BioGecko

Vol 12 Issue 03 2023
ISSN NO: 2230-5807

Evaluation the level of Some Immunological Parameter in patients with hepatitis C virus infection

Moamen Mohamed Shaker[1], Firas Shawqi Algburi[2]
Department of Biology, College of Sciences, Tikrit University, Iraq[1]
College of Medicine, Tikrit University, Iraq[2]
Corresponding authorEmail: moamensh79@gmail.com

Abstract
*Background:*The current study was conducted at Ibn Sina Hospital in Nineveh Governorate, where interleukin 4,10, and 13 were compared with transforming growth factor β1 and C-reactive protein in patients with hepatitis C virus.A total of 90 samples were collected, consisting of 60 samples of patients infected with the hepatitis C virus with 30 control samples.Take 5ml of blood, separate it with a centrifuge, and test the serum. The sample size was equal for Men and Women, and the age range was 18 to 78 years.IL-4, TGFβ1, and CRP were statistically significantWhileIL-10 and IL-13 were not statistically significant.

Key words: Interleukin – 4- Interleukin – 10 - Transforming growth factor β1- hepatitis C virus.

INTRODUCTION:

Hepatitis C virus (HCV) infection is a leadingcause of chronic liver disease and associated morbidity and mortality worldwide (Chigbu *et al*., 2019). Short-term, oral, curable, direct-acting antiviral therapy has transformed the treatment of HCV infectionsincethe2016launchof the first global strategy to eliminate viral hepatitis as a public health threat by 2030 (Indolfi*et al*., 2019). Viral hepatitis (C) was a human problem until1989when the Sherwin-Williams Institute and the CDC announced that scientistshaddiscovered, using polymerase chain reaction (PCR) technology instead ofelectronmicroscopy, thatother virusesbecomehepatitis (non-A -non-B),formerlyviral hepatitis caused by viruses other than (A) or (B) viruses, known as (A) or (B) viruses. Hepatitis viruses aredescribedas aspherical viruses with a relatively small (40-60) nmdiameter,belongingto theFlaviviridaefamily,andhavingan envelopeconsistingof three main components:theviral envelope, the envelope,and single-stranded RNA. The last twoofthesecomponents make up the so-calledviralcore (Hatzakis, *et al* (2021)).

Interleukin – 4 It is a cytokinin that stimulates the differentiation of T cells and the production of antibodies from B cells and plays an important role in the allergic response, so it is expected to play a role in the development of treatment for allergy or its control (Namai*et al*., 2020). After the failure of the innate immunity to work, the adaptive immunity begins to exercise its role from both types of helper T cells (Th1 & Th2). However, T helper 1-associated interleukins have a better response than helper T 2 cells as they are associated with anti-inflammatories (Rasool *etal*., 2022). Viral hepatitis promotes the generation of interleukins, including IL-4, which is involved in cell homeostasis and tissue repair during B-cell signaling and enhances immunity in fighting cancer cells by activating bone marrow-derived tumor suppressor cells as negative regulators of tumorigenic signaling, thus reducing interactions. Immunotherapy and changes in cells, this method are used as a treatment against antibodies that promote cancer (Shabbir *et al*., 2022). It is also an important interleukin in predicting advanced liver cirrhosis in patients with viral hepatitis and factors associated with liver cirrhosis. Studies found that the combination of its level and ferritin level improved diagnostic outcomes and prediction of liver cirrhosis with age (Batsaikhan *et al*., 2019).

Interleukin – 10 It is an interleukin that counteracts excessive inflammatory immune mutations by many cell types, and it exerts its role as an anti-inflammatory by reducing the activity of inflammatory factors and antivirals such as tumor necrosis factor. It regulates the function of transducers and thus provides feedback to proinflammatory T cells, especially Th17 (Zeng *et al*., 2020). Particularly important in

maintaining the intestinal immune homeostasis of microbial antigens as a result of an overactive immune response (Neumann *et al*., 2019). IL-10 plays a very important role as an anti-inflammatory and immunosuppressive agent, and given its immune-regulatory effect, it has been the focus of researchers over the past decade as a focus of study to explore the multiple relationships between it and the risk of viral hepatitis and multiple genetic patterns of infection (Y. Zhang *et al*., 2020). Its level in patients with viral hepatitis had an important role in tracking recovery cases and treatment follow-up, as it showed a difference in levels in the different stages of patients receiving treatment during (12) weeks (Nabeel *et al*., 2022). Interleukin-10 is produced in the form of a drug. Anti-inflammatory and dysregulation in its composition play a role in chronic hepatitis and autoimmune diseases and has a prominent role in the development of viral hepatitis infection in drug users (Noh *et al*., 2021). During chronic hepatitis C virus infection, immune cells become exhausted. Functionally, this thing is reflected in an increase in programmed cell death and an increase in anti-inflammatory levels after the infected patients received treatment. Regular numbers of T cells and regular levels of (IL-10) were observed. T-cell activity and lack of cell exhaustion were observed (Osuch *et al*., 2020).

Interleukin – 13It was previously believed that interleukin-13 is redundant to the body's need, and after the progress of studies, its role has become clear in autoimmune diseases, infections, and fibrosis, and it has a prominent role in allergic diseases and assisting in the functional transformation of helper T cells that show its regulatory role in the immune response (Mao *et al*., 2019) Studies also showed its role in autoimmune liver diseases, lupus erythematosus, psoriasis, and rheumatoid arthritis (R. Wang *et al*., 2018). The increase in interleukin, according to what was studied, was associated with an improvement in the condition of Alzheimer's patients when they started treatment with Dupilumab treatment, as the difference in the ratio of IL-13 had an effective role in the effectiveness of the treatment (Wollenberg *et al*., 2019). It has an important role in inflammatory responses and is similar in some characteristics to (IL-4) and its role with T helper cells of the second type is multidirectional inside the body. It activates macrophages and B cells and produces immunoglobulins and has a role in non-immune cells such as smooth muscle cells and endothelial cells (Marone *et al*., 2019). It also co-operates with IL-4 in its action and gives an evolutionary excess of synergistic immunity (Bieber, 2020). It was noted that it played an important role in the development of the liver condition from a simple injury to liver cancer, especially during treatment, as it was found to have an effective role in patients, and the difference in the therapeutic response of patients was related to its percentage within their bodies and the ability of the immune system to work well and resist the pathogen (Jílková *et al*., 2020). It also has a distinctive role in the therapeutic progress of patients with viral hepatitis, cooperating with vitamin D, because vitamin D also has an anti-inflammatory function and reduces infection with viral hepatitis. Its combination with therapeutic interferons reduces the proliferation of the viral hepatitis virus (Ali *et al*., 2021).

Human TGF β1Transforming growth factor–β1 is a cytokinin central regulator of cell differentiation, migration and proliferation (Frangogiannis, 2020). It is a secreted polypeptide protein with a mass of 25 kD containing 390 amino acids (Tzavlaki& Moustakas, 2020). The mutant is a member of 33 mammalian cells and its signaling contributes to a wide range of physiological and pathological processes and has key roles in growth, immunity, wound healing, cancer, fibrosis, and skeletal and cardiac diseases. Its transcriptional regulation of cellular signaling genes to shape cell behavior (H. Liu & Chen, 2022). The family consists of three major members (TGF β1, TGF β2, and TGF β3) (Lodyga & Hinz, 2020). It performs its action by binding to serine/The dual threonine kinases on the surface of the target cell with intracellular phosphorylated receptors form complexes that then bind to transcription factors and activators to regulate the transcription of target genes and initiate their action (Ning *et al*., 2019). TGFβ1 plays an important role in the development of hepatitis C viral disease by Controlling viral spread and mediating inflammatory responses through virus entry and intracellular replication (Zou *et al*., 2021). Several studies emphasize the relationship between genetic polymorphism and individual differences in clinical features and progression of infection or disease progression due to differences in regulatory regions. Or coding for the TGFβ1 gene could lead to a change in function or loss of the expressed protein (Noh *et al*., 2021).

C-reactive protein (CRP) (0.1-5.0 mg/L blood) is an acute-phase reactant synthesized by hepatocytes in response to an inflammatory response to the host's innate immune response that promotes apoptosis and phagocytosis. In addition, CRP can also activate the classical complement pathway by binding to the C1q protein. CRP production is regulated by pro-inflammatory cytokines such as interleukin 6 (IL-6), IL-1β, and tumor necrosis factor-α. Among these interleukins, IL-6 is the most important inflammatory cytokine regulating CRP gene expression. The CRP gene is located on the chromosome. In hepatocytes, IL-6 induces CRP production at the transcriptional level, which is facilitated by IL-1(Singh et al., 2020)

MATERIALS AND METHODS:

1.Sample collection:From December 1, 2022, to February 30, 2023, patient samples and control samples were collected from Ibn Sina Hospital. The total number of samples was 90, and they were divided into group A of 60 patients. The number of men and women was equal (30 males and 30 females) and Group B had 30 controls (15 males and 15 females). The samples were divided into three groups, the first group was patients and controls, the second group was the sex group, and the third group was the age group. Samples from patients diagnosed with hepatitis C virus were collected from patients with a new sterile syringe of 5 ml Blood was drawn, and each sample was placed in a glass test tube capped with a sealant containing the solidified gel and left at room temperature for two hours. Blood samples were then centrifuged at 1,000g for 20 minutes and serum was stored at -20°C.

2. Methods:

1 - HCV detection: Enzyme-linked immunosorbent assay is used to detect hepatitis C virus antibody (anti-HCV-Ab) (IgG, IgM) in serum or plasma, and to find the binding site of antibody and antibody in the sample. pits ELISA strip Parameter (Ag) combined with horseradish peroxidase (HRP-protein A conjugate) to form an immune enzyme complex characterized by the addition of a substrate solution (TMB-substrate) until etching of the ELISA strip gives a blue color, and then stop. Add stop solution (1N sulfuric acid) to react. The color density (OD) is read at the wavelength (450/620-690nm) by a dedicated reader of the ELISA system. The intensity of the antibody concentration in the sample is directly proportional to the color intensity (Wilber. JC).

2 - Interleukins – 4, 10 and 13:

The working principle is based on a quantitative immunoassay using the sandwich ELISA technique, in which the antigen to be detected is incubated with antibodies added to the test cartridge during the working process. Test wells are coated with the interleukin-specific antibody to be looked for, then added to the sample for study, then a biotin antibody to interleukin after removal of all extraneous compounds, followed by horseradish peroxidase (HRP)-protein A Conjugate) was added after washing the nuclei. (1N for sulfuric acid) To convert the color to yellow, the intensity of the color is proportional to the amount of antibody in the sample and then read at the wavelength (450nm).

3 – TGF β1:The working principle is based on the use of the quantitative immunoassay method using the sandwich ELISA technique, where the antigen that will be detected is incubated with antibodies added during the working method present in the test box. The test pits are coated with antibodies specific to the interleukin to be searched for and then the samples for the research are added, followed by the addition of the biotin antibody for the interleukin after getting rid of any unrelated compounds, then the Horseradish peroxidase enzyme (HRP-Protein A Conjugate) is added after washing the pits. (The sulfuric acid is 1N) to turn the color into yellow, the intensity of the color is directly proportional to the number of antibodies in the sample, then it is read at the wavelength (450 nm).

4 – CRP: In human blood serum or plasma, latex particles marked with (Anti-human CRP) accumulate when mixed with samples that contain protein (C). Agglutination causes a change in absorption depending on the content of the examined sample (CRP), which can be measured by a spectrophotometer. Protein (C) is an acute phase protein found in normal blood serum and increases significantly after tissue infection with bacterial, viral, and other infections and malignancies during tissue necrosis and infections resulting from infection.

3-Statistical analysis:This was done with the software SPSS (Statistical Package for the Social Sciences) (version 26) and Microsoft Office Excel (2016). Data were analyzed using one-way analysis of variance (ANOVA) to determine differences between study groups. Correlation tests are used to

check the correlation between the parameters under study. Results are expressed as mean SD (mean ± SD). The level of statistical significance ($p < 0.01$) and ($p < 0.05$).

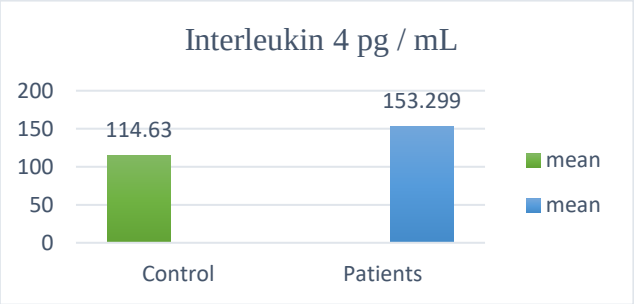
4- Results and Discussion: Human and biochemical parameters for all participants were as shown in Tables:

A – Patients and controls:

Table (1) Comparison between laboratory data of controls and patients

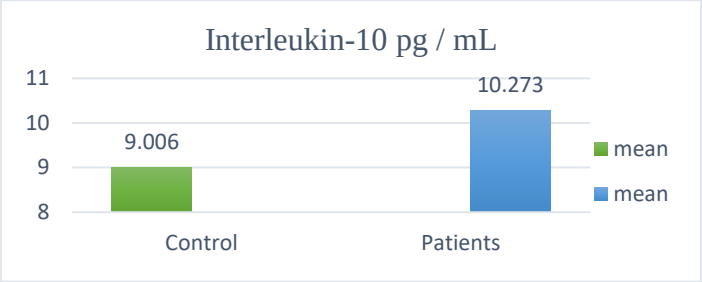
parameters	Control mean ± SD	Patients mean ± SD	p-value
Age	44.8 ± 16.83	37.13 ± 8.45	0.858
IL - 4 pg / mL	114.630±17.343	153.299±71.594	0.010
IL - 10 pg / mL	9.006±2.0263	10.273±4.476	0.210
IL - 13 pg / mL	192.648±32.904	232.650±107.471	0.071
TGF - β1 ng / mL	9.872±1.308	8.713±2.209	0.015
CRP mg/L	3.550±1.252	9.360±4.837	0.000

Interleukin 4 is a spark for the activation of the immune system and the differentiation of its cells, which is why its value increases in pathological conditions. In patients with hepatitis C, interleukin-4 is secreted due to viral activity and changes in metabolic pathways under the influence of viral proteins. Changes in metabolic pathways and activation of the immune system, which plays a role in the differentiation of immune cells to play their role against the virus and hepatitis, current Studyagrees with the study of (Mai *et al*., 2020) and (Radmanić*et al*., 2022).(M. Z. Zhang *et al*., 2017) and his team, as well as a study (Ribeiro *et al*., 2021) mentioned that the high levels of interleukin-4 in patients with hepatitis give different levels during the pathological stage through which the development of viral hepatitis can be inferred, and the results were identical to what was reached in this study.



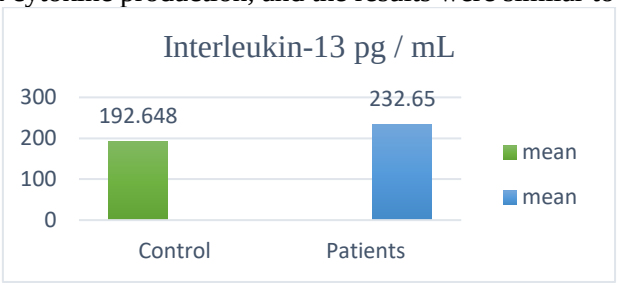
Interleukin- 4Figure (1)

Interleukin-10 has potent anti-inflammatory properties that play a key role in reducing the host's immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue balance by increasing the interleukin ratio. Controls CD8+ levels and produces anti-inflammatory factors by increasing T-cell activity, which promotes cytokine release. This study was consistent with studies by Sultan and his group (Sultan *et al*., 2022) that interleukinis positively correlated with elevated levels of creatinine and urea and their elevation is associated with liver damage. As mentioned in a study (Oft, 2019), the results of his research showed an increase in interleukin-10 in patients compared to healthy patients due to its role in activating immune cells against infection, particularly regulatory T cells, and acting as Antigen against tumors in the body and the development of advanced diseases due to the lack of its secretion in the body. According to a study (Iyer& Cheng, 2012), an increase in interleukin-10 is increasingly associated with advanced stages of cirrhosis. this study showed elevated levels of interleukin-10 as it is anti-inflammatory.



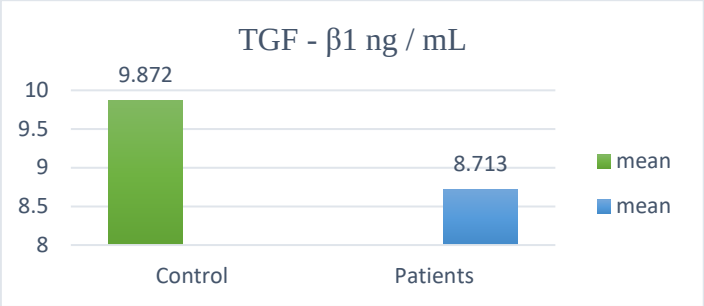
Interleukin - 10 Figure (2)

In the present study, the results of Interleukin - 13 similar to the study (M.Z. Zhang *et al*., 2017) and its group, in which we obtained statistical significance between patients and controls, Interleukin 13 plays a role in macrophage cell polarization in the treatment of kidney damage, particularly acute, therefore it is higher in patients than in healthy people due to its immunoregulatory role and also coincided with a study by Liu, in which he reported an increase in the percentage ofinterleukins in his study (Liu *et al*., 2019). mentioned -13 in patient samples compared to samples for its role in regulating Th2 cell differentiation growth and cytokine production, and the results were similar to those in the study.



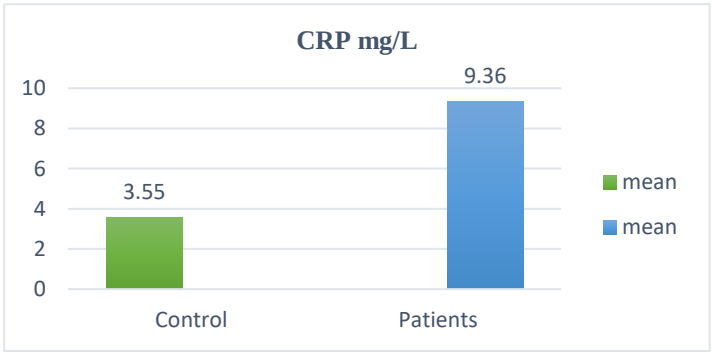
Interleukin - 13 Figure (3)

TGF β1In the study, the relationship was inverse between the percentage of transforming growth factor beta 1 between patients and control, where the percentage of control was higher than that of patients, and there was a statistical significance between control and patient samples. (Gu *et al*., 2020) and his group, the percentage of growth factor is increased in pathological samples compared to control samples. Transforming growth factors and liver fibrosis were considered to be directly associated with liver injuries. Transforming growth factor regulates inflammation in its anti-inflammatory role, and regulates T cells, secretions, cell differentiation, and cellular death. Programmer. In a study (Y. Li *et al*., 2022) it was mentioned that transforming growth factor beta 1 is considered a powerful factor in liver injury and affects liver cells and promotes a rapid response to inflammation by deposition of a large part of it in the cells and its role in the formation of intracellular complexes Accelerating the process of cirrhosis of the liver and the therapeutic possibilities emphasize reducing its activity within the body.The second study matched the current study of a decrease in the percentage of transforming growth factor beta 1 in patients compared to control samples, as the study (Azar *et al*., 2000) in patients exposed to type 1 diabetes, which is considered an autoimmune disease resulting from a genetic and hormonal immune reaction that It affected the level of transforming growth factor beta 1 and also produced kidney diseases resulting from diabetes mellitus. Also, a study (by Olivieri *et al*., 2010) mentioned a decrease in the level of transforming growth factor beta-1 if it is secreted into the body in an inactive form before it reaches its site of action and is affected by insulin activity. The percentages will decrease in patient samples compared to control samples.



TGF - β1 Figure (4)

CRP incurrent study agreed with the study of (Singh *et al*., 2021) and his group and the study of (de Souza Pires-Neto *et al*., 2020), where the percentage of protein C increased in patient samples to initiate an inflammatory response inside the body that is beneficial to the host against the viral pathogen to release cytokines To control stress and reduce damage to liver cells against viral infections as they are more accurate in fighting viral infections by activating hepatic defensive immune cells. The current study also agreed with the study of (Cosentino *et al*., 2019) that the percentage of protein C was elevated in liver injuries compared to healthy people, as the percentage of high protein C was among the indications of the initial diagnosis of acute liver injuries, and Murashima mentioned in his study (Murashima *et al*., 2019) The role of protein C and its high percentage in patients by determining kidney damage in hemodialysis patients, where an increase in protein C levels was observed, corresponding to a decrease in albumin levels due to nephropathy.



CRP mg/LFigure (5)

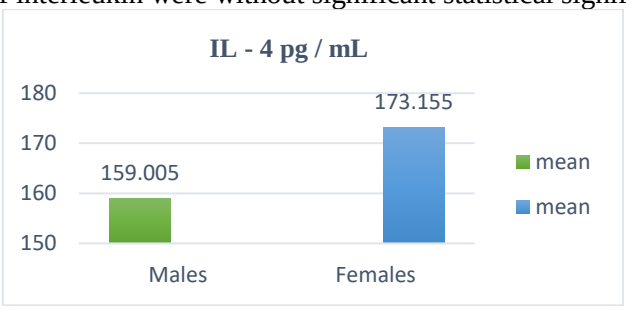
B - Males and Females:

Table (2) Comparison between laboratory data of Males and Females

parameters	Malesmean ± SD	Females mean ± SD	p-value
Age	43.83±6.35	46.4±5.47	0.32
IL - 4 pg / mL	159.005±67.834	173.155±73.139	0.506
IL - 10 pg / mL	12.559±5.151	11.453±4.221	0.330

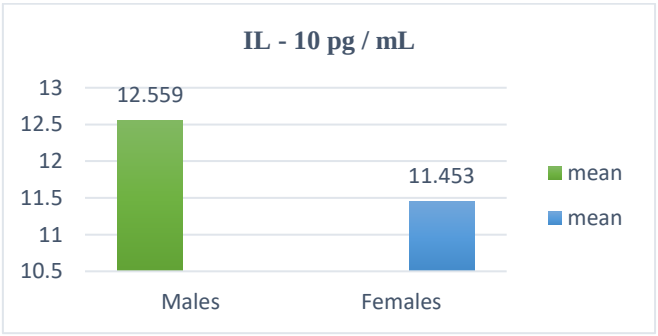
IL - 13 pg / mL	260.332±143.076	268.934±114.819	0.738
TGF - β1 ng / mL	7.651±2.50	8.501±2.356	0.181
CRP mg/L	7.78±3.94	10.35±5.01	0.110

Interleukin-4 was affected by sex (male and female). There was no significant difference between these groups in our study, which matched the following study (Becerra-Díaz *et al*., 2021). Gender affects the increase in interleukin-4 only in some diseases such as asthma, and its rate rises due to hormonal activity. Female physiology, especially premenstrual periods. Likewise, Kimbro's study (Kimbro *et al*., 2022) showed the general effect of gender on the ratio of interleukin-4. As for the age groups, the differences in the ratios of interleukin were without significant statistical significance.



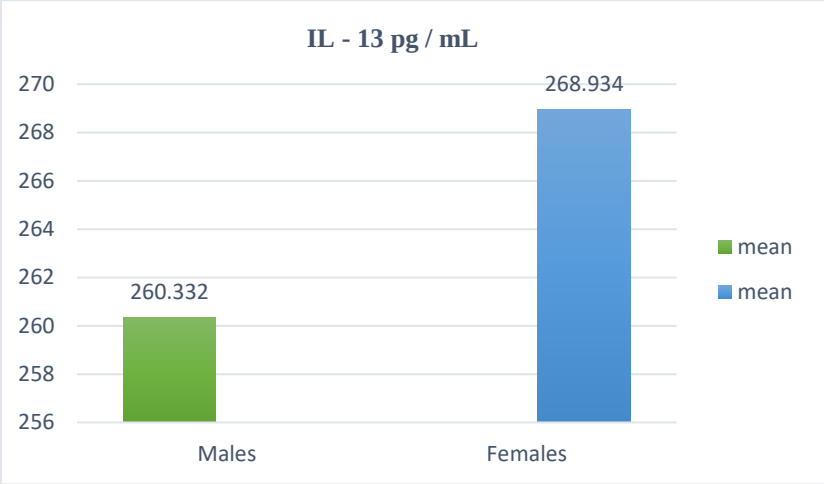
Interleukin-4 Figure (6)

Interleukin-10in terms of sex (male and female), there is an increase in males compared to females without a significant difference or statistical significance, and this is similar to the study of Maria Gabriela (Torcia*et al*., 2012) due to the increase in male hormonal activity that is a reason for the increase in interleukin-10 secretion by a slight increase compared to females. In some pathological cases, the result was opposite to our study, where the interleukin level was higher in females than in males, as in the study (Conway *et al*., 2015), where it was noted that there was less recovery from strokes for females compared to males due to the high level of interleukin 10. And in the study (Chistyakov *et al*., 2018) It was reported that there was no significance of gender in the ratio of interleukin-10.



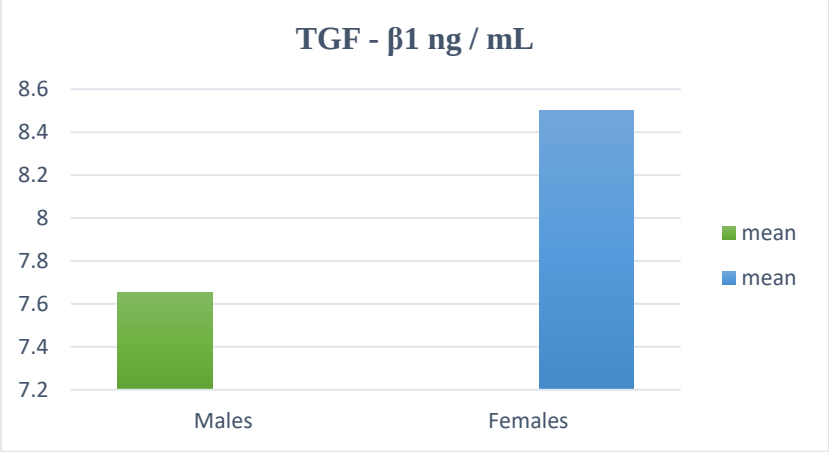
Interleukin- 10 Figure (7)

There is no statistical evidence of the effect of sex on the increase or decrease in the percentage of Interleukin-13, and it is similar to the study (Simard & Gingras, 2001), where there is no effect of high moral value, but there is a slight increase in the percentage of interleukin in females compared to males due to female hormones such as estrogen.



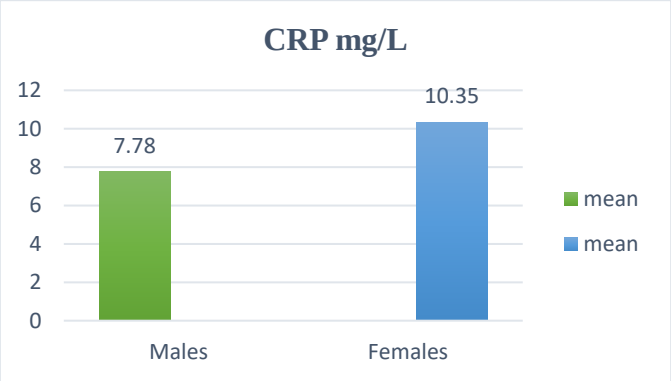
Interleukin - 13 Figure (8)

In terms of sex there is no statistical relationship or significant significance of TGF β 1, and our current study matched the following studies (Roohi *et al*., 2014) and (Abbasi *et al*., 2012).



TGF - β1 Figure (9)

In terms of gender (male and female), the current study showed a higher percentage of protein in females compared to males, but there is no significant correlation or statistical significance between the sexes, according to the study (Jha *et al*., 2019) and the study (Hamann *et al*., 2019), where it is The difference is due to the different physiological conditions of females during different age periods, or it may be related to the average body mass index, where the percentage of protein C in females is higher than that of males, according to the study (Cohen *et al*., 2021), and these studies all agreed with the results of our current study.



CRP Figure (10)

Correlations:

Table (3) Correlations between Parameters

Parameter	Correlations	IL - 4	IL - 10	IL - 13	TGF - β1	CRP
IL - 4 pg / mL	Person cor.	1				
	P value	---				
IL - 10 pg / mL	Person cor.	0.225	1			
	P value	0.083	---			
IL - 13 pg / mL	Person cor.	0.406 *	0.512 *	1		
	P value	0.001	0.000	---		
TGF - β1 ng / mL	Person cor.	0.023	0.025	0.049	1	
	P value	0.862	0.852	0.711	---	
CRP mg/L	Person cor.	0.052	0.034	0.021	0.094	1
	P value	0.042	0.797	0.876	0.475	---

- Correlations betweenIL - 4 and IL –13:

There is a positive relationship between them and there is a statistical significance, as they have a parallel role in the activation of Type 2 immunity through direct and indirect interactions by activating T cells and accelerating the differentiation of immune cells(Gurram & Zhu, 2019)(Zhao et al., 2020).

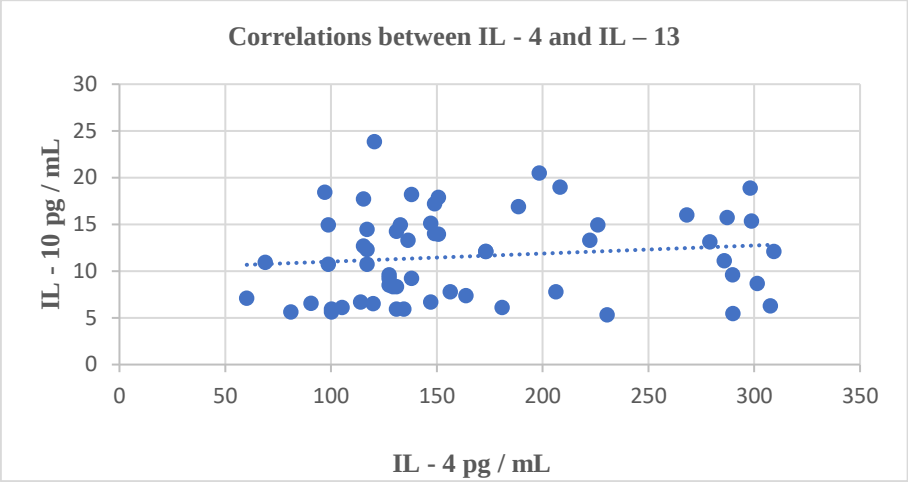


Figure (11) Correlations between IL - 4 andIL – 13

- Correlations between IL - 10 and IL – 13:

There is a positive, statistically significant relationship between the two, due to their synergistic role in the outcome of hepatitis infection, pathological development, and therapeutic response during the stages of the disease(Edwards-Smith *et al*., 1999)(Knapp *et al*., 2003) .

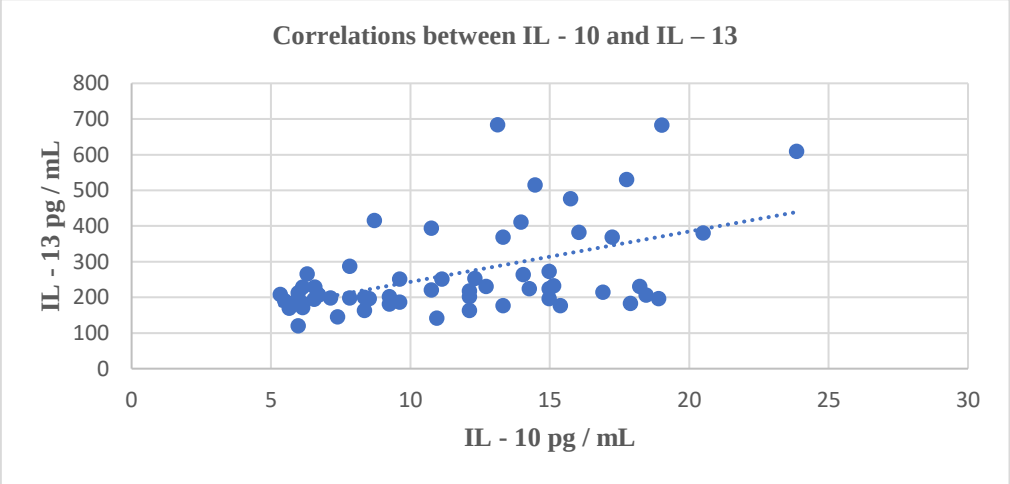


Figure (12) Correlations between IL - 10 and IL – 13

References

1. Abbasi, F., Amiri, P., Sayahpour, F. A., Pirmoradi, S., Abolhalaj, M., Larijani, B., ... & Amoli, M. M. (2012). TGF-β and IL-23 gene expression in unstimulated PBMCs of patients with diabetes. Endocrine, 41, 430-434.
2. Ali, M. E., Halby, H. M., Ali, M. Y., Hassan, E. A., El-Mokhtar, M. A., Sayed, I. M., ... & Mahran, Z. G. (2021). Role of Serum Vitamin D, Interleukin 13, and microRNA-135a in Hepatocellular Carcinoma and Treatment Failure in Egyptian HCV-Infected Patients Receiving Direct Antiviral Agents. Viruses, 13(10), 2008.

3. Azar, S. T., Salti, I., Zantout, M. S., & Major, S. (2000). Alterations in plasma transforming growth factor β in normoalbuminuric type 1 and type 2 diabetic patients. The Journal of Clinical Endocrinology & Metabolism, 85(12), 4680-4682.
4. Batsaikhan, B., Lu, M. Y., Yeh, M. L., Huang, C. I., Huang, C. F., Lin, Z. Y., ... & Dai, C. Y. (2019). Elevated interleukin-4 levels predicted advanced fibrosis in chronic hepatitis C. Journal of the Chinese Medical Association, 82(4), 277-281.
5. Becerra-Díaz, M., Lerner, A. D., Diana, H. Y., Thiboutot, J. P., Liu, M. C., Yarmus, L. B., ... & Heller, N. M. (2021). Sex differences in M2 polarization, chemokine and IL-4 receptors in monocytes and macrophages from asthmatics. Cellular immunology, 360, 104252.
6. Bieber, T. (2020). Interleukin-13: targeting an underestimated cytokine in atopic dermatitis. Allergy, 75(1), 54-62.
7. Chigbu, D. I., Loonawat, R., Sehgal, M., Patel, D., & Jain, P. (2019). Hepatitis C virus infection: host–virus interaction and mechanisms of viral persistence. Cells, 8(4), 376.
8. Chistyakov, D. V., Azbukina, N. V., Astakhova, A. A., Goriainov, S. V., Chistyakov, V. V., & Sergeeva, M. G. (2018). Sex-mediated differences in lps induced alterations of TNFα, IL-10 expression, and prostaglandin synthesis in primary astrocytes. International journal of molecular sciences, 19(9), 2793.
9. Cohen, E., Margalit, I., Shochat, T., Goldberg, E., & Krause, I. (2021). Markers of chronic inflammation in overweight and obese individuals and the role of gender: a cross-sectional study of a large cohort. Journal of Inflammation Research, 14, 567.
10. Conway, S. E., Roy-O'Reilly, M., Friedler, B., Staff, I., Fortunato, G., & McCullough, L. D. (2015). Sex differences and the role of IL-10 in ischemic stroke recovery. Biology of sex differences, 6(1), 1-5.
11. Cosentino, N., Genovese, S., Campodonico, J., Bonomi, A., Lucci, C., Milazzo, V., ... &Marenzi, G. (2019). High-sensitivity C-reactive protein and acute kidney injury in patients with acute myocardial infarction: a prospective observational study. Journal of Clinical Medicine, 8(12), 2192.
12. Crouchet, E., Bandiera, S., Fujiwara, N., Li, S., El Saghire, H., Sun, X., ... & Baumert, T. (2020). Single cell RNA-seq of patient liver tissues uncover HRH2+/CLEC5a high/macro low macrophages as therapeutic target for the treatment of liver fibrosis and cancer prevention. *Journal of Hepatology*, *73*, S97.
13. de Souza Pires-Neto, O., Amoras, E. D. S. G., Queiroz, M. A. F., Demachki, S., da Silva Conde, S. R., Ishak, R., ... &Vallinoto, A. C. R. (2020). Hepatic TLR4, MBL, and CRP gene expression levels are associated with chronic hepatitis C. Infection, Genetics and Evolution, 80, 104200.
14. Edwards-Smith, C. J., Jonsson, J. R., Purdie, D. M., Bansal, A., Shorthouse, C., & Powell, E. E. (1999). Interleukin-10 promoter polymorphism predicts the initial response of chronic hepatitis C to interferon alfa. *Hepatology*, *30*(2), 526-530.
15. Frangogiannis, N. G. (2020). Transforming growth factor–β in tissue fibrosis. Journal of Experimental Medicine, 217(3).
16. Gu, Y. Y., Liu, X. S., Huang, X. R., Yu, X. Q., & Lan, H. Y. (2020). Diverse role of TGF-β in kidney disease. Frontiers in cell and developmental biology, 8, 123.
17. Gurram, R. K., & Zhu, J. (2019). Orchestration between ILC2s and Th2 cells in shaping type 2 immune responses. *Cellular & molecular immunology*, *16*(3), 225-235.
18. Hamann, P. D., Shaddick, G., Hyrich, K., Green, A., McHugh, N., Pauling, J. D., & BSRBR-RA Contributors Group. (2019). Gender stratified adjustment of the DAS28-CRP improves inter-score agreement with the DAS28-ESR in rheumatoid arthritis. Rheumatology, 58(5), 831-835.
19. Hatzakis, A. (Ed.). (2021). Hepatitis C: Epidemiology, prevention and elimination. Springer.

20. Indolfi, G., Easterbrook, P., Dusheiko, G., El-Sayed, M. H., Jonas, M. M., Thorne, C., ... &Penazzato, M. (2019). Hepatitis C virus infection in children and adolescents. The lancet Gastroenterology & hepatology, 4(6), 477-487.
21. Iyer, S. S., & Cheng, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Critical Reviews™ in Immunology, 32(1).
22. Jha, M. K., Minhajuddin, A., Chin-Fatt, C., Greer, T. L., Carmody, T. J., & Trivedi, M. H. (2019). Sex differences in the association of baseline c-reactive protein (CRP) and acute-phase treatment outcomes in major depressive disorder: Findings from the EMBARC study. Journal of psychiatric research, 113, 165-171.
23. Kimbro, K. S., Wamble, E., & Williams, A. (2022). Sex differences in IL-3, IL-4, and IL-7 correlation with cholesterol and triglyceride levels in African Americans with high HbA1c. The Journal of Immunology, 208(1_Supplement), 45-13.
24. Knapp, S., Hennig, B. J., Frodsham, A. J., Zhang, L., Hellier, S., Wright, M., ... &Thursz, M. R. (2003). Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. *Immunogenetics*, *55*, 362-369.
25. Li, Y., Fan, W., Link, F., Wang, S., & Dooley, S. (2022). Transforming growth factor β latency: A mechanism of cytokine storage and signalling regulation in liver homeostasis and disease. *JHEP Reports*, *4*(2), 100397.
26. Liu, C., Qin, L., Ding, J., Zhou, L., Gao, C., Zhang, T., ... & Xu, Y. (2019). Group 2 innate lymphoid cells participate in renal fibrosis in diabetic kidney disease partly via TGF-β1 signal pathway. Journal of Diabetes Research, 2019.
27. Lodyga, M., & Hinz, B. (2020, May). TGF-β1–a truly transforming growth factor in fibrosis and immunity. In *Seminars in cell & developmental biology* (Vol. 101, pp. 123-139). Academic Press.
28. Macek Jílková, Z., Seigneurin, A., Coppard, C., Ouaguia, L., Aspord, C., Marche, P. N., ... &Decaens, T. (2020). Circulating IL-13 is associated with de novo development of HCC in HCV-infected patients responding to direct-acting antivirals. Cancers, 12(12), 3820.
29. Mai, M., Jiang, Y., Wu, X., Liu, G., Zhu, Y., & Zhu, W. (2020). Association of TGF-β1, IL-4, and IL-10 polymorphisms with chronic kidney disease susceptibility: a meta-analysis. *Frontiers in genetics*, *11*, 79.
30. Mao, Y. M., Zhao, C. N., Leng, J., Leng, R. X., Ye, D. Q., Zheng, S. G., & Pan, H. F. (2019). Interleukin-13: A promising therapeutic target for autoimmune disease. Cytokine & Growth Factor Reviews, 45, 9-23.
31. Marone, G., Granata, F., Pucino, V., Pecoraro, A., Heffler, E., Loffredo, S., ... &Varricchi, G. (2019). The intriguing role of interleukin 13 in the pathophysiology of asthma. Frontiers in pharmacology, 10, 1387.
32. Murashima, M., Nishimoto, M., Kokubu, M., Hamano, T., Matsui, M., Eriguchi, M., ... & Tsuruya, K. (2019). Inflammation as a predictor of acute kidney injury and mediator of higher mortality after acute kidney injury in non-cardiac surgery. Scientific reports, 9(1), 20260.
33. Nabeel, M. M., Darwish, R. K., Alakel, W., Maher, R., Mostafa, H., Hashem, A., ... & Marie, M. S. (2022). Changes in serum interferon gamma and interleukin-10 in relation to direct-acting antiviral therapy of chronic hepatitis C genotype 4: a pilot study. Journal of Clinical and Experimental Hepatology, 12(2), 428-434.
34. Namai, F., Shigemori, S., Ogita, T., Sato, T., & Shimosato, T. (2020). Construction of genetically modified Lactococcus lactis that produces bioactive anti-interleukin-4 single-chain fragment variable. Molecular Biology Reports, 47, 7039-7047.
35. Neumann, C., Scheffold, A., & Rutz, S. (2019, August). Functions and regulation of T cell-derived interleukin-10. In Seminars in immunology (Vol. 44, p. 101344). Academic Press.

36. Ning, J., Zhao, Y., Ye, Y., & Yu, J. (2019). Opposing roles and potential antagonistic mechanism between TGF-β and BMP pathways: Implications for cancer progression. *EBioMedicine*, *41*, 702-710.
37. Noh, I. C., Ahmad, I., Suraiya, S., Musa, N. F., Nurul, A. A., &Ruzilawati, A. B. (2021). Cytokine (IL-10, IL-6, TNF-α and TGF-β1) Gene Polymorphisms in Chronic Hepatitis C Virus Infection among Malay Male Drug Abusers. Biomedicines, 9(9), 1115.
38. Oft, M. (2019, August). Immune regulation and cytotoxic T cell activation of IL-10 agonists–Preclinical and clinical experience. In Seminars in immunology (Vol. 44, p. 101325). Academic Press.
39. Olivieri, A., De Angelis, S., Dionisi, S., D'Annunzio, G., Locatelli, M., Marinaro, M., ... &Boirivant, M. (2010). Serum transforming growth factor β1 during diabetes development in non-obese diabetic mice and humans. Clinical & Experimental Immunology, 162(3), 407-414.
40. Osuch, S., Laskus, T., Berak, H., Perlejewski, K., Metzner, K. J., Paciorek, M., ... & Caraballo Cortés, K. (2020). Decrease of T-cells exhaustion markers programmed cell death-1 and T-cell immunoglobulin and mucin domain-containing protein 3 and plasma IL-10 levels after successful treatment of chronic hepatitis C. Scientific Reports, 10(1), 16060.
41. Radmanić, L., Bodulić, K., Šimičić, P., Vince, A., &ŽidovecLepej, S. (2022). The effect of treatment-induced viral eradication on cytokine and growth factor expression in chronic hepatitis C. Viruses, 14(8), 1613.
42. Rasool, K. H., Alubadi, A. E. M., & Al-Bayati, I. F. I. (2022). The role of Serum Interleukin-4 and Interleukin-6 in Helicobacter pylori-infected patients. Microbial Pathogenesis, 162, 105362.
43. Ribeiro, C. R. D. A., de Almeida, N. A. A., Martinelli, K. G., Pires, M. A., Mello, C. E. B., Barros, J. J., & De Paula, V. S. (2021). Cytokine profile during occult hepatitis B virus infection in chronic hepatitis C patients. Virology Journal, 18(1), 1-11.
44. Roohi, A., Tabrizi, M., Abbasi, F., Ataie-Jafari, A., Nikbin, B., Larijani, B., ... & Maleki, A. (2014). Serum IL-17, IL-23, and TGF-β levels in type 1 and type 2 diabetic patients and age-matched healthy controls. BioMed research international, 2014.
45. Shabbir, M., Badshah, Y., Khan, K., Trembley, J. H., Rizwan, A., Faraz, F., ... & Razak, S. (2022). Association of CTLA-4 and IL-4 polymorphisms in viral induced liver cancer. BMC cancer, 22(1), 518.
46. Simard, J., & Gingras, S. (2001). Crucial role of cytokines in sex steroid formation in normal and tumoral tissues. Molecular and cellular endocrinology, 171(1-2), 25-40.
47. Singh, S., Bansal, A., & Kumar, P. (2021). CRP levels in viral hepatitis: a meta-analysis study. International Journal of Infection, 8(1).
48. Sultan, R. H., Abdallah, M., Ali, T. M., Ahmed, A. E., Assal, H. H., Elesawy, B. H., & Ahmed, O. M. (2022). The associations between cytokine levels, kidney and heart function biomarkers, and expression levels of angiotensin-converting enzyme-2 and neuropilin-1 in covid-19 patients. Vaccines, 10(7), 1045.
49. Torcia, M. G., Nencioni, L., Clemente, A. M., Civitelli, L., Celestino, I., Limongi, D., ... & Palamara, A. T. (2012). Sex differences in the response to viral infections: TLR8 and TLR9 ligand stimulation induce higher IL10 production in males. PloS one, 7(6), e39853.
50. Tzavlaki, K., & Moustakas, A. (2020). TGF-β Signaling. Biomolecules, 10(3), 487.
51. Wang, R., Lu, Y. L., Huang, H. T., Qin, H. M., Lan, Y., Wang, J. L., ... & Wei, Y. S. (2018). Association of interleukin 13 gene polymorphisms and plasma IL 13 level with risk of systemic lupus erythematosus. *Cytokine*, *104*, 92-97.
52. Wollenberg, A., Howell, M. D., Guttman-Yassky, E., Silverberg, J. I., Kell, C., Ranade, K., ... & van der Merwe, R. (2019). Treatment of atopic dermatitis with tralokinumab, an anti–IL-13 mAb. Journal of Allergy and Clinical Immunology, 143(1), 135-141.

53. Zeng, C., Zhang, C., Walker, P. G., & Dong, Y. (2020). Current topics in microbiology and immunology. Formulation and Delivery Technologies for mRNA Vaccines, 1-40.
54. Zhang, M. Z., Wang, X., Wang, Y., Niu, A., Wang, S., Zou, C., & Harris, R. C. (2017). IL-4/IL-13–mediated polarization of renal macrophages/dendritic cells to an M2a phenotype is essential for recovery from acute kidney injury. Kidney international, 91(2), 375-386.
55. Zhao, W., Tan, C., Yu, X., Yu, R., Mei, Q., & Cheng, Y. (2020). A 7-amino acid peptide mimic from hepatitis C virus hypervariable region 1 inhibits mouse lung Th9 cell differentiation by blocking CD81 signaling during allergic lung inflammation. Journal of Immunology Research, 2020.
56. Zou, L. L., Li, J. R., Li, H., Tan, J. L., Wang, M. X., Liu, N. N., ... & Peng, Z. G. (2021). TGF-β isoforms inhibit hepatitis C virus propagation in transforming growth factor beta/SMAD protein signalling pathway dependent and independent manners. Journal of Cellular and Molecular Medicine, 25(7), 3498-3510.