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Serum Inducible Protein -10 chemokine as a biomarker for clearance of HCV with and without treatment in Egyptian patients

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ABSTRACT

Background:
Hepatitis C virus (HCV) is a major health problem worldwide particularly in Egypt. Chemokine IP-10 may be a good prognostic marker for the outcome of HCV treatment

Aim:
Assessment the potential predictive value of Serum Inducible Protein -10 chemokine (IP-10) in the clearance of HCV RNA in Egyptian patients with and without treatment

Materials and Methods: Ninety Egyptian individuals were involved in the current study where, 20 (23%) patients were chronic HCV chronic (positive HCV antibodies and positive HCV RNA without treatment, 20 (22%) were healthy individuals (negative for both HCV antibodies and HCV RNA, 20 cases (22%) were natural clearance (positive HCV antibodies and negative for HCV RNA without treatment), 20 (22%) were achieved SVR after treatment (responder group, HCV positive and negative for HCV RNA after treatment) and 10 (11%) were non responder (positive HCV antibodies and still positive HCV RNA after treatment. HCV RNA was quantitated by real time PCR for and serum IP10 level was measured by commercial ELISA kit. All biochemical and hematological examinations included liver function, CBC and alphefeto protein were assessed.

Results: The mean serum levels of IP-10 were significantly higher ($p< 0.001$) in CHC patients (345.4±100) pg/ml than healthy control group (101.5±31.4) and natural clearance group (103.2±40.7). Also serum levels of IP-10 was significantly elevated in non responder group (257.4±52.5) compared with each of SVR group (103.5± 43.5) ($p< 0.001$) and healthy group (101.5±31.4), ($p< 0.001$). Prediction of a clinical response based on a combination of these chemokines revealed high sensitivity (82%), specificity (85%), negative predictive value (95%), and area under the curve (1.00). Moreover, there is no correlation ((R= 0.05), P value $p< 0.795$) between serum level of IP-10 and HCV viral load.

Conclusion: IP10 is a useful non-invasive biomarker for viral clearance and might be used to apply patients according to the predictable treatment outcome. Accordingly, patients who are unlikely to respond to treatment would avoid unnecessary exposure to medication that is related with high morbidity.

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INTRODUCTION

HCV has been exposed to be the most common origin of chronic liver disease and hepatocellular carcinoma all over the world [1]. Hepatitis C virus (HCV) infects more than 185 million people, occurring among individuals of all ages, sexes ,races and regions of the world[2]. The brutality of chronic inflammation and the level of liver disease progression differ significantly Whereas 20–33% of chronic hepatitis C (CHC) patients progress to cirrhosis over 20–30 years, the remains have mild chronic hepatitis that does not progress or developments very slowly[3].Spontaneous HCV elimination during the acute phase obliges forceful CD4 and CD8 responses against multiple viral epitopes[4]. Egypt has been classified that has highest prevalence rate of HCV in the world(15-20%)[5]. It is broadly putative that the routine of mass parenteral antischistosomal therapy involved application of tartar emetic injections (from 1950s to 1980s) led to extensive infection with (15–20%) o f adult patients have HCV antibodies in Egypt[6]. (Makhzangy et al. 2009). Although about 30% of patients may clear the virus spontaneously, the mainstream of patients who develop chronic HCV have been reflected the main health problem [7]. In this patient population, cirrhosis may progress within 20 years of infection. With hepatic decompensation and hepatocellular carcinoma, these longstanding consequences have put more hindrances on resources in an already overstressed Egyptian healthcare system [8]. The four is the most public genotype of HCV in the Middle East and Africa, mostly Egypt which is the reason for more than 90% of HCV infections[9].

The accomplishment of a Sustained virolgical response (SVR) is the purpose of giving treatment to chronic HCV patients to overcome hepatitis C Virus with undetectable HCV RNA at 12 weeks after finalising treatment course [10].

Many direct acting antiviral agents have been developed in this era showing vigorous activity with higher rates of sustained virological response [11].The dose of both sofosbuvir and daclatasvir is taken orally once per day, in a dose of 400 mg and 60 mg, correspondingly and this is according to the protocol of Egyptian National Committee for Controlling HCV (NCCVH) and the guidelines of European Association for the Study of the Liver [12]. Also SOF-DCV in patients with chronic HCV-G4 was verified to be safe and related with a high SVR12 rate (95.1%), in patients with different stages of fibrosis [13].Thus, recognizing factors that predict SVR become fundamental option which will improve clinical decision making, from the economic point of view to economize treatment cost and increase its efficacy [14]. It has been noticed the immune factors other than antibodies may play a defensive role in HCV infection. T cells have been confirmed to be important in the control of many viral infections. Vigorous and polyfunctional HCV-specific T cell responses have been related with the spontaneous clearance of infection [15]. Chemokines and cytokines regulate inflammation and immunity in HCV infection they are considered engaging biomarker for treatment and play vital role in clearance of the virus [16]. Chemokines (CKs) are considered a family of small

proteins secreted by the cells. Their name (derivative from chemoattractant cytokines) is due to their capacity to induce directed chemotaxis in close responsive cells. Some CKs are involved during an immune response to recuilt cells of the immune system cells to a site of infection, so they can target and destroy invading bodies such as microbes [17]. While others are considered homeostatic and are implicated in controlling the migration of cells during normal processes of tissue maintenance or development [18]. so they are assumed to be attractive biomarkers for treatment consequence. Many chemokine are influenced by exogenous interferon and play important roles in clearance of the virus. The immune system of responders inclines to have a lower baseline activation before starting treatment that is markedly induced in response to treatment by IFN [19]. Interferon-gamma inducible protein 10 kDa (IP-10); also known as chemokine (c-x-c) motif ligand 10 (CXCL10) which formed in its mature form of 77 amino acids [20]. CXCL10 has been assigned to various roles, for instance chemoattraction for monocytes/macrophages, T cells, NK cells, and dendritic cells, promotion of T cell adhesion to endothelial cells, antitumor activity, and inhibition of bone marrow colony development and angiogenesis [21].

IP- 10 is interferon stimulating gene produced by different cells, including hepatocytes and non parenchymal liver cells during CHC [22].The Intrahepatic production of IP10 and other non-ELR chemokines recruits a pro-inflammatory, anti-viral immune response to the liver by binding chemokine receptor CXCR3 on CD4+ TH1, CD8+ Tc , and natural killer cells [23]. And this in turn stimulate the innate and adaptive immune response, So the non-ELR CXC chemokines specifically CXCL10, assistance in coordinate the hepatic inflammatory response of chronic hepatitis C [24]. It has been proposed that CXCL10 in the serum of chronic HCV patients may not denote the biologically active form. Because it was found as truncated form, resulting from the N-terminal cleavage of two aminoacids by the protease dipeptidylpeptidase 4 (DPP4, orCD26). So, the truncated form of CXCL10 retains CXCR3 binding, but does not induce signaling [25]**.** As such, it acts as antagonist effect .and this shows that, presence of high level of IP10 in chronic patients and decrease in individuals whose clear the virus [26]. Also IP10 decreased with DAA therapy until they reached levels similar to healthy donors obviously suggesting a re-orchestration of innate and adaptive immune cells with prospective consequences for inflammatory processes [25]. Therefore, assessment of pre-treatment IP-10 may be useful in response prediction in chronic HCV infection. And become good prognostic marker for the outcome of HCV treatment. The current study aimed at evaluation of the value of pre-treatment serum IP level in the prediction of the likelihood of SVR in Egyptian chronic HCV patients receiving SOF-DCV combination therapy.

Materials and methods:

The present study recruited 90 Cases (48 males, 42 females) and their ages ranged from 23 to 65 years. The study excluded the patients who met the following criteria (pregnancy, liver cirrhosis, liver

transplantation, heart disease, renal failure, autoimmune disease or co-infection with HBV or HIV). The study protocol was approved by Local Ethics Committee . An informed written consent was taken from each patient before inclusion in this study. The study involved five cohorts as the following: 1st cohort (control): twenty healthy volunteers had negative HCV Ab by Elisa and negative HCV RNA by PCR. 2nd cohort (Natural Clearance); Twenty patients had positive HCV Ab and negative HCV RNA and they didn't receive any anti-HCV treatment. 3rd cohort (Chronic Hepatitis C) Twenty patients had a positive HCV Ab and positive HCV RNA. 4th cohort (sustained virological responders after treatment; Twenty patients had a negative HCV RNA after 12 weeks of treatment. 5th cohort (Non-responders after treatment); ten patients had a positive HCV RNA after 12 weeks of treatment.

Antiviral regimen

The antiviral treatment regimen consisted of one tablet containing 400 mg of SOF (Sovaldi®; Gilead Sciences Inc., Foster City, CA, USA) once daily and one tablet containing 60 mg of DCV (Daklinza®; Bristol-Myers Squibb, New York, NY, USA) once daily. The treatment period was 12 weeks. Other contraindications included in the Standard Product Characteristics of SOF and DCV, especially drug–drug interactions, were respected. Sustained virological response (SVR) was assessed as HCV RNA negativity 12 weeks post treatment.

Sampling:

Venous blood samples of about 10 ml were collected from each patient, required volumes were put into sterile vaccutainer tube contained EDTA for complete blood count, sodium citrate for prothrombin time and gel for assessment of Hepatitis B surface Antigen (HBsAg), Hepatitis C Virus Antibody (HCVAb), Human immunodeficiency virus (Anti – HIV), ALT, AST, Bilirubin), Alkaline phosphatase, Albumin, Tumor marker Alpha Feto Protein (AFP), CXCL 10 and HCV RNA PCR.

HCV-RNA Quantification:

HCV RNA was assessed by the Roche COBAS® AmpliPrep/COBAS TaqMan® HCV Quantitative Test v2.0 (Roche Molecular Systems Inc., Branchburg, NJ, USA). The lower detection limit was 15 IU/ml. HCV-RNA levels were assessed in all cohorts

IP-10 Quantification:

Serum IP-10 level was measured in all cohorts by the commercially enzyme-linked immunosorbent assay according to manual procedures (Human CXCL10/ IP-10; Quantikine ® ELISA R&D Systems, Minneapolis, USA). The minimum detectable level is 1.67 pg/mL.

Measurement of serum Liver function tests:

Serum ALT, AST, albumin and alkaline Phospahtase were measured by spectrophotometer using kits supplied by SPINREA CT,S.A.U. -Ctra. Santa Coloma, Girona Spain. Serum total and direct bilirubin were measured by spectrophotometer using kits (Diammond, Cairo, Egypt).

Tumor marker alpha feto protein:

Was measured in all cohorts by the commercially available enzyme-linked immunosorbent assay (Invitrogen, USA).

Complete blood count:

For all cohorts were done by automated cell counter alpha SWE Lab, Sweden.

Statistical analysis:

All parametric values are expressed as means (SD). Different groups were compared using the student's t test. The spearman coefficient was used to evaluate correlations between variables. Variables included in the analyses were age, sex, aspartate transaminase (AST), alanine aminotransferase (ALT), alpha fetoprotein (AFP), hematological tests, baseline serum IP-10 level, and viral load. Statistical

analyses were performed by SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA); p values lower than 0.05 were considered significant. Moreover, The sensitivity and specificity, Efficiency, Positive Predictive Value, Negative Predictive Value of IP -10 in the positive and negative HCV RNA was measured as shown in the table (1).

Results:

Ninety Egyptian individuals were involved in the current study where, 20 (23%) patients were chronic HCV chronic (positive HCV antibodies and positive HCV RNA without treatment, 20 (22%) were healthy individuals (negative for both HCV antibodies and HCV RNA, 20 cases (22%) were natural clearance (positive HCV antibodies and negative for HCV RNA without treatment), 20 (22%) were achieved SVR after treatment (responders group, HCV positive and negative for HCV RNA after treatment) and 10 (11%) were non responders (positive HCV antibodies and still positive HCV RNA after treatment figure (1).

The mean serum levels of IP-10 chronic HCV, natural clearance, responders and no responders groups

The mean serum levels of IP-10 were significantly higher ($p< 0.001$) in CHC patients (345.4±100) pg/ml than healthy control group (101.5±31.4) and natural clearance group (103.2±40.7). Also serum levels of IP-10 was significantly elevated in non responder group (257.4±52.5) compared with each of SVR group (103.5± 43.5) ($p< 0.001$) and healthy group (101.5±31.4), ($p< 0.001$) as shown in figure (2).

The sensitivity and specificity of IP -10 in the positive and negative HCV RNA with and without treatment

Assessment of serum level of IP-10 by using Elisa has showed discrimination between chronic HCV infection and HCV spontaneous clearance also between responders and non responders patients to new DAAs. Prediction of a clinical response based on serum level of IP- 10 chemokine demonstrated high sensitivity (82%), specificity (85%), negative predictive value (95%), table 1. The ROC curve of IP10 showed an AUC of 1.00 (95% confidence interval 1.00-1.00). At a cut off value 194.565pg/ml for predicting SVR, at point the sensitivity was 100% and specificity was 100%) as shown in figure (3). However, there is no correlation (($R= 0.05$), P value $p< 0.795$) between serum level of IP-10 and HCV viral load as shown in figure (4)

Biochemical parameters (liver functions, Alphafetoprotein) of the studied groups and serum level of IP-10

There is significant difference (P value < 0.001) among five groups for biochemical parameter where the chronic HCV Patients and Non responders patients had high level of ALT, AST, ALP and AFP more than responders, natural clearance as shown in table 2. Moreover, there is non positive correlation between IP10 level with alkaline phosphatase, and albumin as shown in Figure (5). There is positive correlation between IP10 level and each of hemoglobin and RBCs and negative correlation between IP10 level and each of HCT and platelets as shown in Figure (6).

Discussion

HCV infection is characterized by an enlarged production of chemokines and cytokines[22]**.** Chemokines, moreover being inflammatory mediators, may as well be useful as markers of treatment consequence. Furthermore, IP10 is a chemokine that targets T lymphocytes and monocytes . IP10 has a chemotazactic function on different cell types

(monocytes,macrophages ,natural killer cells, activated T lymphocytes and dendritic cells) by binding with specific receptor CXCR3[27]. High pre-treatment IP10 levels reflect the therapeutic nonresponse. This outcome be unique for IP10 because the other non-ELR CXC or CC chemokines studied haven't prognostic value for treatment response in HCV infection [19]. This study was designed to assess the role of chemokine IP10 for stratify the patients who go to HCV spontaneous clearance without treatment and who are expected to be responder to the treatment of HCV Egyptian patients. In this study, the mean age of chronic HCV patients and Non responder to treatment is 49±12 and 47±13 respectively This is agreement with the result of [28]**.** where the mean age of hepatitis c infected patients was 49.19±11.50 .And the lower mean ages were found in healthy (38+11), spontaneous clearance (41±10) and responder to treatment (42± 11). In this study healthy individuals, natural clearance and responders had BMI less than chronic patients and Non responders which agree with [27] which reported that patients with SVR were younger and had lower BMI.

The main finding of this study is significant association of serum IP10 levels in spontaneous clearance compared with HCV chronic patients without treatment and IP10 levels in non responders (NR) to treatment compared with treatment responders .The results show higher IP 10 level in chronic patients (345.4±100) pg/ml) compared with spontaneous clearance (103±40.7pg/ml) and compared with healthy controls (101±31.4pg/ml) and IP10 levels were higher in non responders (257.4±52.5 pg/ml) compered with responders (103.5± 43.5 pg/ml) which is agreement with results of [29] which 586 and 392 pg/ml respectively. It has been reported that plasma CXCL10 is treated by dipeptidylpeptidase DPP4; also known as CD26) thus leading to the generation of an antagonist form. [30] supported the NH_2-truncation of CXCL10 (short form) which is capable of binding CXCR3 but does not induce signaling [25]**.** Confirming that CXCL10 levels are increased in chronic and non responder patients to treatment of HCV [31].

The current results showed a positive correlation between IP10 levels with AST&ALT and this is consistent with [20] who mention that the elevated IP10 level was positively related with liver damage as indicated by high liver fibrosis score and high liver enzymes level. Moreover, this disagree with [27] which reported that the correlation of serum IP10 level with AST and ALT levels didn't reach to significant levels and this may be attributed to small sample size. In the current study, there wasn't a significant correlation between IP10 level and HCV RNA levels which may be due to low sample size, and most patients have almost nearly viral load and this is consistent with [27] and [32]. and disagree with [16] who reported that IP10 levels significantly decrease in patients reaching SVR compered with non responders suggesting that decline of this chemockine could be an indicator of disruption of interahepatic virus –host interaction .and this attributed to DAAs persuade rapid decrease in HCV replication with a consequent reduction of IP10 plasma level. Moreover, the present data showed negative correlation between IP1 0 plasma level and AFP .And this disagree with [28] and this attributed to his patients grade were cirrhosis and our patients are chronic and non responder and didn't reach to cirrhosis state where (AFP) is a tumor marker of hepatocellular carcinoma (HCC) [33] .Also slight raises of AFP can be seen in benign liver diseases such as virus related acute and chronic hepatitis [34].

ROC curve analysis showed a suitable IP10 cutoff level in predicting the SVR to DAAs treatment the best sensitivity and specificity for identifying SVR was 194 pg/ml while all the responder to treatment in this study had IP10 level less than 194

pg/ml (specificity 100%) and all Non responders had IP10 level more than 194pg/ml with (sensitivity 100%) [35]. reported that the pretreatment IP10 level for predicting SVR was 499.02pg/ml with specificity 100% and sensitivity 82.6% And[36]reported that pretreatment threshold IP10 level with the best settlement sensitivity and specificity to identify non-responder was 359 pg/ml , 81.8% of non responders (NR) were identified by IP10 more than 359 pg/ml and 45.2% of SVR had IP10 level less than 359pg/ml. One of the several factors causative to virological response in chronic hepatitis C (CHC) is interferon-gamma-inducible protein-10 (IP-10). Its level reflects the status of interferon-stimulated genes, which in turn is related with virological response to antiviral therapy as reported by [37].

Conclusion

IP10 is a useful non-invasive biomarker for viral clearance and it became beneficial for predicting the promising virological response before beginning the treatment. Subsequently, patients who are unlikely to respond to the treatment would avoid needless exposure to medication that is related with high morbidity

References

1. Gálvez, José A., Rafael Clavería-Gimeno, Juan J. Galano-Frutos, Javier Sancho, Adrian Velázquez-Campoy, Olga Abian, and María D. Díaz-de-Villegas. 2019. "Stereoselective Synthesis and Biological Evaluation as Inhibitors of Hepatitis C Virus RNA Polymerase of GSK3082 Analogues with Structural Diversity at the 5-Position." *European Journal of Medicinal Chemistry* 171.
2. Lu, Y., L. Y. Lin, J. G. Tan, H. P. Deng, X. H. Li, Z. Zhang, Y. Li, Z. Zhou, X. Xu, X. Xie, and S. J. Mei. 2017. "A Correlation Study between Gene Polymorphism of Th Cell Expressed Chemokine Receptor CXCR3 and Its Ligand Levels with HCV Infection Prognosis." *European Review for Medical and Pharmacological Sciences* 21(6):1290–95.
3. Elgharably, Ahmed, Asmaa I. Gomaa, Mary M. E. Crossey, Peter J. Norsworthy, Imam Waked, and Simon D. Taylor-Robinson. 2017. "Hepatitis C in Egypt - Past, Present, and Future." *International Journal of General Medicine*.
4. Waked, Imam, Waheed Doss, Manal Hamdy El-Sayed, Chris Estes, Homie Razavi, Gamal Shiha, Ayman Yosry, and Gamal Esmat. 2014. "The Current and Future Disease Burden of Chronic Hepatitis C Virus Infection in Egypt." *Arab Journal of Gastroenterology* 15(2):45–52.
5. Omran, Dalia, Mohamed Alboraie, Rania A. Zayed, Mohamed Naguib Wifi, Mervat Naguib, Mohamed Eltabbakh, Mohamed Abdellah, Ahmed Fouad Sherief, Sahar Maklad, Heba Hamdy Eldemellawy, Omar Khalid Saad, Doaa Mohamed Khamiss, and Mohamed El Kassas. 2018. "Towards Hepatitis C Virus Elimination: Egyptian Experience, Achievements and Limitations." *World Journal of Gastroenterology* 24(38):4330–40.
6. Makhzangy, Hesham El, Gamal Esmat, Mohamed Said, Maissa ElRaziky, Soheir Shouman, Rasha Refai, Claire Rekacewicz, Rita Raafat Gad, Nicolas Vignier, Mohamed Abdel-Hamid, Khaled Zalata, Pierre Bedossa, Stanislas Pol, Arnaud Fontanet, and Mostafa K. Mohamed. 2009. "Response to Pegylated Interferon Alfa-2a and Ribavirin in Chronic Hepatitis C Genotype 4." *Journal of Medical Virology* 81(9):1576–83.
7. Abelhafez, Tawfeek H., Ashraf A. Tabll, Mostafa K. El-Awady, Mohammad M. Mashaly, Reem El Shenawy, Yasmine S. El-Abd, Maysa H. Shaker, and Camelia A. Abdel Malak. 2017. "Naturalizing Activity and Safety of Human Monoclonal Antibodies against of Hepatitis C Virus." *Human Antibodies* 26:1–9.
8. El-Sokkary, Rehab H., Rehab M. Elsai. Tash, Takwa E. Meawed, Omnia S. El Seifi, and Eman M. Mortada. 2017. "Detection of Hepatitis C Virus (HCV) among Health Care Providers in an Egyptian University Hospital: Different Diagnostic Modalities." *Infection and Drug Resistance* 10:357–64.

9. Kamal, Sanaa M. and Imad A. Nasser. 2008. "Hepatitis C Genotype 4: What We Know and What We Don't yet Know." *Hepatology* 47(4):1371–83.
10. Terrault, Norah A. and Tarek I. Hassanein. 2016. "Management of the Patient with SVR." *Journal of Hepatology* 65(1):S120–29.
11. Abdo, Mahmoud, Yehia ElShazly, Sameh Seif, Gamal Esmat, Mohamed Korany, Imam Waked, Wahid Doss, Hadeel Gamal Eldeen, Wafaa Elakel, Manal Hamdy El-Sayed, Ayman Yosry, Samy Zaky, Magdy El Serafy, Mohamed Said, Rasha Eletreby, Maissa El Raziky, Mohamed El Kassas, Rabab Fouad, Siham Abdel Rehim, and Tamer Elbaz. 2016. "Real Life Egyptian Experience of Efficacy and Safety of Simeprevir/Sofosbuvir Therapy in 6211 Chronic HCV Genotype IV Infected Patients." *Liver International* 37(4): 534–41.
12. EASL. 2016. "EASL Recommendations on Treatment of Hepatitis C European Association for the Study of the Liver." *J Hepatology* (September):1–23.
13. Omar, H., W. El Akel, T. Elbaz, M. El Kassas, K. Elsaeed, H. El Shazly, M. Said, M. Yousif, A. A. Gomaa, A. Nasr, M. AbdAllah, M. Korany, S. A. Ismail, M. K. Shaker, W. Doss, G. Esmat, I. Waked, and Y. El Shazly. 2018. "Generic Daclatasvir plus Sofosbuvir, with or without Ribavirin, in Treatment of Chronic Hepatitis C: Real-World Results from 18 378 Patients in Egypt." *Alimentary Pharmacology and Therapeutics* 47(3):421–31.
14. Stepanova, Maria. 2017. "E c o n o m i c B u Rd e n o f Hepatitis C Infection." 21:22042.
15. Zhong, Jin, Dongmei Li, Yonghong Zhang, Xinyue Chen, Zhengkun Tu, Yuanyuan Cui, Hongqing Yan, Shasha Wang, Chao Zhang, Xia Jin, Pei Hao, Rui Hua, and Junqi Niu. 2017. "Comprehensive Mapping of Antigen Specific T Cell Responses in Hepatitis C Virus Infected Patients with or without Spontaneous Viral Clearance." *Plos One* 12(2):e0171217.
16. Mascia, Claudia, Serena Vita, Paola Zuccalà, Raffaella Marocco, Tiziana Tieghi, Stefano Savinelli, Raffaella Rossi, Marco Iannetta, Irene Pozzetto, Caterina Furlan, Fabio Mengoni, Claudio Maria Mastroianni, Vincenzo Vullo, and Miriam Lichtner. 2017. "Changes in Inflammatory Biomarkers in HCV-Infected Patients Undergoing Direct Acting Antiviral-Containing Regimens with or without Interferon." *PLoS ONE* 12(6):1–14.
17. Salem, Ahmed M., Reham M. Dawood, Noha G. Bader El Din, Marwa K. Ibrahim, Reem El-Shenawy, Mostafa M. Elhady, Mostafa K. El Awady, and Sally Farouk. 2017. " The Synergistic Effect of TNFα -308 G/A and TGFβ1 -509 C/T Polymorphisms on Hepatic Fibrosis Progression in Hepatitis C Virus Genotype 4 Patients ." *Viral Immunology* 30(2):127–35.
18. Thanapirom, Kessarin, Sirinporn Suksawatamnuay, Wattana Sukeepaisarnjaroen, Pisit Tangkijvanich, Sombat Treeprasertsuk, Panarat Thaimai, Rujipat Wasitthankasem, Yong Poovorawan, and Piyawat Komolmit. 2015. "Association between CXCL10 and DPP4 Gene Polymorphisms and a Complementary Role for Unfavorable IL28B Genotype in Prediction of Treatment Response in Thai Patients with Chronic Hepatitis C Virus Infection." *PLoS ONE* 10(9).
19. Neesgaard, Bastian, Morten Ruhwald, and Nina Weis. 2017. "Inducible Protein-10 as a Predictive Marker of Antiviral Hepatitis C Treatment: A Systematic Review." *World Journal of Hepatology* 9(14):677–88.
20. Lagging, Martin, Galia Askarieh, Francesco Negro, Stephanie Bibert, Jonas Söderholm, Johan Westin, Magnus Lindh, Ana Romero, Gabriele Missale, Carlo Ferrari, Avidan U. Neumann, Jean Michel Pawlotsky, Bart L. Haagmans, Stefan Zeuzem, Pierre Yves Bochud, and Kristoffer Hellstrand. 2011. "Response Prediction in Chronic Hepatitis c by Assessment of IP-10 and IL28B-Related Single Nucleotide Polymorphisms." *PLoS ONE* 6(2).

21. Zeremski, M., R. B. Dimova, S. Benjamin, M. S. Penney, M. C. Botfield, and A. H. Talal. 2015. "Intrahepatic and Peripheral CXCL10 Expression in Hepatitis C Virus-Infected Patients Treated with Telaprevir, Pegylated Interferon, and Ribavirin." *Journal of Infectious Diseases* 211(11):1795–99.
22. Sánchez-Ruano, Juan José, María Ángeles Jiménez-Sousa, Ana Zaida Gómez-Moreno, Tomas Artaza-Varasa, Sonia Vázquez-Morón, Salvador Resino, José Saura-Montalban, Daniel Pineda-Tenor, Pablo Ryan, Luz Maria Medrano, and Amanda Fernández-Rodríguez. 2017. "CXCL9-11 Polymorphisms Are Associated with Liver Fibrosis in Patients with Chronic Hepatitis C: A Cross-Sectional Study." *Clinical and Translational Medicine* 6(1):0–9.
23. Meissner, Eric G., Jérémie Decalf, Armanda Casrouge, Henry Masur, Shyam Kottilil, Matthew L. Albert, and Darragh Duffy. 2015. "Dynamic Changes of Post-Translationally Modified Forms of CXCL10 and Soluble DPP4 in HCV Subjects Receiving Interferon-Free Therapy." *PLoS ONE* 10(7):1–9.
24. Brownell, Jessica and Stephen J. Polyak. 2013. "Molecular Pathways: Hepatitis C Virus, CXCL10, and the Inflammatory Road to Liver Cancer." *Clinical Cancer Research* 19(6):1347–52.
25. Riva, Antonio, Melissa Laird, Armanda Casrouge, Arvydas Ambrozaitis, Roger Williams, Nikolai V. Naoumov, Matthew L. Albert, and Shilpa Chokshi. 2014. "Truncated CXCL10 Is Associated with Failure to Achieve Spontaneous Clearance of Acute Hepatitis C Infection." *Hepatology* 60(2):487–96.
26. Decalf, Jérémie, Kristin V Tarbell, Armanda Casrouge, Jeffrey D. Price, Grace Linder, Estelle Mottez, Philippe Sultanik, Vincent Mallet, Stanislas Pol, Darragh Duffy, and Matthew L. Albert. 2016. "Inhibition of DPP 4 Activity in Humans Establishes Its in Vivo Role in CXCL 10 Post-Translational Modification : Prospective Placebo-Controlled Clinical Studies." 1–5.
27. Azab, Naglaa and Naglaa Azab. 2017a. "Interferon Inducible Protein-10 Level and Il28b Gene Polymorphism as Predictors of the Response to Pegylated Interferon / Ribavirin Therapy in Egyptian HCV Patients ." (January).
28. Ghada R. El-Hendawya, Ahmed A. Salamaa, Elsayed I. Elshaybb, Nancy R. Ahmed Elhosseny. 2018. "Study of Interferon-γ-inducible Protein-10 Levels during Antiviral Therapy of Hepatitis C Patients with Sofosbuvir plus Ribavirin and Interferon in Menoufia Hospitals." *Menoufia Medical Journal* 30:997–1004.
29. Aref, Ahmed Mostafa, Mohamed Shamrouh Othman, and Samah Mamdouh. 2016. "New Biomarkers for Response to Treatment of HCV Infected Patients Based on IP-10 and IL 28B Polymorphism Analysis." 6(5): 122–29.
30. Charles, Edgar D. and Lynn B. Dustin. 2010. "Chemokine Antagonism in Chronic Hepatitis C Virus Infection." *J Clin Invest* 107(11):25–27.
31. Ragab, Gaafar and Mohamed A. Hussein. 2017. "Vasculitic Syndromes in Hepatitis C Virus: A Review." *Journal of Advanced Research* 8(2):99–111.
32. Aljumah, Abdulrahman A, Faisal Abaalkhail, Hamad Al-Ashgar, Abdullah Assiri, Mohamed Babatin, Faleh Al-Faleh, Abdullah Alghamdi, Raafat Al-Hakeem, Almoataz Hashim, Adel Alqutub, Homie Razavi, FaisalM Sanai, Khalid Al-Swat, Jonathan Schmelzer, and Ibrahim Altraif. 2016. "Epidemiology, Disease Burden, and Treatment Strategies of Chronic Hepatitis C Virus Infections in Saudi Arabia in the New Treatment Paradigm Shift." *Saudi Journal of Gastroenterology* 22(4):269.
33. Taura, Naota, Sachiko Fukuda, Tatsuki Ichikawa, Hisamitsu Miyaaki, Hidetaka Shibata, Takuya Honda, Tohei Yamaguchi, Yoko Kubota, Shinjiro Uchida, Yasuhiro Kamo, Emi Yoshimura, Hajime Isomoto, Takehiro Matsumoto, Fuminao Takeshima,

Takuya Tsutsumi, Shotaro Tsuruta, and Kazuhiko Nakao. 2012. "Relationship of α-Fetoprotein Levels and Development of Hepatocellular Carcinoma in Hepatitis C Patients with Liver Cirrhosis." *Experimental and Therapeutic Medicine* 4(6):972–76.

34. Abd-elfatah, Sabry and Farag Khalil. 2014. "Evaluation of the role of alpha-fetoprotein (afp) levels in chronic viral hepatitis C patients, without hepatocellular carcinoma (HCC)." (January): 130–54.
35. Azab, Naglaa and Naglaa Azab. 2017b. "Interferon Inducible Protein-10 Level and Il28b Gene Polymorphism as Predictors of the Response to Pegylated Interferon / Ribavirin Therapy in Egyptian HCV Patients ." (February).
36. Al Ashgar, Hamad I., Mohammed Q. Khan, Mohammed Al-Ahdal, Sahar Al Thawadi, Ahmad Salem Helmy, Ahmed Al Qahtani, and Faisal M. Sanai. 2013. "Hepatitis C Genotype 4: Genotypic Diversity, Epidemiological Profile, and Clinical Relevance of Subtypes in Saudi Arabia." *Saudi Journal of Gastroenterology* 19(1):28–33.
37. Crisan, Dana, Mircea Dan Grigorescu, Corina Radu, Alina Suciu, and Mircea Grigorescu. 2017. "Interferon-γ-Inducible Protein-10 in Chronic Hepatitis C: Correlations with Insulin Resistance, Histological Features & Sustained Virological Response." *Indian Journal of Medical Research* 145(April):543–50.

TABLES

Table (1) : The sensitivity and specificity of IP -10 in the positive and negative HCV RNA

Reference test	Evaluated test		Total
	+ve	-ve	
+ve	True +ve (a)	False-ve (c)	a+c
-ve	False +ve (b)	True –ve (d)	b+d
Total	a+b	c+d	a+b+c+d

Sensitivity: A/(A+C)X100= 28/ (28+2)X100= 93%

Specificity :D/(D+B)X100=58/(58+2)X100=96%

Efficiency: (a+c)/ (a+b+c+d)X100=95.5%

Positive Predictive Value :A/ (A+B)X100=28/(28+2)=93%

Negative Predictive Value :D/(D+C)X100=58/(58+2)=96%

Table (2): The biochemical parameters profile among cohort study

Parameters	**Healthy controls 20**	**Chronic 20**	**Natural clearance 20**	**Responders 20**	**Non-Responders 10**	**P value**
AFP (ng/ml)	1.64 ±0.91	4.95±4.86	4.86 ±1.02	2.67 ±2.82	8.22 ±3.22	<0.001
Direct Bilirubin(mg/dl)	0.18 ±0.03	0.23±0.13	0.13 ±0.02	0.18 ±0.05	0.28 ±0.13	0.007
Total Bilirubin (mg/dl)	0.53 ±0.11	1.28±0.76	0.57 ±0.10	0.59 ±0.16	0.91 ±0.26	0.333
PT (seconds)	12.1 ±0.2	12.6±0.4	12.3 ±0.2	12.2 ±0.3	13.0 ±0.6	<0.001
Albumin (g/L)	4.41 ±0.26	4.19±0.24	4.31 ±0.30	4.35 ±0.21	3.79 ±0.44	<0.001
ALP (U/L)	73 ±13	88±14	14 ±12	70 ±13	93 ±9	<0.001
AST (U/L)	25 ± 6	45 ± 18	29 ± 7	30 ± 13	49 ± 13	<0.001
ALT (U/L)	24 ±6	47±20	20 ±8	31 ±17	53 ±12	<0.001

Where AFP (ng/ml) :Alpha Feto protein : up to 10, Direct Bilirubin(mg/dl) : Up to 0.25, Total Bilirubin (mg/dl) : Up to 1.0, PT (seconds) : prothrombine time : 11.5 – 12.0, Albumin (g/L) : 3.5 – 5.2 , ALP (U/L): Alkaline Phosphatase : Up to 127, ALT (U/L) : Glutamate pyruvate transaminase : Up to 40, AST (U/L): Glutamate Oxaloacetete transaminase : Up to 40

Table (3): The hematological parameters profile among cohort study:

Parameter	Healthy control (n=20)	Chronic group (n=20)	Natural clearance group (n=20)	Responders group (n=20)	Non- Responders group (n=10)	P value
Age (years)	38±11	49±12	42±11	41±10	47±13	0.027
Sex (M)	10 (50.0%)	14 (70.0%)	11 (55.0%)	6 (30.0%)	7 (70.0%)	0.098
(F)	10 (50.0%)	6 (30.0%)	9 (45.0%)	14 (70.0%)	3 (30.0%)	
BMI (kg/m)	23.0 ±3.49	27.1 ±4.40	24.6 ±4.38	24.9±4.69	28.2 ±2.60	<0.001
RBCs (10^6/mm^3)	4.61 ±0.61	4.81 ± 0.41	4.74 ±0.54	4.47 ±0.45	4.66 ±0.40	0.262
Hb (g/L)	12.56 ±1.80	13.07 ± 1.39	12.61 ±1.77	11.79 ±1.25	12.49 ±1.36	0.145
HCT (%)	37.71 ±5.42	39.27 ± 3.97	38.07 ±5.53	35.58 ±4.13	38.00 ±4.27	0.187
TLC (10^3/mm^3)	7.16 ±1.50	7.16 ±1.98	7.91 ±1.53	8.42 ±1.75	5.26 ±0.86	<0.001
Neutrophils (%)	54.40 ±7.26	47.12 ±14.90	54.15±6.75	55.20 ±6.54	48.50 ±7.53	0.027
Lymphocytes (%)	38 ±8	44 ±9	38 ±7	37 ±6	44 ±8	0.023
Monocytes (%)	7 ±3	6 ±2	6 ±2	6 ±3	6 ±2	0.836
PLT (10^3/mm^3)	241 ±44	170 ±59	199 ±44	212 ±38	108 ± 37	<0.001

Where RBCs (10^6/mm^3) : red blood cells : Male 4.5 – 5.6 & Female 3.8– 5.0, Hb (g/L) : Hemoglobin : Male 13.0 – 17.0 & Female 12.0 – 16.0, HCT (%) : Hematocrite : Male 40.0 – 52.0 & Female 36.0 – 42.0, TLC (10^3/mm^3) : Total Leukocyte count : 4.0 – 11.0, Neutrophils (%) : 40 – 60, Lymphocytes (%) : 20 – 40, Monocytes (%) : 2.0 – 8.0, PLT (10^3/mm^3) : Platelets count : 150 – 400

FIGURES

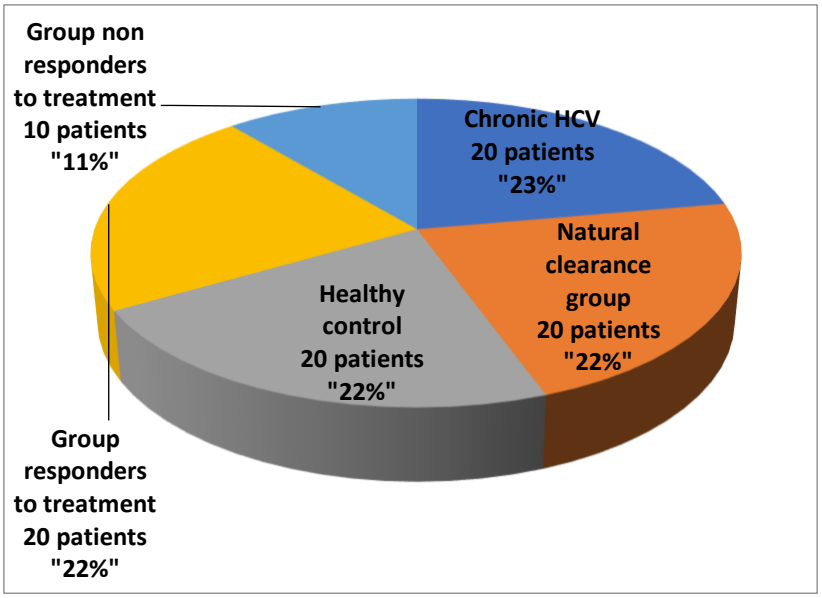

Figure (1): Distribution of the enrolled patients in the present study.

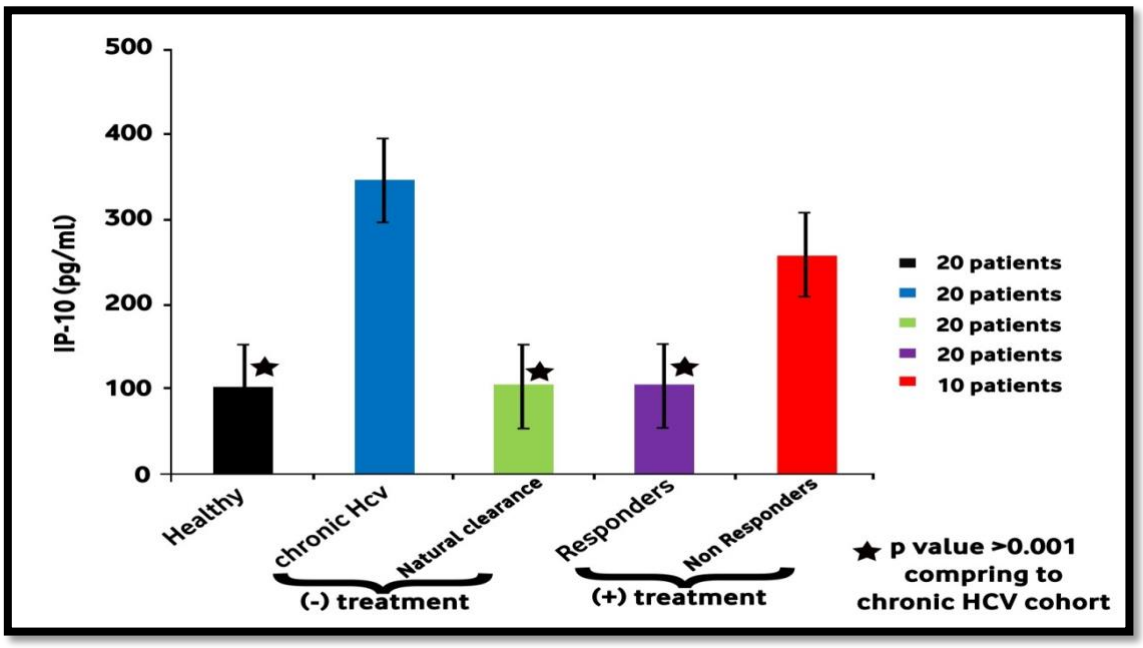

Figure (2): serum level of IP-10 in chronic HCV, natural clearance and responders and non-responders patients as measured by ELISA

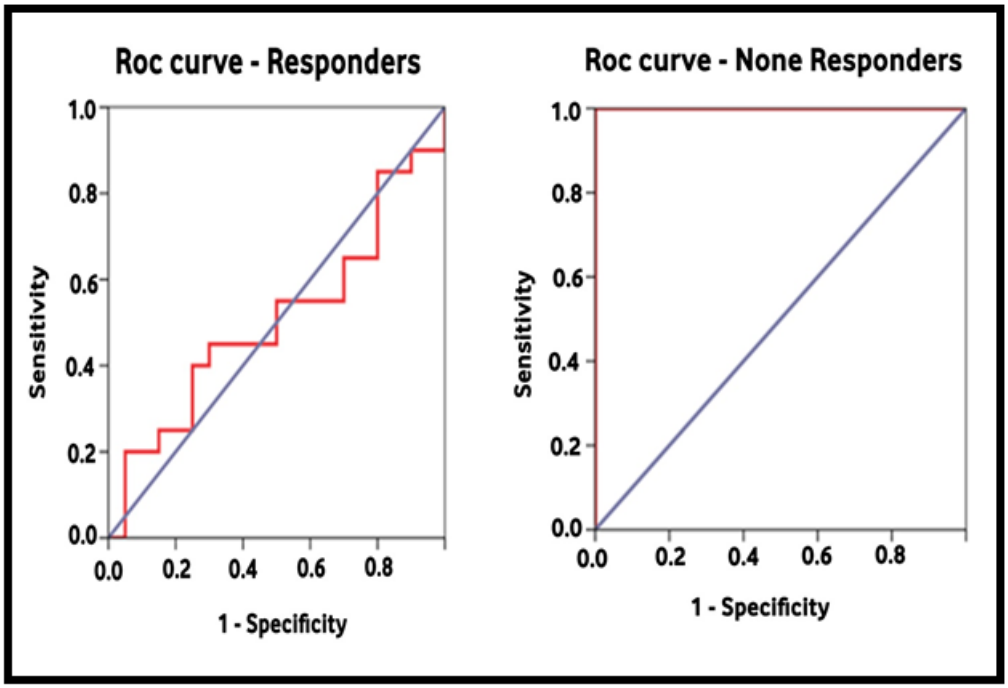

Figure (3): ROC analysis of serum level of IP-10 in responders and non-responders HCV patients to DAA treatment.

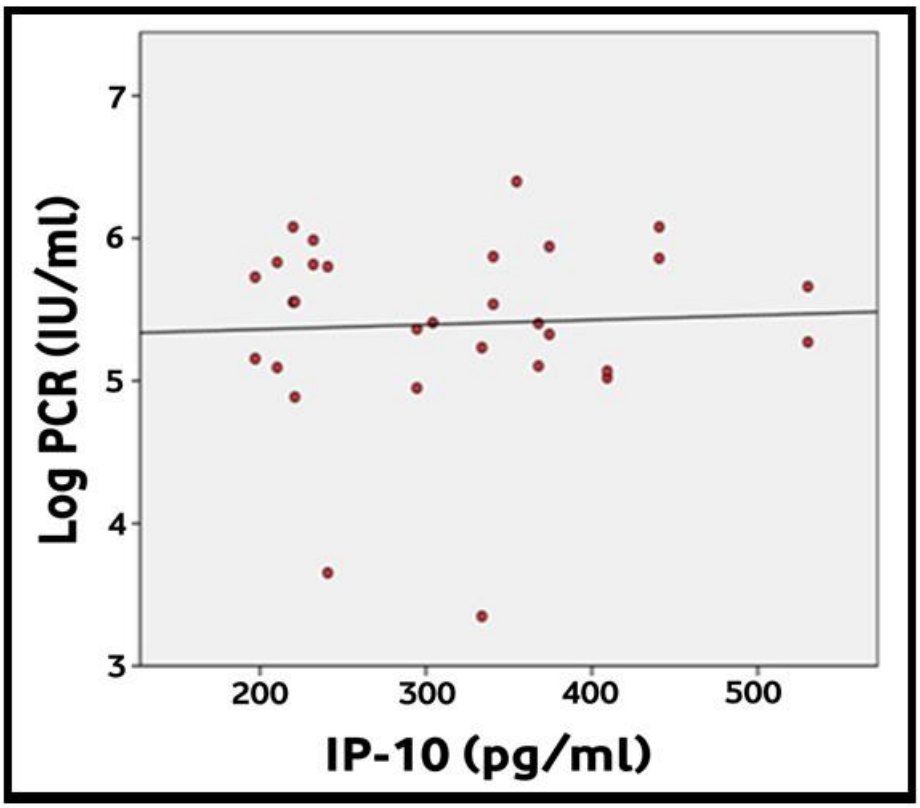

Figure (4): The correlation of serum level of IP-10 and HCV HCV viral load

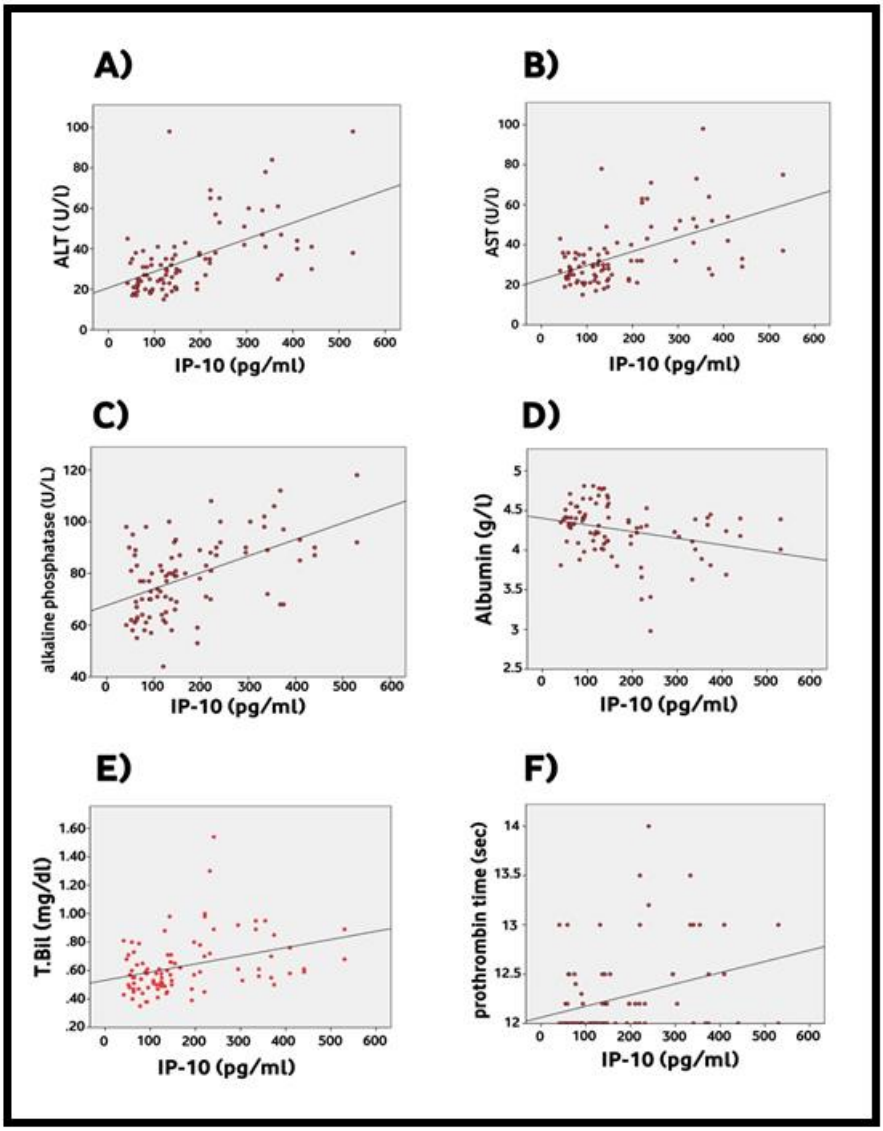

Figure (5): Correlation among IP-10 level, liver function parameters**.**

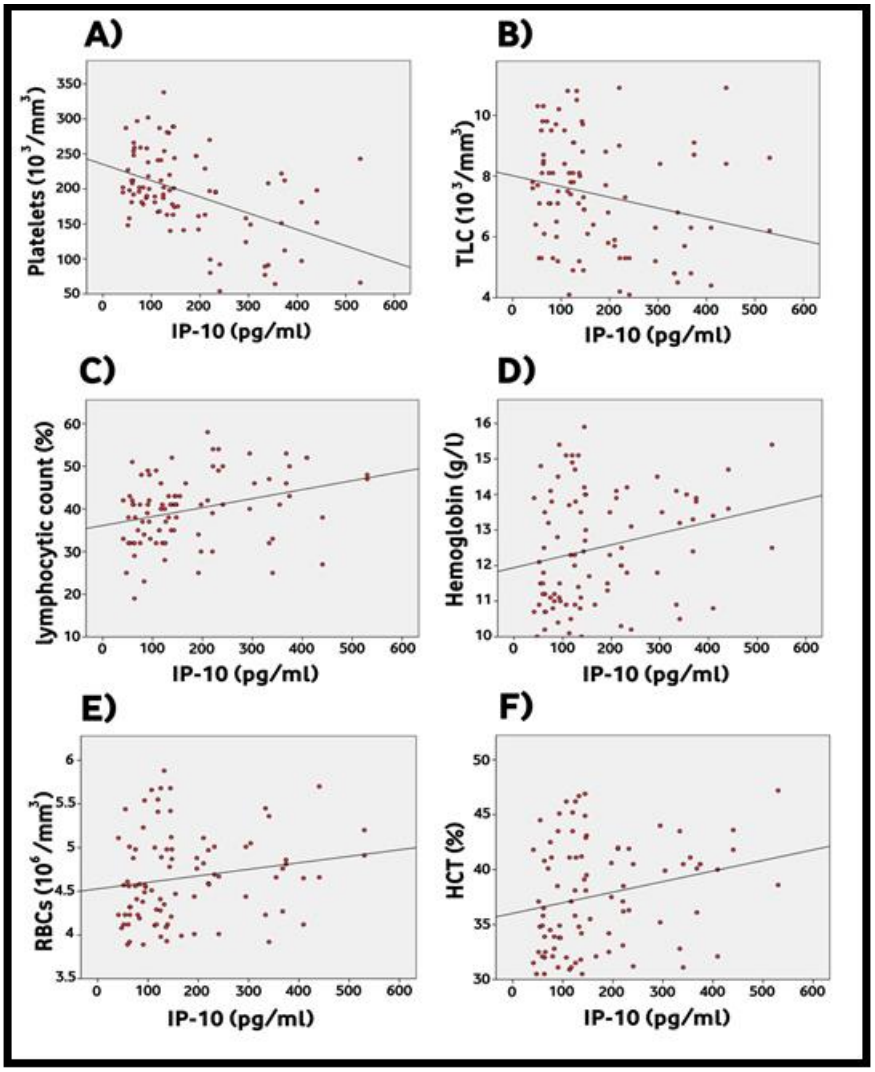

Figure (6): Correlation among IP-10 level, Hematological parameters.