer's guidelines by using the Strip reader Stat Fax USA.

Statistical analysis of the data

Data was fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The values with $P < 0.05$ were considered significant.

3. RESULTS

TNF- α ranged from 7.0 – 9.12 (**pg/ml**) in the control group with a mean of 7.66 ± 0.59 (**pg/ml**), while in group 2 HCV patients it ranged from 10.40 – 127.8 (**pg/ml**) with mean of 30.07 ± 31.09 (**pg/ml**), in group 3 HCV+HCC patients it ranged from 9.50 – 15.84 **pg/ml** with mean of 12.04 ± 1.65 **pg/ml**, in group 4 HBV patients it ranged from 9.12 – 1010.8 **pg/ml** with mean of 61.05 ± 199.1 **pg/ml**, while in group 5 HBV+HCC patients it ranged from 10.10 – 19.70 **pg/ml** with mean of 14.36 ± 2.18 **pg/ml**, there was statistically significant difference between the five groups with p-value $< 0.001^*$,

Compared to the control group the four groups HCV (G2), HCV+HCC (G3), HBV (G4), and HBV+HCC (G5) have the same statistically significant difference with the same p-value $< 0.001^*$ which was significant. The serum TNF- α expression level HCV group (G2) was significantly elevated than **HCV+HCC** (G3) with a p-value of 0.010^* significant. Still, the Serum TNF- α expression level of the HBV group (G4) was higher than **HBV+HCC** (G5) (61.05 ± 199.1 , 14.36 ± 2.18 respectively) with p-value 0.960.

Comparing between **HCV** and **HBV** groups Serum TNF- α was highly increased in **HBV** than **HCV** group (61.05 ± 199.1 , 30.07 ± 31.09 respectively) with p-value 0.747 not significant.

But the serum TNF- α level in (**HBV+HCC**) group was higher than (**HCV+HCC**) with a p-value of 0.045^* significant, as shown in Table (1) and Figures (1,2).

Table (1): Comparison between the different studied groups according to TNF- α

	Control (n = 25)	HCV (n = 25)	HCV+HCC (n = 25)	HBV (n = 25)	HBV+HCC (n = 15)	Test of Sig.	p
TNF-α (pg/ml)							
Min. – Max.	7.0 – 9.12	10.40 – 127.8	9.50 – 15.84	9.12 – 1010.8	10.10 – 19.70		
Mean \pm SD.	7.66 \pm 0.59	30.07 \pm 31.09	12.04 \pm 1.65	61.05 \pm 199.1	14.36 \pm 2.18	H= 66.518*	<0.001*
Median (IQR)	7.40 (7.22 – 8.11)	14.90 (11.86 – 29.60)	11.90 (10.90 – 12.70)	14.10 (12.30 – 19.80)	14.30 (13.80 – 15.40)		
p₁		<0.001*	<0.001*	<0.001*	<0.001*		
Sig. bet. Grps.		p ₂ =0.010*, p ₃ =0.747, p ₄ =0.818, p ₅ =0.024*, p ₆ =0.045*, p ₇ =0.960					

IQR: Inter quartile range

SD: Standard deviation.

F: F for One-way ANOVA test, pairwise comparison bet. Each 2 groups were done using a **Post Hoc Test (Tukey)**H: H for **Kruskal Wallis test**, pairwise comparison bet. Each 2 groups were done using a **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p-value for comparing the different studied groups.

p₁: p-value for comparing between **Control** and **each other groups**.p₂: p-value for comparing between **HCV** and **HCV+HCC**.p₃: p-value for comparing between **HCV** and **HBV**p₄: p-value for comparing between **HCV** and **HBV+HCC**.p₅: p-value for comparing between **HCV+HCC** and **HBV**p₆: p-value for comparing between **HCV+HCC** and **HBV+HCC**.p₇: p-value for comparing between **HBV** and **HBV+HCC**.*: Statistically significant at p \leq 0.05

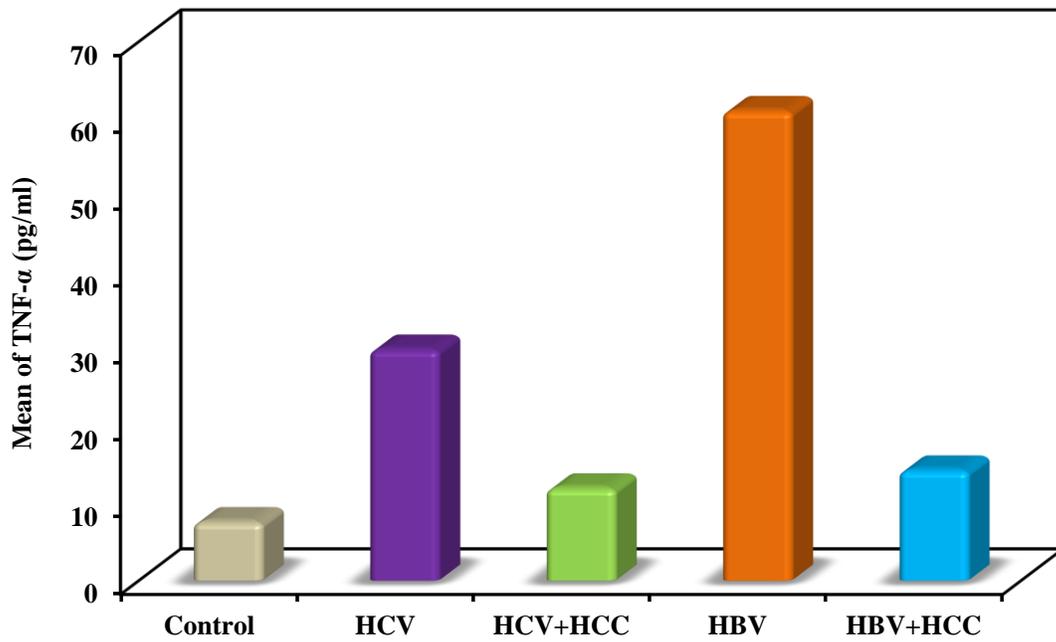


Figure (1): Levels of serum TNF- α in all studied five groups.

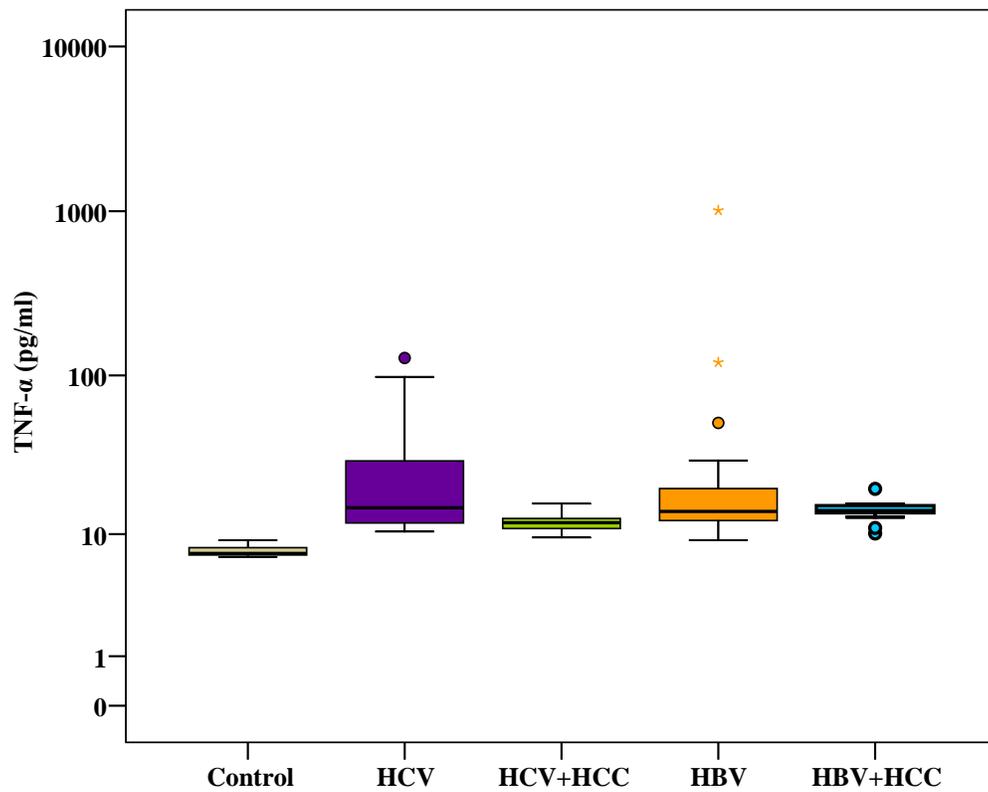


Figure (2): Levels of serum TNF- α in all studied five groups.

4. DISCUSSION:

Tumor Necrosis Factor- (TNF- α) is a key inflammatory cytokine in the progression of liver disease. This cytokine has been linked to hepatic damage, cirrhosis, and the promotion of hepatocellular cancer (9), TNF- α interacts with its receptor on the cell membrane and triggers intracellular downstream signaling (13).

The results of this study showed TNF- α ranged from 7.0 – 9.12 (pg/ml) in the control group with a mean of 7.66 ± 0.59 (pg/ml), while in group 2 HCV patients it ranged from 10.40 – 127.8 (pg/ml) with mean of 30.07 ± 31.09 (pg/ml), in group 3 HCV+HCC patients it ranged from 9.50 – 15.84 pg/ml with mean of 12.04 ± 1.65 pg/ml, in group 4 HBV patients it ranged from 9.12 – 1010.8 pg/ml with mean of 61.05 ± 199.1 pg/ml, while in group 5 HBV+HCC patients it ranged from 10.10 – 19.70 pg/ml with mean of 14.36 ± 2.18 pg/, there was statistically significant difference between the five groups with p value $<0.001^*$ also, Compared to control group the four groups HCV (G2), HCV+HCC (G3), HBV (G4), HBV+HCC (G5) have higher serum TNF- α levels than the healthy control group with same a statistically significant difference and same p-value $<0.001^*$ which was significant to differentiate the healthy control group from infected four groups.

Coinciding with our findings Tilg H et al., reported that TNF- α serum levels are elevated in fulminant hepatitis patients. Furthermore, blood TNF- α levels were found to be considerably greater in patients with fulminant hepatitis who died compared to individuals who survived. TNF- α levels were shown to be significantly higher in individuals with chronic liver disease of various etiologies, including hepatitis B and C, autoimmune hepatitis, alcoholic hepatitis, primary biliary cirrhosis, and hemochromatosis (14).

However, these findings differed from those obtained by Che Noh I et al., whose investigation was unable to

produce a statistically significant result. They found that circulating TNF- α was not linked to HCV infection. The discrepancies in the findings might be related to the sample size, research population, and participants' ethnic backgrounds (15).

High TNF- α production is linked to an increase in pro-inflammatory cytokine secretion, the activation of proto-oncogenes, and the activation of numerous genes involved in cell proliferation, invasion, and cancer cell metastasis (11, 12). Excess TNF- α production can also result in the creation of free radicals in the form of Reactive Oxygen Species, which can cause further liver damage and genomic instability (16). High TNF- α expression is also considered to be an independent indicator of poor survival in HCC patients (17).

The results of this current study also showed Serum TNF- α expression level HCV group (G2) was significantly elevated than HCV+HCC (G3) with a p-value of 0.010^* significant, but the Serum TNF- α expression level of the HBV group (G4) was higher than HBV+HCC (G5) (61.05 ± 199.1 , 14.36 ± 2.18 respectively) with p-value 0.960.

Comparing between HCV and HBV groups Serum TNF- α was highly increased in HBV than HCV group (61.05 ± 199.1 , 30.07 ± 31.09 respectively) with p-value 0.747 not significant.

However, the serum TNF- α level in (HBV+HCC) group was higher than (HCV+HCC) with a p-value of 0.045^* significance.

SNPs on the TNF- α promoter can affect TNF- levels. They caused elevated and constitutive TNF- α expression and were linked to an increased risk of HCC. (18). TNF- α is a powerful pro-inflammatory cytokine in and of itself (19). Necroinflammation in hepatocytes causes mutagenesis and oncogene activation from proto-oncogenes in host cells, resulting in HCC (20). TNF- α is also known to

generate HCC via the chronic inflammatory route by activating and differentiating hepatic progenitor cells (21).

Funding:

This research received no external funding.

Conflicts of Interest:

The authors affirm that there are no conflicts of interest.

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