

## Correlation of CXCL10 and TGFB1 levels with response to direct-acting antiviral in chronic HCV patients: A guide for drug responsiveness in cases of undetectable viral load



Hend Moness<sup>1</sup>, Wafaa Abdelhamid<sup>2</sup>, Hanaa Fathelbab<sup>2</sup>, Nadia Ismail<sup>3</sup>, Esraa Alaa Ahmed<sup>1</sup>, Omima M Mohamed<sup>1</sup>, Noha M. Abdullah<sup>1\*</sup>

<sup>1</sup>Clinical Pathology Department, Faculty of Medicine, Minia University, Minia, Egypt.

<sup>2</sup>Department of endemic diseases, Faculty of Medicine, Minia University, Minia, Egypt.

<sup>3</sup>Internal Medicine, Faculty of Medicine, Minia University, Minia, Egypt

**Abstract**— Hepatitis C virus (HCV) infection poses a significant public health problem in Egypt and developing countries, accounting for the highest prevalence globally. This study aimed to measure the level of CXCL-10 and TGF-B1 in chronic HCV patients before and following treatment with direct-acting antiviral therapy (DAAs). The study included 50 subjects, divided into two groups: thirty individuals with HCV infection and twenty HCV-negative individuals who were considered to be in good health and served as the control group. We selected 30 HCV-positive patients who did not start treatment from all HCV-positive patients who attended an endemic clinic. Patients were followed up for 12 or 24 weeks after their DAA treatment. Then, based on treatment response, we classified these patients into (15 HCV-positive patients who responded to treatment) and (15 HCV-positive patients who did not respond to treatment). CXCL10 and TGFB1 were measured by enzyme immunoassay (EIA). There was a significant positive correlation between CXCL10 and TGFB1 levels in all studied groups. Compared to responders and control groups, CXCL10 and TGFB1 levels were significantly higher in HCV patients and non-responders who did not initiate therapy. Therefore, CXCL10 and TGFB1 could be combined with HCVPCR to improve the sensitivity and specificity of treatment response detection.

**Keywords:** CXCL10, TGFB1, HCV, direct acting antiviral therapy (DAAs)

### 1. Introduction

Hepatitis C virus (HCV) infection is a significant cause of chronic liver disease. Globally, an estimated 58 million individuals have chronic HCV infection, with approximately 1.5 million new infections occurring annually. About 3.2 million adolescents and children are infected with chronic hepatitis C [1]. For decades, Egypt had the highest prevalence of HCV infection worldwide. Nonetheless, significant progress has been made to eradicate the disease. These measures resulted in the mass treatment of approximately 2.5 million Egyptian HCV patients using the most effective regimens of direct-acting antiviral therapy [2]. Between October 2018 and April 2019, nearly 50 million of the population were screened, and those who tested positive were referred for treatment [3]. Recent studies have demonstrated that the use of new direct-acting antiviral drugs (DAAs) yields better outcomes with fewer side effects and shorter treatment durations, some as short as eight weeks [4]. In the past, a combination of pegylated-interferon (PEG-IFN) alpha and ribavirin (RBV) was utilized. Over time, the clinician used direct-acting antiviral therapy such as simeprevir, sofosbuvir, and Ledipasvir [5]. These drugs target various points of the HCV viral replication cycle by directly binding to components of the replicase complex or by initiating Ribonucleic acid (RNA) chain termination [6].

Several cytokines and chemokines may be associated with the persistence of HCV infection and the development of fibrosis, including transforming growth factor (TGF)-1 and C-X-C motif chemokine ligand 10 (CXCL10). During HCV infection, CXCL10 is essential for the coordination of intrahepatic inflammatory responses and the transition from innate to adaptive immunity. During HCV infection, hepatocytes and intrahepatic lymphocytes are the primary producers of CXCL10 [7]. Patients with chronic hepatitis C have elevated peripheral and intrahepatic CXCL10 levels, which are indicative of increased liver inflammation and advanced fibrosis [8]. Consequently, the determination of high CXCL10 levels in peripheral blood can be used as a marker of T-helper (1) orientated immune response as its activation causes an increased IFN- $\gamma$  and TNF- $\alpha$  production that stimulates the target cells to secrete CXCL10, thereby stimulating the immune cascade [9]. Moreover, the primary source of TGF $\beta$ 1 is Kupffer cells [10], which are activated by the continuous liver injury caused by HCV infection. Additionally, it was reported that HCV-infected hepatocytes secrete TGF $\beta$ 1 in response to the elevation of reactive oxygen species and the presence of HCV core protein [11]. Increased levels of TGF- $\beta$ 1 mRNA have been identified in patients with chronic liver disease with a positive correlation with levels of the amino-terminal peptide of type III procollagen, a serum marker of hepatic fibrogenesis [12]. Studies have demonstrated that individuals undergoing anti-HCV therapy who do not exhibit a positive response tend to experience an elevation in their levels. In contrast, individuals who responded to the therapy demonstrated decreased levels [13]. Therefore, this study aimed to measure the level of CXCL-10 and TGF- $\beta$ 1 in chronic HCV patients to determine their role in responsiveness to direct-acting antiviral therapy.

## **2. Methods**

### **2.1 Study Design**

The study was conducted at the Clinical Pathology Department, Faculty of Medicine, Minia University, from September 2020 to February 2021. This study was approved by the ethics committee of Minia University. Informed consent was obtained from all participants (Approval No.622:5/2020). A total of 50 subjects participated in that study; they were divided into 30 HCV-positive patients and 20 HCV-negative apparently healthy people as a control group (Group III). We selected 30 HCV-positive patients who did not start treatment (Group I) from those who attended the endemic clinic. Patients were followed up after treatment for 12 or 24 weeks, according to (the National Committee for Control of Viral Hepatitis). The treatment protocol was Sofosbuvir, Daclatasvir, and Ribavirin for 12 weeks or Sofosbuvir and Daclatasvir for 24 weeks. According to treatment response, we categorized patients into group IIa (15 HCV-positive patients who responded to treatment) and group IIb (15 patients who did not respond to treatment). Patients with fatty liver disease, HBV infection, child score C, hepatocellular carcinoma, extrahepatic malignancy, alcohol hepatitis, type 1 diabetes, Graves' ophthalmology, systemic rheumatological disorders, and pregnancy were excluded from the study. All subjects were subjected to full history taking, including risk factors of HCV infection, abdominal examination, and ultrasonography exam.

### **2.2 Laboratory Investigations**

First, 8 ml of venous blood was withdrawn from every subject using a disposable plastic syringe. This sample was divided as follows: 1ml in Ethylene Diamine Tetra acetic Acid (EDTA) containing tube for CBC. After that, 1.8 ml of blood was placed on a tube containing 0.2 ml trisodium citrate to detect prothrombin concentration (dilution 1:9). Then, 5 ml of venous blood was transferred into two plain tubes. Each tube was allowed to clot in the incubator for 2.5 ml and then centrifuged. The expressed serum was used to determine liver function and HBsAg and the other for determination of CXCL10 and TGF $\beta$ 1. Complete blood count (CBC) was determined by an automated cell counter (Celltac ES, Nihon Kohden Corporation, Automated hematology analyzer, Japan). Liver function tests were

assayed using a fully automated clinical chemistry auto-analyzer system (Auto-analyzer SelectraproM, ELITech Group, clinical chemistry automation systems, Finland). Prothrombin concentration was determined using a fully automated coagulometerStago compact max (France). HBsAg was detected by chemiluminescence using (Cobas e411 analyzer, HITACHI, Germany). Finally, CXCL10 and TGFB1 were measured by EIA. For CXCL10, we used (Human CXCL10/IP-10 Quantikine ELISA Kit, catalog no: DIP100, R&D Systems, USA). TGFB1 we used (SinoGeneClon Biotech Co., Ltd, catalog no:19060) according to the manufacturer's recommendations.

### **2.3 Statistical Analysis**

Statistical analysis was conducted using version 20 of Statistical Package for the Social Sciences (SPSS). Quantitative data were summarized as mean, standard deviation (SD) for normally distributed data, and median for abnormally distributed data. Qualitative data were expressed as frequency (%). Quantitative results were compared using either Mann-Whitney or Analysis Of Variance (ANOVA) followed by Bonferroni correction. Qualitative data were analyzed using the Chi-square test (X<sup>2</sup>) and Fisher exact test. For correlation studies, Spearman and Pearson correlations were used as appropriate. Receiver operating characteristic (ROC) curve analysis was done to calculate the area under the curve (AUC), optimal cutoff point, sensitivity, specificity, positive and negative predictive values, and accuracy of variables predicting cases. Statistical significance was defined for all analyses as p-values  $\leq 0.05$  and highly significant if less than 0.001.

### **3. Results**

Regarding the demographic data for studied subjects, there were no statistically significant differences between the studied groups regarding age or sex, as shown in (Table 1). Hematological parameters revealed a statistically significant decrease in the hemoglobin level in patients who did not initiate therapy and exhibited statistically significant increases in direct and total bilirubin. Nonnon-responders demonstrated statistically significant increases in direct and total bilirubin (p=0.010 and 0.008, respectively). Non-respondents' AST and ALT levels were significantly higher than respondents (p= 0.011 and 0.030, respectively). In addition, direct bilirubin, total bilirubin, AST, and ALT were significantly higher in the non-responder group than in the control group (p-values of 0.049 and 0.007, respectively, for INR). In contrast, the platelet count was statistically significantly lower when a group of patients did not initiate therapy compared to the control and non-responder groups(p-values of 0.009 and 0.008, respectively) (Table 2).

In comparison to the responder group (p= 0.007 and 0.004) and the control group (p=m0.030 and 0.003), patients who did not begin treatment had a statistically significant increase in direct and total bilirubin, as measured by liver function tests. Regarding responders and non-responders, direct and total bilirubin significantly increased among non-responders (p=0.010, 0.008) when compared with responders. Both AST and ALT were statistically elevated in non-responders compared to respondents (p= 0.011, 0.030). In addition, direct bilirubin, total bilirubin, AST, and ALT were significantly higher in the non-responder group compared to the control group (p= 0.039, 0.008, 0.012, 0.031) (Table 3). Finally, there was a statistically significant decrease in albumin when compared to responders (p= 0.023).

Regarding viral RNA, there were statistically significant differences between patients before treatment and (responder, non-responder and control) (p-values of 0.02, 0.018, and 0.011, respectively). In addition, there was a statistically significant difference between non-responders and both responders and controls (at a p-value <0.0001). There was no statistically significant difference between responder and control viral RNA levels (p-value = 1.0) (Table 4).

CXCL-10 was found to have the highest concentration in both patients before treatment and non-responder groups. (Table 5 and Figure 1). In addition, all groups demonstrated statistically significant differences in CXCL-10 levels. Concerning the level of TGFB1, it also showed the highest levels in patients before treatment and non-responder groups (Fig. 2). The level of statistical significance was set at a p-value of  $< 0.0001$ .

There was a strong positive correlation between CXCL10 and TGFB1 concentrations in Group I ( $r = 0.874$ ,  $p\text{-value} = 0.299$ ) in Group IIa ( $r = 0.750$ ,  $p\text{-value} = 0.354$ ) and Group IIb ( $r = 0.936$ ,  $p\text{-value} = 0.730$ ) (Fig. 3a,b&c). Additionally, hematological and liver function tests were correlated with CXCL10 and TGFB1 levels among the various study groups. For AST in Group I ( $r = 0.908$ ,  $p\text{-value} = 0.327$ ), in Group IIa ( $r = 0.953$ ,  $p\text{-value} = 0.735$ ), and in group IIb ( $r = 0.917$ ,  $p\text{-value} = 0.197$ ) (Fig 4a,b&c). The correlation coefficient ( $r$ ) for ALT was found to be 0.904 ( $p\text{-value} = 0.489$ ) in Group I, 0.911 ( $p\text{-value} = 0.215$ ) in Group IIa, and 0.915 ( $p\text{-value} = 0.180$ ) in Group Iib (Fig 5a, b &c). Furthermore, TGFB1 exhibited strong positive associations with AST and ALT levels in both pre-treatment patients and non-responders. In contrast, it demonstrated a moderate correlation with responders. In the AST group, the correlation coefficient ( $r$ ) was found to be 0.835 with a  $p\text{-value}$  of 0.937. Similarly, in group IIa, the correlation coefficient was 0.713, with a  $p\text{-value}$  of 0.954. In Group IIB, the correlation coefficient was 0.870 with a  $p\text{-value}$  of 0.465 (Figures 6a, 6b, and 6c). Regarding ALT, the correlation coefficient in Group I was 0.833, with a  $p\text{-value}$  of 0.063. In Group IIa, the correlation coefficient was 0.691, with a  $p\text{-value}$  of 0.861. In group IIb, the correlation coefficient was 0.868 with a  $p\text{-value}$  of 0.490 (Fig 8a & b, Fig 9 a & b, Supplementary Tables S. 4 & 5). In the pre-treatment group, a positive correlation was observed between the concentration of TGFB1, CXCL10, and the RNA level of HCV ( $r = 0.560$ ,  $p\text{-value} = 0.929$ ) for TGFB1 ( $r = 0.798$ ,  $p\text{-value} = 0.167$ ) for CXCL10. Similarly, in the non-responder group, a positive correlation was found between the concentration of TGFB1 and CXCL10 and the RNA level of HCV ( $r = 0.612$ ,  $p\text{-value} = 0.679$ ,  $r = 0.869$ ,  $p\text{-value} = 0.459$ ) respectively (Fig 8a & b, Fig 9 a & b, Supplementary tables S. 4 & 5).

In the non-responder group, ROC curve analysis showed that the AUC was 0.887 (95% confidence interval [CI] = 0.796 – 0.978), while the AUC for TGFB1 was 0.921 (95% CI = 0.849 – 0.993). The sensitivity of CXCL10 was found to be higher (93.33%, 86.67%) compared to TGFB1, while its specificity was lower (74.29%, 82.86%), respectively. The study revealed that CXCL10 exhibited higher sensitivity and specificity compared to TGFB1 in responders. Conversely, in non-responders, TGFB1 demonstrated greater specificity but lower sensitivity when compared to CXCL10 (Fig. 10 and supplementary table S. 6).

#### 4. Discussion

Chronic HCV infection is one of the most prevalent causes of cirrhosis and a significant global health concern. Chronic HCV infection has also been linked to an increased risk of hepatocellular carcinoma [14]. Egypt had the highest prevalence of chronic hepatitis C infection worldwide, with more than 90% of HCV patients infected with genotype 4 virus type [15]. The 2018 updated guidelines of WHO recommended pan-genotypic direct-acting antivirals for treatment. These medications are highly effective and interferon- and ribavirin-free for the majority of hepatitis C patients [16]. DAAs drugs have demonstrated efficacy in treating the majority of HCV infections. However, the eradication of HCV with DAAs treatment partially restores immune cell function [17]. Viral resistance to HCV direct-acting antiviral drugs has emerged as a crucial factor in determining the optimal use of DAAs for treating HCV infection. Resistance-associated substitutions (RASs) may develop during therapy or be present at baseline, thereby increasing the likelihood of treatment

failure[18].

In order to determine the role of CXCL-10 and TGF-B1 in the responsiveness of chronic HCV patients to direct-acting antiviral therapy, we aimed to measure their levels in this study. Concerning CXCL10, there was a significant increase in CXCL10 levels in pre-treatment and non-responder patients compared to responder and control groups. This was consistent with the findings of Mascia et al., who reported that CXCL10 levels in HCV-infected patients were significantly higher than in healthy donors ( $p= 0.0001$ ). This is caused by the rebound of viral replication and significantly decreased in patients reaching Sustained virologic response after 12 weeks but did not reach normal values and remained significantly higher than in healthy donors ( $p = 0.001$ ). This finding suggests the persistence of residual inflammation. Nevertheless, it contradicts our finding as CXCL10 retained its normal levels compared to the control group ( $p= 0.410$ ). The decline of this chemokine could indicate disruption of the intrahepatic virus-host interaction. Furthermore, Ferrari et al., 2019 suggested that CXCL10 shows a significant increase in non-responders and patients with fibrosis compared to responders [19-20]. Moreover, Zeremski et al. suggested a decline in peripheral CXCL10 levels after 1 and 2 weeks of DAAs therapy, suggesting it to be a marker in predicting viral clearance [21]. Moreover, El-Ahwany et al., 2015 reported that CXCL10 levels significantly increased in the non-responder group either before treatment, after 12 weeks, or after 24 weeks compared to responders [15].

Conversely, Afzal et al. were against the current study. They reported no significant difference in either peripheral or intrahepatic CXCL10 expression at baseline between treatment responders and non-responders. The association with HCV RNA levels was of borderline significance. Due to its classification as an IFN-stimulated gene, it is anticipated that CXCL10 will experience significant induction following the initiation of Pegylated-Interferon/Ribavirin (PEG-IFN/RBV) therapy. Nevertheless, it is worth noting that the rapid decrease in CXCL10 levels observed in patients treated with DAAs could potentially be attributed to the swift decay of the virus itself, as viral replication is known to be a potent stimulator of CXCL10 production. Moreover, it has been observed that the levels of HCV RNA decrease significantly or become undetectable within a few days in a considerable number of patients undergoing treatment with DAA regimens. As a result, the expression of CXCL10 declines at a faster rate compared to patients treated with pegylated interferon and PEG-IFN/RBV [22]. In cases of undetectable viral load, HCV can replicate intracellularly without being detectable in peripheral blood. Therefore, we can establish a new score for assessing DDA therapy responsiveness by utilizing an equation involving calculations between HCV RNA, CXCL10, and TGFβ1.

TGFβ1 levels were significantly higher in patients and non-responders before treatment than in responders and control subjects. This finding is consistent with the findings of Kotsiri et al., who reported that only in responders did TGF-1 levels decrease significantly at the end of treatment and during the six-month post-treatment follow-up period compared to baseline. In contrast, the TGF-1 levels of non-responders decreased numerically but not statistically significantly from baseline to the end of treatment or six months after treatment [23]. Flisiak et al. also illustrated that TGF-β1 levels after treatment with Peg-IFNα and ribavirin decreased significantly compared to baseline in a small number of patients, regardless of SVR, reaching levels observed in healthy volunteers. However, TGF-β1 levels increased significantly 24 weeks after treatment in non-responders compared to patients who achieved SVR [24]. Moreover, El-Ahwany et al. reported that the TGFβ1 level significantly increased significantly in the non-responder group either before treatment or after 12 or 24 weeks compared to responders [15]. Janczewska et al. also found that serum TGF-β1 levels decreased after IFNα and ribavirin treatment in responders and non-responders. The decreased levels

of TGFB1 in non-responders were different from our study. These differences might reflect different populations, stages of the disease, or the methodology of cytokine assessment [25].

Concerning levels of ALT and AST, there was a significant increase in patients before treatment and non-responders compared to responder and control groups. This finding is in agreement with Kotsiri et al., who analyzed this by ALT, a cytosolic enzyme found in its highest concentrations in the liver and is more specific to the liver [23]. Zeremski et al. and El-Ahwany et al. reported a significant increase in ALT and AST levels in before-treatment and non-responder patients compared to responders and control groups [15, 26]. A strong positive correlation was reported between CXCL10 with AST and ALT in all our studied groups. Additionally, all our study groups showed a strong positive correlation between TGFB1, AST, and ALT. In addition, there was a strong positive correlation between CXCL10 and TGFB1 in all the study groups.

ROC curve study analysis revealed that TGFB1 was more specific and less sensitive than CXCL10 in non-responders. This is first study that detects sensitivity and specificity for CXCL10 and TGFB1 in non-responder patients. Measuring the levels of these two immunological biomarkers at various stages of liver fibrosis was a limitation of the study. The comparison of CXCL10 expression between hepatic tissue and peripheral blood levels was not feasible due to limited financial resources. The chemokine CXCL10 and the cytokine TGFB1 both play significant roles in the regulation of cell growth, fibrogenesis, and necroinflammation. Consequently, the severity of HCV-induced liver damage is positively associated with the levels of these molecules. Moreover, the reduced sample size can be attributed to the temporal context of the study, during this period we had few cases because of COVID-19 pandemic.

## 5. Conclusion

Compared to responders and control groups, CXCL10 and TGFB1 levels were significantly higher in non-responders and patients who did not initiate therapy. After the successful DAAs therapy of CHC, the circulating CXCL10 and TGFB1 levels declined to the same level as in a healthy volunteer. CXCL10 and TGFB1 could potentially be sensitive prognostic markers for HCV clearance and responsiveness to DAA therapy.

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**Availability of data and material:** the datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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**Conflict of interest:** The authors have no competing interests to declare to the content of this article.

**Correspondence\*:** Noha M Abdullah / MD, PhD. Department of Clinical Pathology. Minia University  
Minia, Egypt .

## 6. References

- [1] World Health Organization; Hepatitis, C. Key Facts. Available online: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-c>.
- [2] Elsharkawy A, El-Raziky M, El-Akel W, El-Saeed K, Eleteby R, Hassany M, et al. Planning and prioritizing direct-acting antivirals treatment for HCV patients in countries with limited resources: Lessons from the Egyptian experience. *J Hepatol.* 2018; 68 (4): 691–698. <https://doi.org/10.1016/j.jhep.2017.11.034>.
- [3] Waked I, Esmat G, Elsharkawy A, El-Serafy M, Abdel-Razek W, Ghalab R, et al. Screening and Treatment Program to Eliminate Hepatitis C in Egypt. *N Engl J Med*; 2020;382(12):1166–1174. <https://doi.org/10.1056/nejmsr1912628>.
- [4] Mayberry J. and Lee W. M. The revolution in treatment of hepatitis C. *Medical Clinics*, (2019): 103(1), 43-55. <https://doi.org/10.1016/j.mcna.2018.08.007>.
- [5] Zeuzem S., Ghalib R., Reddy K. R., Pockros P. J., Ari Z. B., Zhao Y. and Buttertton J. R. Grazoprevir–elbasvir combination therapy for treatment-naïve cirrhotic and noncirrhotic patients with chronic hepatitis C virus genotype 1, 4, or 6 infection: a randomized trial. *Annals of internal medicine*, (2015): 163(1), 1-13. <https://doi.org/10.7326/M15-0785>.
- [6] Kish T., Aziz A. and Sorio M. Hepatitis C in a new era: a review of current therapies. *Pharmacy and Therapeutics*, (2017): 42(5), 316.
- [7] Li, Kui, et al. "Activation of chemokine and inflammatory cytokine response in hepatitis C virus–infected hepatocytes depends on toll-like receptor 3 sensing of hepatitis C virus double-stranded RNA intermediates." *Hepatology* 55.3 (2012): 666-675. <https://doi.org/10.1002/hep.24763>.
- [8] Matsuura, Kentaro, et al. "Serum interferon-gamma-inducible protein-10 concentrations and IL28B genotype associated with responses to pegylated interferon plus ribavirin with and without telaprevir for chronic hepatitis C." *Hepatology Research* 44.12 (2014): 1208-1216. <https://doi.org/10.1111/hepr.12294>.
- [9] Antonelli, Alessandro, et al. "Chemokine (C–X–C motif) ligand (CXCL) 10 in autoimmune diseases." *Autoimmunity reviews* 13.3 (2014): 272-280. <https://doi.org/10.1016/j.autrev.2013.10.010>.
- [10] Choi, Jinah, and J-H. James Ou. "Mechanisms of liver injury.III. Oxidative stress in the pathogenesis of hepatitis C virus." *American Journal of Physiology-Gastrointestinal and Liver Physiology* 290.5 (2006): G847-G851. <https://doi.org/10.1152/ajpgi.00522.2005>.
- [11] Dixon, Laura J., et al. "Kupffer cells in the liver." *Comprehensive Physiology* 3.2 (2013): 785. <https://doi.org/10.1002%2Fcp.120026>.
- [12] Tanikawa, Aline Aki, et al. "Platelet-derived growth factor A mRNA in platelets is associated with the degree of hepatic fibrosis in chronic hepatitis C." *Revista da Sociedade Brasileira de Medicina Tropical* 50 (2017): 113-116. <https://doi.org/10.1590/0037-8682-0302-2016>.
- [13] Pawlotsky J.-M., Aghemo A., Back D., Dusheiko G., Forns X., Puoti M. and Sarrazin C (2015) EASL recommendations on treatment of hepatitis C 2015. *J hepatol* 63(1), 199-236.
- [14] Webster D.P. and Klenerman P. Dusheiko GM. Hepatitis C. *Lancet*, (2015): 385(9973), 1124-1135
- [15] El-Ahwany E. G., Nagy F. M., Zoheiry M. K., El Ghannam M. T., El Khashab M. N., Ahmadi W. and Shaker Z. A. role of t regulatory cells in chronic HCV infected egyptian patients and their impact on the response to pegylated interferon therapy. *Journal of the Egyptian Society of Parasitology*, (2015): 45(2), 345-55. <https://doi.org/10.21608/jesp.2015.89917>.
- [16] Pozza R., McCoy-Hill C., Hall K., Hefner A., Wilgers K., Tapelband J. and Hassanein T.

- Eradicating hepatitis C virus: The APRN's role. *The Nurse Practitioner*, (2019): 44(11), 16-27. <https://doi.org/10.1097/01.npr.0000586008.23422.2c>.
- [17] Wu X., Roberto J. B., Knupp A., Greninger A. L., Truong C. D., Hollingshead N. and Crispe I. N. Liver immune abnormalities persist after cure of Hepatitis C Virus by anti-viral therapy. *medRxiv*, (2020): 347. <https://doi.org/10.1101/2020.12.22.20248755>.
- [18] Pawlotsky J.-M. Hepatitis C virus resistance to direct-acting antiviral drugs in interferon-free regimens. *Gastroenterology*, (2016): 151(1), 70-86. <https://doi.org/10.1053/j.gastro.2016.04.003>.
- [19] Mascia C., Vita S., Zuccalà P., Marocco R., Tieghi T., Savinelli S. and Lichtner M. Changes in inflammatory biomarkers in HCV-infected patients undergoing direct acting antiviral-containing regimens with or without interferon. *PLoS One*, (2017): 12(6), e0179400. <https://doi.org/10.1371/journal.pone.0179400>.
- [20] Ferrari S. M., Fallahi P., Ruffilli I., Elia G., Ragusa F., Paparo S. R. and Antonelli A. Immunomodulation of CXCL10 secretion by hepatitis C Virus: Could CXCL10 be a prognostic marker of chronic hepatitis C?. *Journal of immunology research*, (2019): 348, 2314-8861. <https://doi.org/10.1155/2019/5878960>.
- [21] Zeremski M., Hooker G., Shu M. A., Winkelstein E., Brown Q., Des Jarlais D. C. and Edlin B. R. Induction of CXCR3-and CCR5-associated chemokines during acute hepatitis C virus infection. *Journal of hepatology*, (2011): 55(3), 545-53. <https://doi.org/10.1016/j.jhep.2010.12.033>.
- [22] Afzal M., Ali A., Sheikh N., Rafique S. and Idrees M. Peripheral Expression of CXCL10 Gene in Chronic Hepatitis C Patients Treated with Sofosbuvir, Daclatasvir, and Ribavirin. *Journal of Interferon & Cytokine Research*, (2020): 40(6), 301-9. <https://doi.org/10.1089/jir.2019.0185>.
- [23] Kotsiri I., Hadziyannis E., Georgiou A., Papageorgiou M.V., Vlachogiannakos I. and Papatheodoridis G. Changes in serum transforming growth factor- $\beta$ 1 levels in chronic hepatitis C patients under antiviral therapy. *Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology*, (2016): 29(1), 79.
- [24] Flisiak R., Jaroszewicz J., Lapinski TW, Flisiak I and Prokopowicz D Effect of pegylated interferon alpha 2b plus ribavirin treatment on plasma transforming growth factor-beta 1, metalloproteinase-1, and tissue metalloproteinase inhibitor-1 in patients with chronic hepatitis C. *World J Gastroenterol*; (2005): 11:6833–8. <https://doi.org/10.3748%2Fwjg.v11.i43.6833>.
- [25] Janczewska-Kazek E., Marek B., Kajdaniuk D. and Borgiel-Marek H. Effect of interferon alpha and ribavirin treatment on serum levels of transforming growth factor- $\beta$ 1, vascular endothelial growth factor, and basic fibroblast growth factor in patients with chronic hepatitis C. *World J Gastroenterol*. (2006): 12:961–5. <https://doi.org/10.3748%2Fwjg.v12.i6.961>.
- [26] Zeremski, Marija, et al. "Intrahepatic and peripheral CXCL10 expression in hepatitis C virus–infected patients treated with telaprevir, pegylated interferon, and ribavirin." *The Journal of Infectious Diseases* 211.11 (2015): 1795-1799. <https://doi.org/10.1093/infdis/jiu807>.



Figures

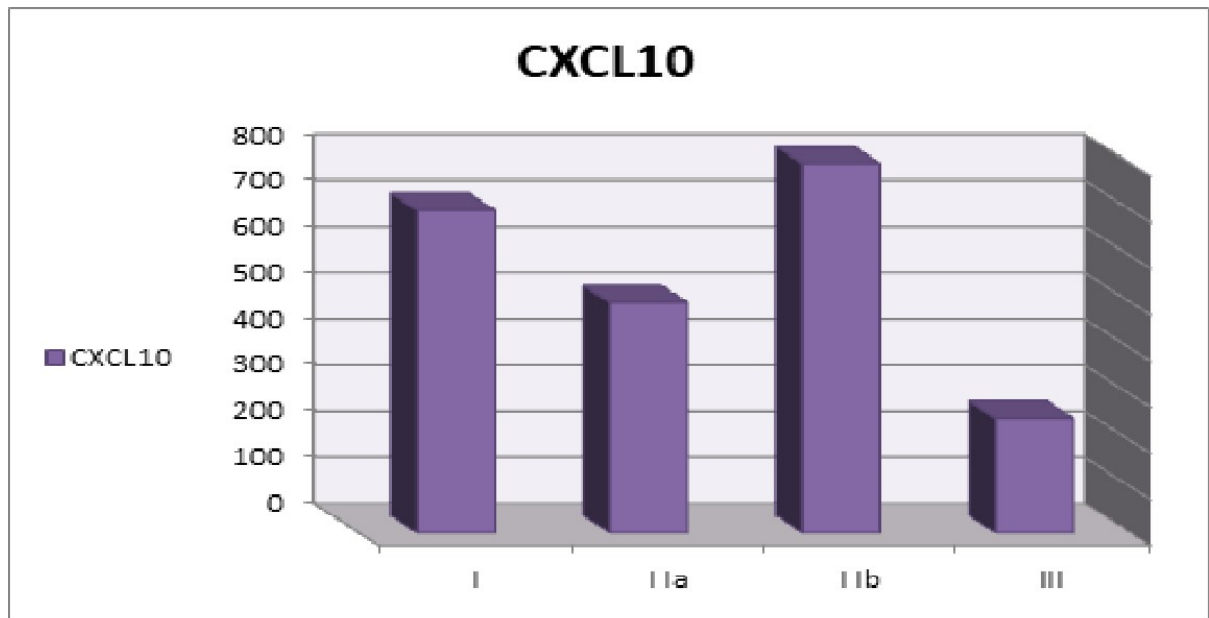


Figure 1 Comparison between levels of CXCL10 among different studied groups.

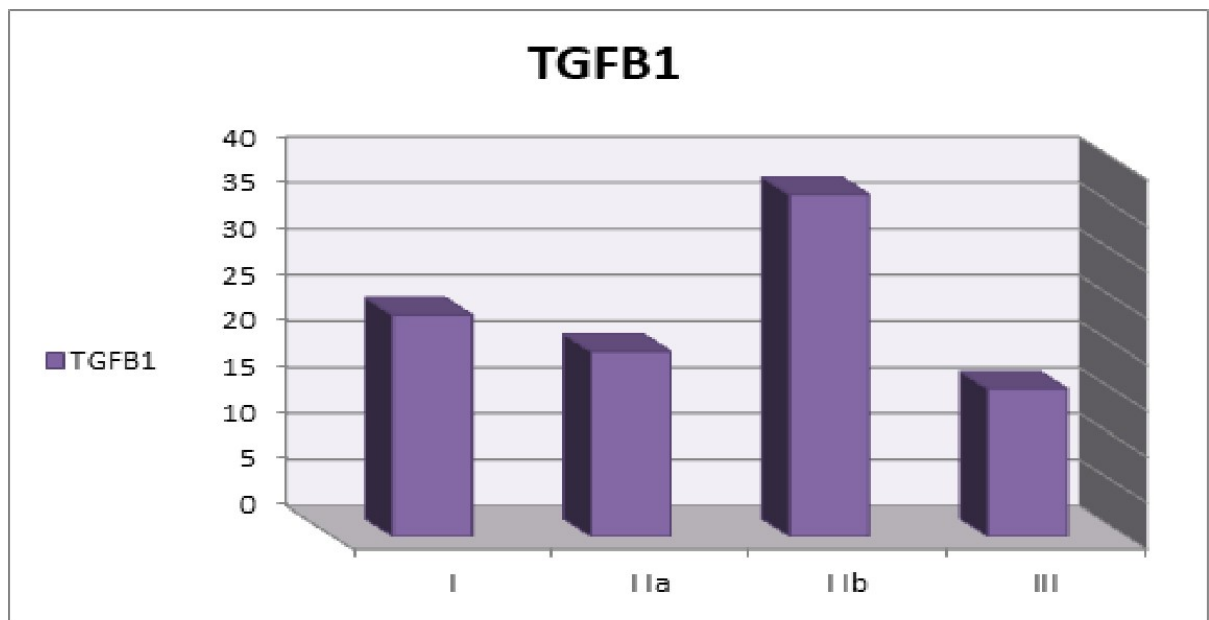
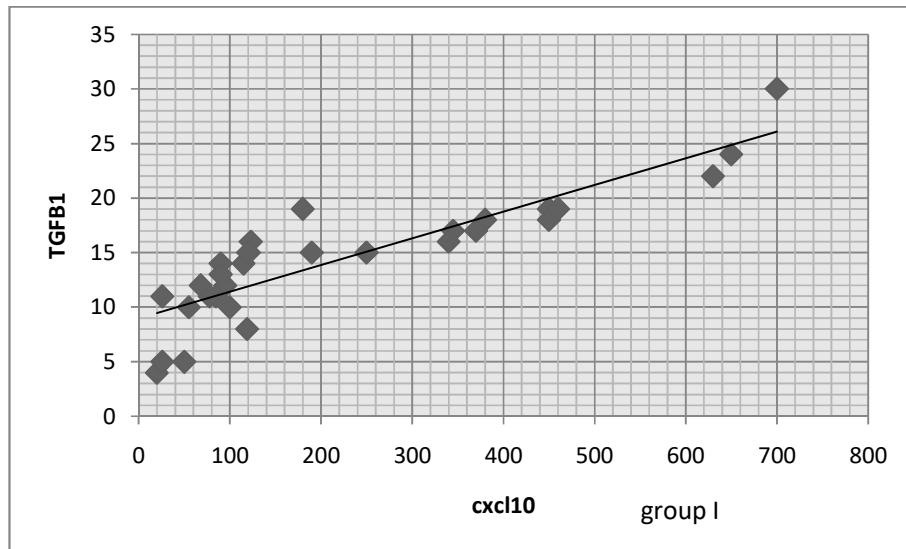
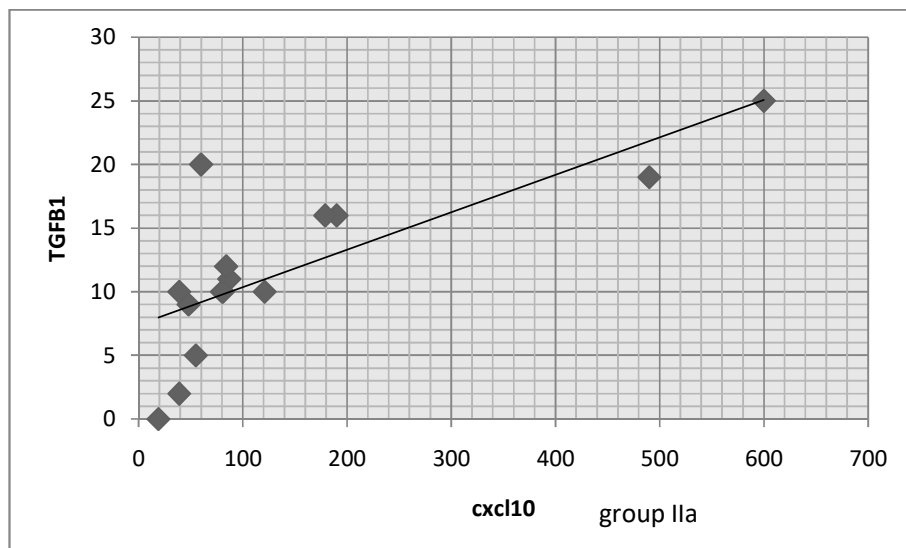


Figure 2 Comparison between levels of TGFB1 among different studied groups .



**Figure 3a:** correlation between TGFB1 and CXCL10 among group I



**Figure 3b:** correlation between TGFB1 and CXCL10 among group IIa

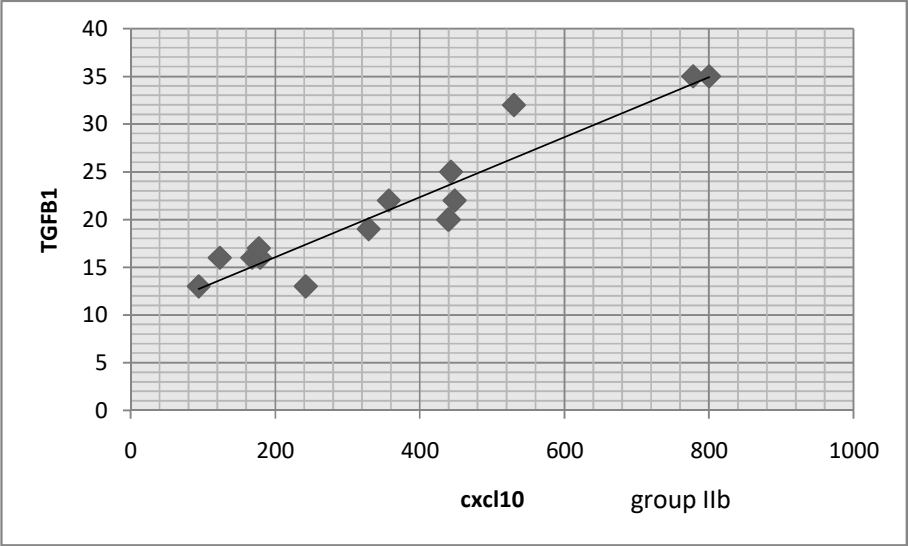


Figure 3c: correlation between TGFβ1 and CXCL10 among group IIb

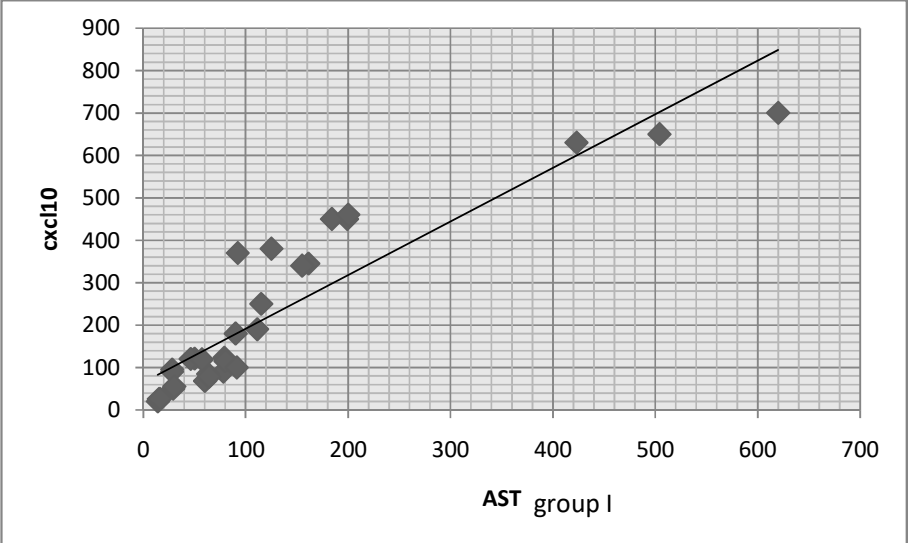
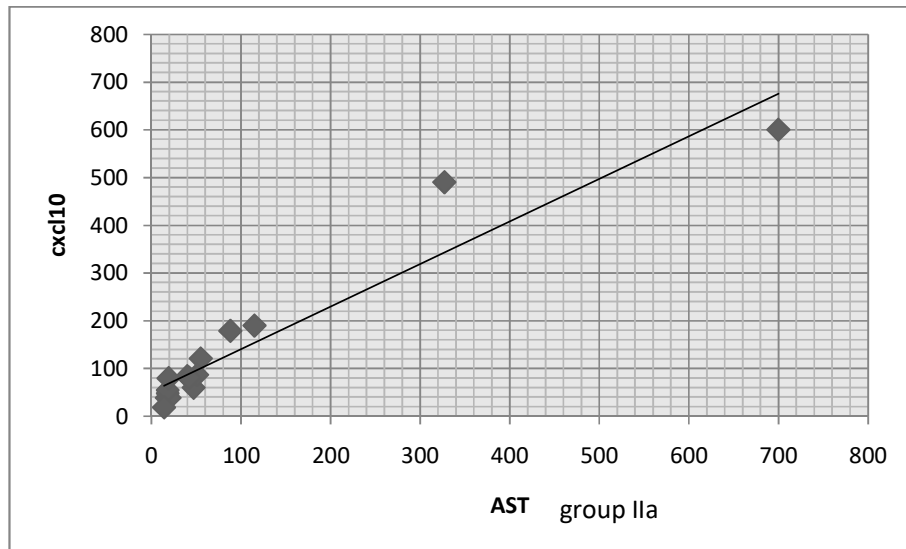
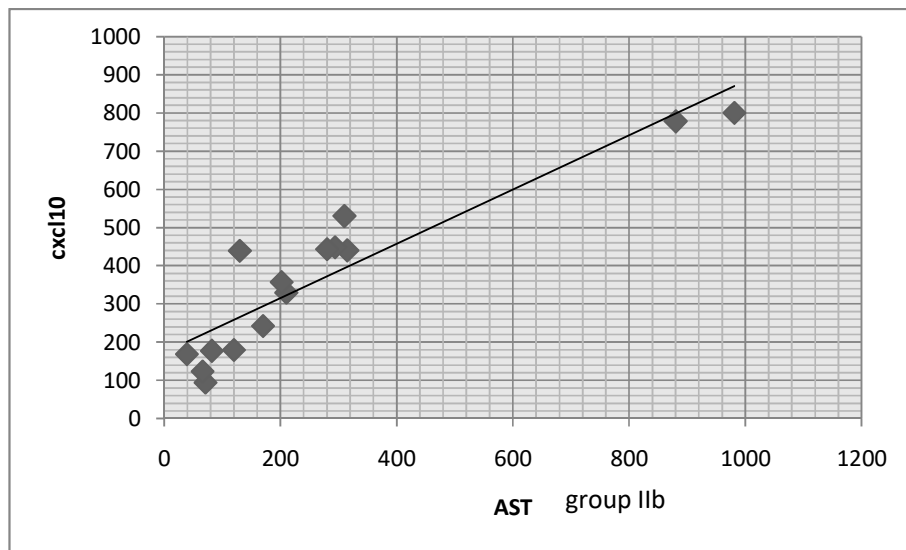


Figure 4a: correlation between CXCL10 and AST among group I



**Figure 4b:** correlation between CXCL10 and AST among group IIa



**Figure 4c:** correlation between CXCL10 and AST among group IIb

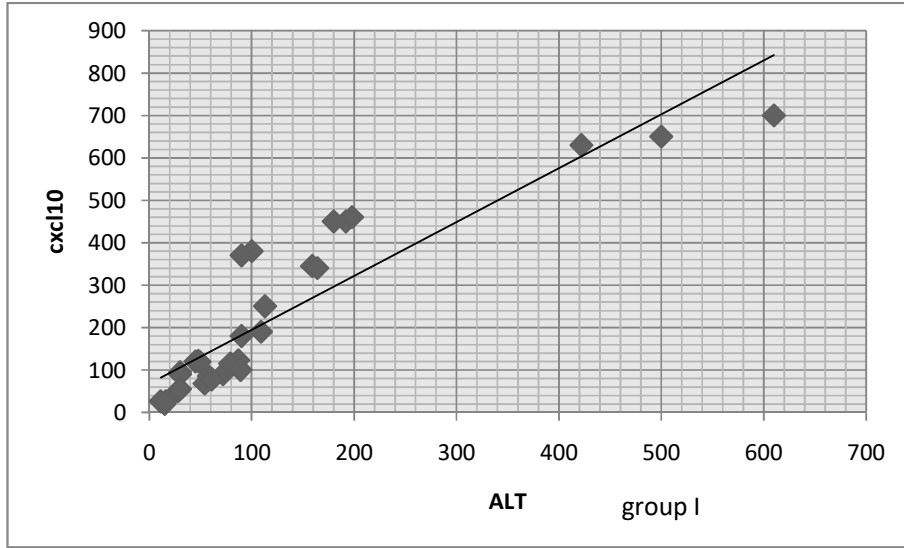


Figure 5a: correlation between CXCL10 and ALT among group I

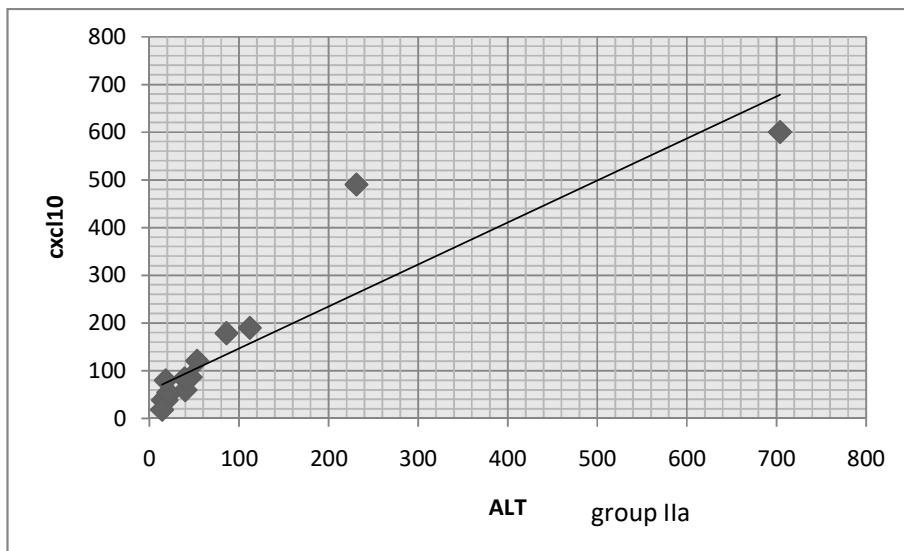
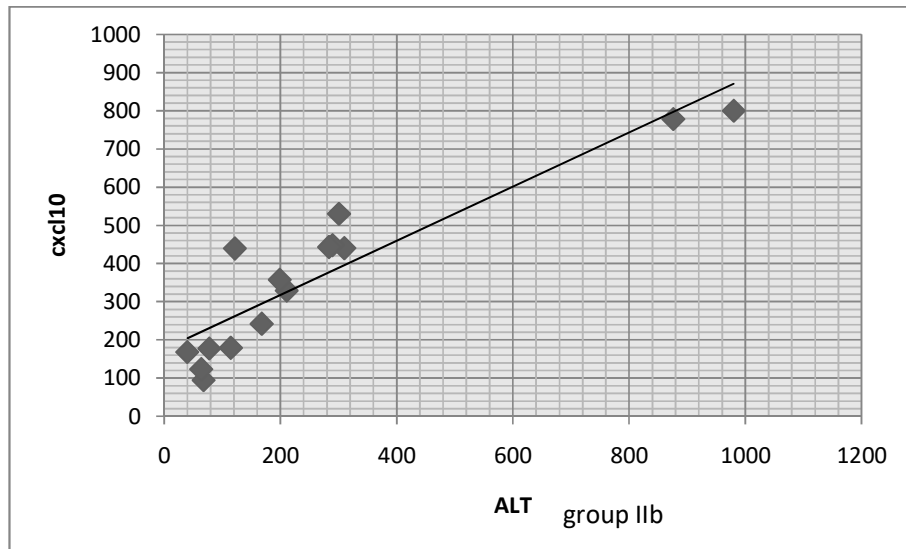
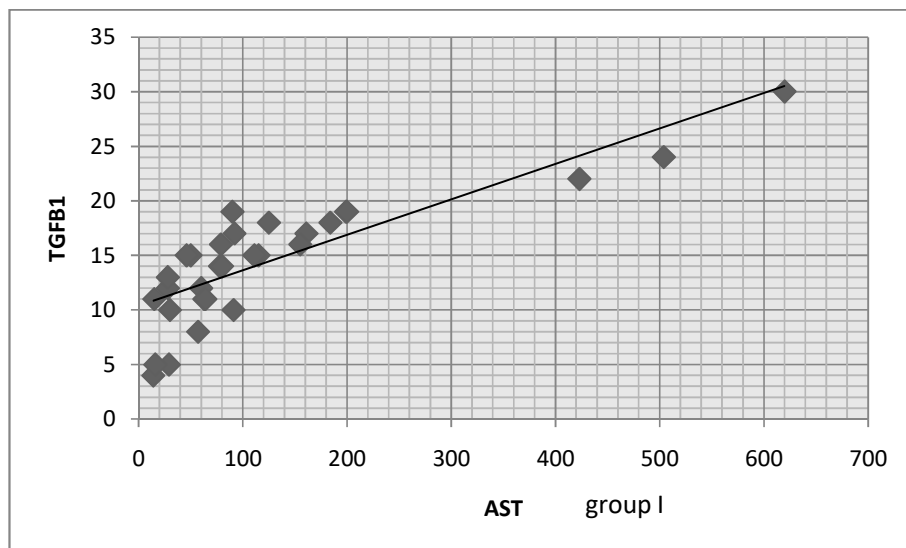


Figure 5b: correlation between CXCL10 and ALT among group IIa



**Figure 5c:** correlation between CXCL10 and ALT among group IIb



**Figure 6a:** correlation between TGFB1 and AST among group I

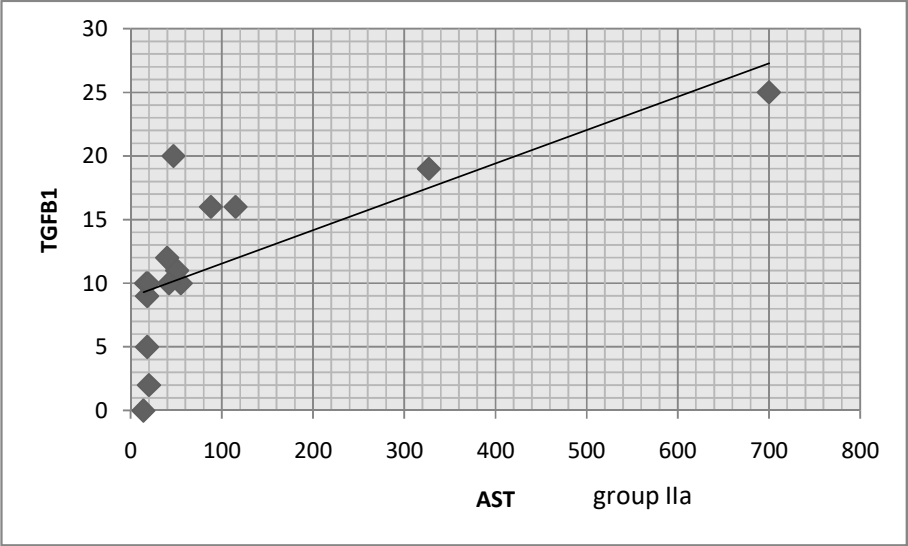


Figure 6b: correlation between TGFB1 and AST among group IIa

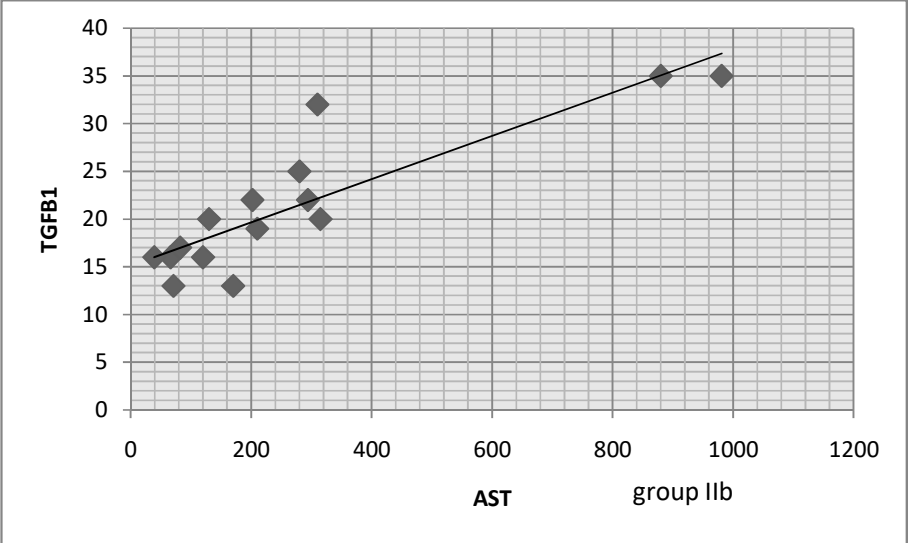
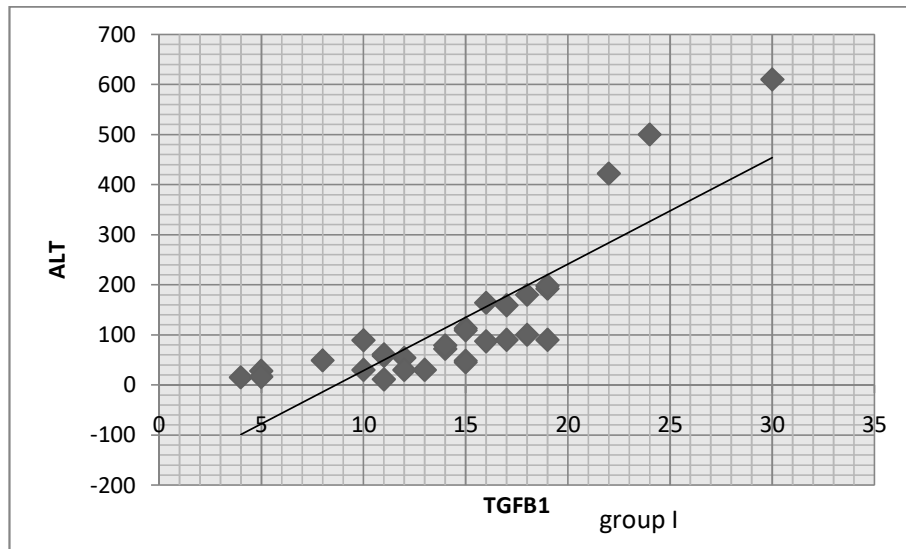
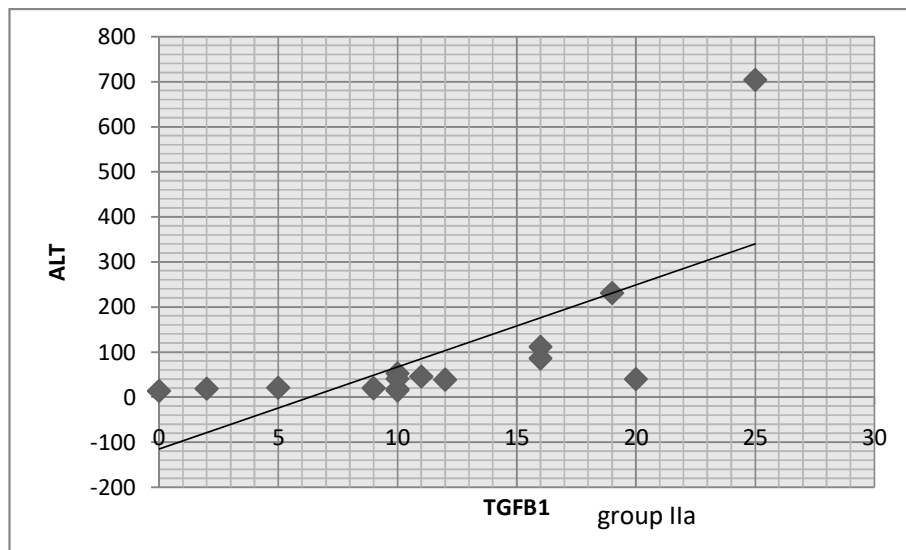


Figure 6c: correlation between TGFB1 and AST among group IIb



**Figure 7a:** correlation between TGFB1 and ALT among group I



**Figure 7b:** correlation between TGFB1 and ALT among group IIa

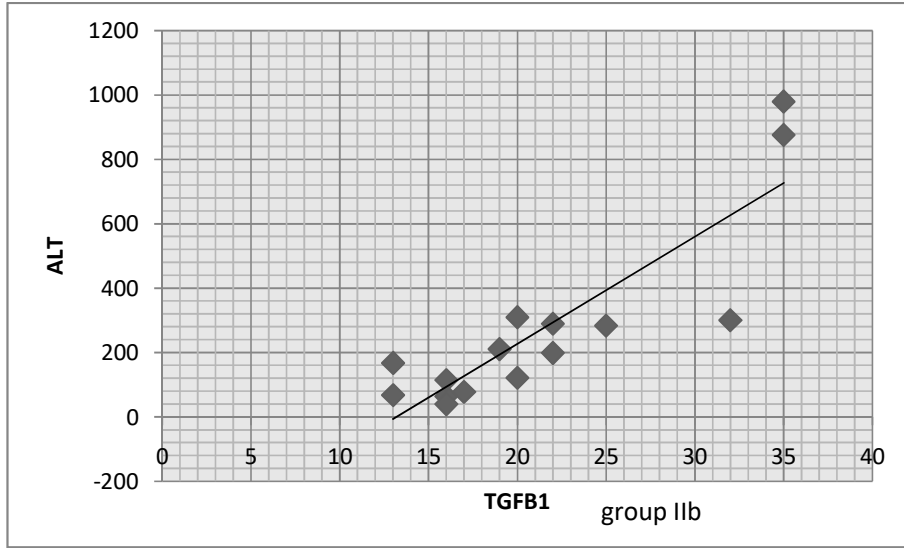
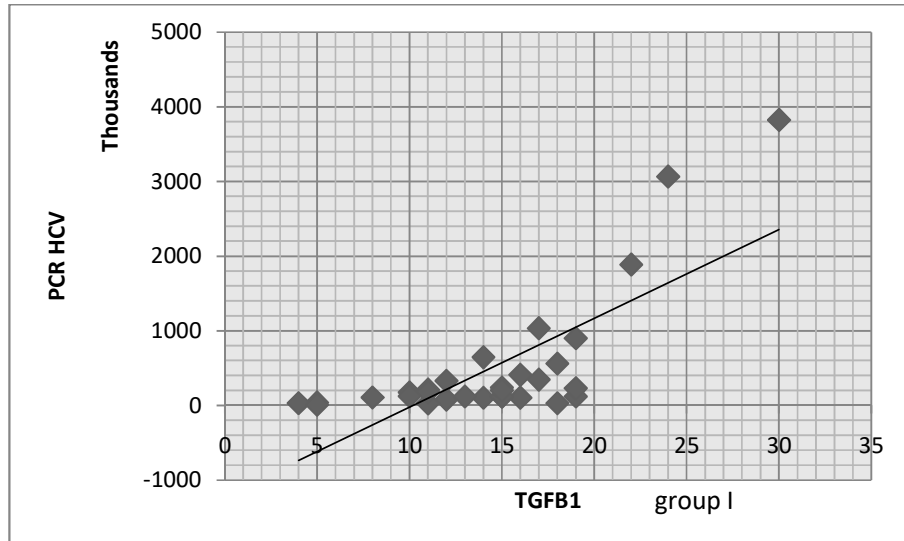
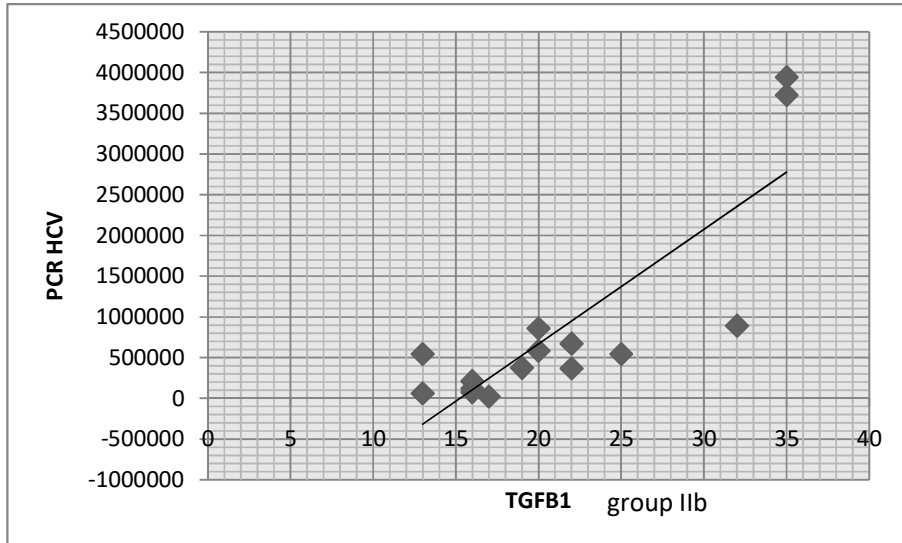


Figure 7c: correlation between TGFB1 and ALT among group IIb



**Figure 8a:** Correlation between TGFB1 and viral RNA among group I.



**Figure 8b**Correlation between TGFB1 and viral RNA among group I Ib.

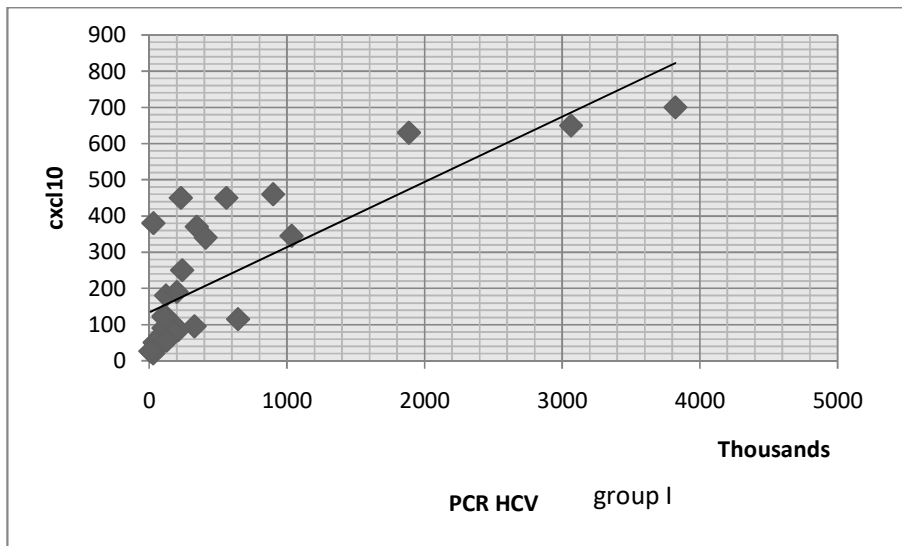


Figure 9a: Correlation between CXCL10 and viral RNA among group I

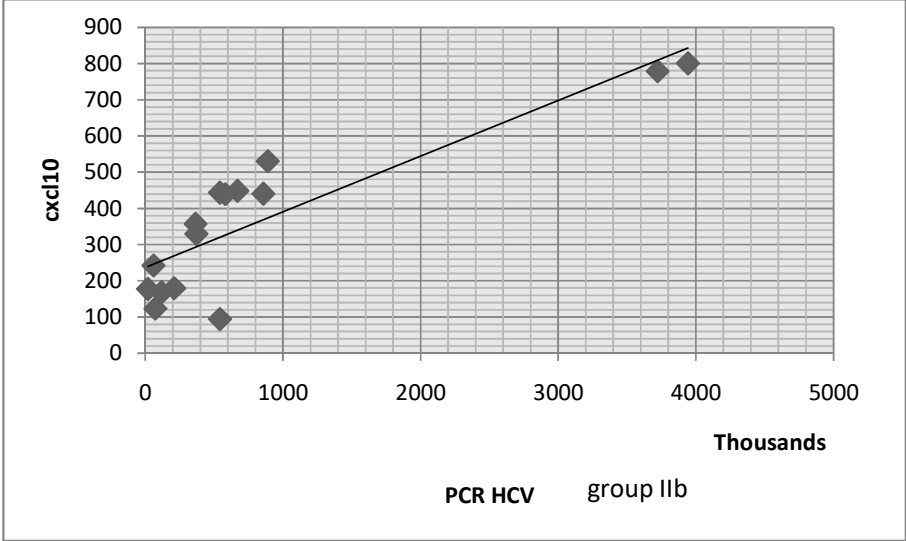


Figure 9b: Correlation between CXCL10 and viral RNA among group IIb.

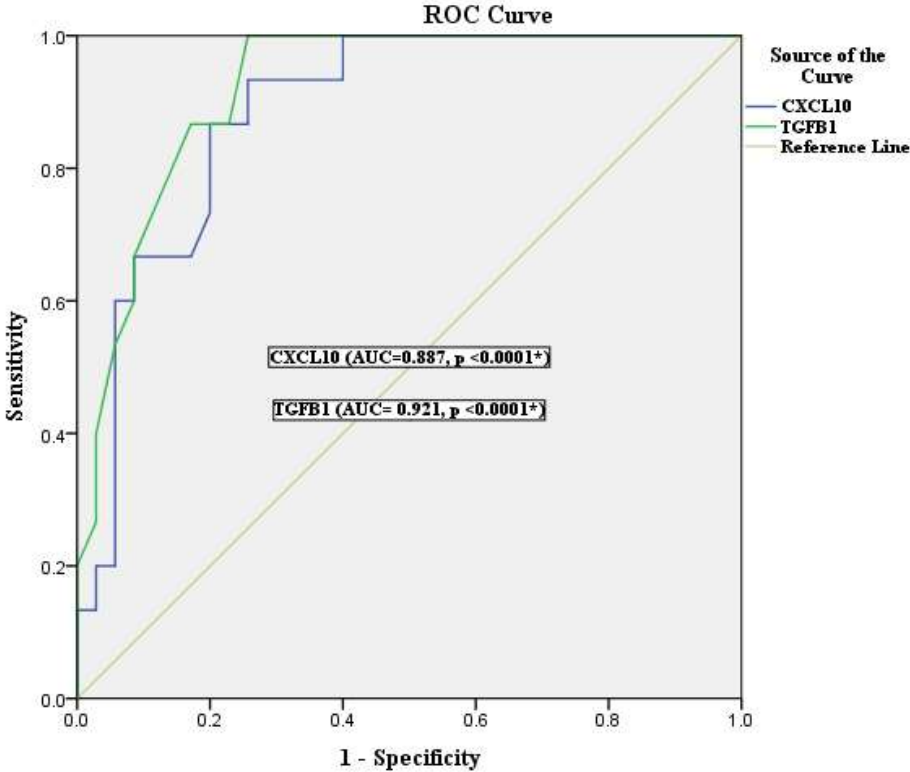


Figure 10: ROC curve analysis for CXCL10 and TGFB1 among non-responder patients

**Tabels**

**Table (1): Demographic data of the different studied groups:-**

Characteristic	Group (I)	Group (II)		Group (III)	p value
		Group (IIa)	Group (IIb)		
<b>Age (years)</b>					
<b>Mean ± SD</b>	54.26 ± 16.59	53.80 ± 17.13	57.06 ± 17.26	47.75 ± 19.34	0.431
<b>Range</b>	26-80	26-75	27-81	21-75	
<b>Gender</b>					
<b>Male</b>	15(50.0%)	7(46.7%)	8(53.3%)	10(50.0%)	0.988
<b>Female</b>	15(50.0%)	8(53.3%)	7(46.7%)	10(50.0%)	

**Table (2): Comparison of different studied groups regarding to hematological parameters:-**

Characteristic	Group (I)	Group (II)		Group (III)	p value						
		Group (IIa)	Group (IIb)		(I)vs(II a)	(I)vs(II b)	(I)vs(II I)	(IIa)vs (IIb)	(IIa)vs (III)	(IIb)vs (III)	
<b>Hb (g/dl)</b>											
<b>Mean ± SD</b>	10.94 ± 2.08	11.36 ± <b>1.54</b>	11.65 ± <b>2.67</b>	12.40 ± <b>1.19</b>							
<b>Range</b>	8.8-13.02	9.8-12.9	8.9-14.3	11.2-13.5	<b>0.502</b>	<b>0.253</b>	<b>0.011*</b>	<b>0.680</b>	<b>0.119</b>	<b>0.260</b>	
<b>WBCs (x10<sup>3</sup> /µl)</b>											
<b>Mean ± SD</b>	9.41 ± <b>4.18</b>	7.26 ± <b>1.54</b>	9.71 ± <b>5.93</b>	6.91 ± <b>1.95</b>							
<b>Range</b>	5.31-13.5	5.72-8.8	3.78-15.6	4.69-8.86	<b>0.079</b>	<b>0.805</b>	<b>0.026*</b>	<b>0.083</b>	<b>0.792</b>	<b>0.035*</b>	
<b>PLT (x10<sup>3</sup> /µl)</b>											
<b>Mean ± SD</b>	219.67 ± 91.54	261.33 ± 81.53	208.73 ± 56.32	278.95 ± 55.59							
<b>Range</b>	128-311	179-342	152-265	223-334	<b>0.087</b>	<b>0.651</b>	<b>0.009*</b>	<b>0.062</b>	<b>0.500</b>	<b>0.008*</b>	
<b>INR</b>											
<b>Mean ± SD</b>	1.21 ± 0.27	1.12 ± 0.11	1.31 ± .47	1.06 ± 0.07							
<b>Range</b>	0.94-1.28	1.01-1.23	0.84-1.78	0.99-1.13	<b>0.274</b>	<b>0.239</b>	<b>0.049*</b>	<b>0.051</b>	<b>0.503</b>	<b>0.007*</b>	

Hb: hemoglobin, PLTs: platelets, WBCs: white blood cells, INR: international normalized ratio. \* Statistical significance p< 0.05

**Table (3): Comparison between different studied groups regarding liver function teststests:-**

Characteristic	Group (I)	Group (II)		Group (III)	p value					
		Group (IIa)	Group (IIb)		(I)vs(Ia)	(I)vs(IIb)	(I)vs(II I)	(IIa)vs(IIb)	(IIa)vs(III)	(IIb)vs(III)
<b>DB (mg/dl)</b> Mean ± SD Range	0.43 ± 0.27 0.16-0.7	0.21 ± 0.09 0.12-0.3	0.44 ± 0.31 0.13-0.75	0.28 ± 0.18 0.1-0.46	<b>0.007*</b>	<b>0.802</b>	<b>0.030*</b>	<b>0.010*</b>	<b>0.473</b>	<b>0.039*</b>
<b>TB (mg/dl)</b> Mean ± SD Range	1.25 ± 0.69 0.56-1.94	0.72 ± 0.21 0.49-0.93	1.27 ± 0.72 0.55-1.99	0.76 ± 0.26 0.5-1.02	<b>0.004*</b>	<b>0.878</b>	<b>0.003*</b>	<b>0.008*</b>	<b>0.845</b>	<b>0.008*</b>
<b>AST (U/L)</b> Mean ± SD Range	80.96 ± 25.59 47-115	18.0 ± 5.03 14-28	180.33 ± 59.77 91-299	16.0 ± 4.98 12-25	<b>0.616</b>	<b>0.015*</b>	<b>0.761</b>	<b>0.011*</b>	<b>0.835</b>	<b>0.012*</b>
<b>ALT (U/L)</b> Mean ± SD Range	67.76 ± 20.70 40-99	18.80 ± 1.86 17-23	122.20 ± 40.15 67-228	19.70 ± 1.92 18-21	<b>0.181</b>	<b>0.099</b>	<b>0.241</b>	<b>0.030*</b>	<b>0.872</b>	<b>0.031*</b>
<b>ALB (g/dl)</b> Mean ± SD Range	3.35 ± 0.64 2.7-4.0	3.76 ± 0.33 3.4-4.0	3.39 ± 0.62 2.7-4.0	3.63 ± 0.49 3.1-4.3	<b>0.023*</b>	<b>0.829</b>	<b>0.096</b>	<b>0.073</b>	<b>0.475</b>	<b>0.224</b>

DB: direct bilirubin, TB: total bilirubin, AST: aspartate transaminase, ALT: alanine transaminase, ALB: albumin.\* Statistical significance p< 0.05.

**Table 4 Comparison of PCR level of HCV in different studied groups**

Characteristic	Group (I)	Group (II)		Group (III)	p value					
		Group(IIa)	Group (IIb)		(I)vs(IIa)	(I)vs(IIb)	(I)vs(II I)	(IIa)vs(IIb)	(IIa)vs(III)	(IIb)vs(III)
<b>PCR of HCV ( Iu/ml )</b> Mean ± SD	127030.69 ± 220887.01	000000	266432.50 ± 241692.22	000000	<0.00001*					
					<b>0.02*</b>	<b>0.018*</b>	<b>0.011*</b>	<b>&lt;0.0001*</b>	<b>1.00</b>	<b>&lt;0.0001*</b>

\* Statistical significance p< 0.05.

**Table 4: Comparison of CXCL10 and TGFB1 in the different studied groups:-**

characteristic	Cases			Group (III)	p value					
	Group (I)	Group (IIa)	Group (IIb)		(I)vs(IIa)	(I)vs(IIb)	(I)vs(III)	(IIa)vs(IIb)	(IIa)vs(III)	(IIb)vs(IIa)
<b>CXCL10 (pg/ml)</b>	225.87	144.80	369.80±	95.25 ±	< 0.0001*					
<b>Mean ± SD</b>	± 201.24	± 170.77	218.43	64.41	0.014	0.011*	0.012	0.001*	0.410	<
<b>Range</b>	20-700	19-500	94-800	<b>0.05-249</b>	7*		*			0.0001*
<b>TGFB1 (ng/ml)</b>	12.10	8.52 ±	25.26 ±	7.65 ±	< 0.0001*					
<b>Mean ± SD</b>	± 5.72	9.64	7.42	6.23	0.11	< 0.0001*	0.033	<	0.719	<
<b>Range</b>	<b>0.04-24</b>	<b>0.003-20</b>	13-37	<b>0.05-16</b>	6		*	0.0001*		0.0001*

CXCL10: C-X-C motif chemokine ligand 10, TGFB1: Transforming Growth Factor Beta 1.\*

Statistical significance p< 0.05.