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Correlation between CXCL-motif-10 and IFN- γ on Hemodialysis Patients with HCV under Treatment

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ABSTRACT: ***Background:*** **Hepatitis C virus is remain a contagious disease specifically in development countries. Several cytokines were determined as marker of chronic hepatitis C infection through treatment stages.** ***Objective:*** **This study was aimed to investigate the relationship between variations of treatment efficacy on serum levels of cytokines for dialysis patients with chronic hepatitis C infection.** ***Methods:*** **We evaluate 60 dialysis patients presumably with positive CHC and 24 healthy control using anti- HCV antibodies ELISA kit. ALP chemical serum levels were detected using kit of an automated chemical Analyzer. CXCL-10 and IFN-γ serum level was demonstrated using ELISA technique. Correlation between parameters was evaluated using SPSS version 16.0.** ***Results:*** **CXCL-10 and ALP serum levels were raised in RIT patients while IFN-γ serum level elevated in SVR and RIT patients. There were significant correlation coefficient between dependent variables and drug status with $P < 0.01$ value. There were no significant relations between dependent variables, sex and age groups. There were interrelationships between SVR and RIT groups according to the variables under study.** ***Conclusions:*** **CXCL-10 serum level may be used as a marker to detect CHC patients under treatment. IFN- γ serum level has no indication with dialysis patients who take the drug regularly or intermittently and the same thing applies with ALP serum level.**

Keyword: ALP, CXCL-10, HCV, Hemodialysis, IFN.

I. INTRODUCTION

Hepatitis C virus is a highly contagious disease related to the family *Flaviviridae* with a positive sense single strand RNA genome. Genomic RNA exists as at least 6 genotypes with 50 subtypes [11]. The seriousness of the virus is transferred to dialysis patients. HCV causes of morbidity and mortality in hemodialysis (HD) patients [8]. The prevalence of HCV infection ranges from 1-3% in general population, but this ratio is higher in dialysis patients from 3- 23% due to the fact that these patients have risk factors for acquisition of HCV, such as receipt of blood transfusions and use of illicit intravenous drug [18, 19]. Because there is no vaccine for the virus, attention has been drawn to finding a suitable drug. Current therapies for hepatitis C pursue to limit development of persistent inflammation by reducing systemic viral load [3]. The treatment is currently adjusted around the combination between pegylated interferon alpha (Peg IFN/α) and the non-specific anti-viral Ribavirin [11, 17]. Unfortunately, treatment program fails to eliminate the infection in roughly 50% of patients. Interferon-gamma (IFN-γ) is an antiviral activity. It is one of the T- helper related cytokine. Previous reports had shown that IFN-γ might inhibit virus replication and mediate liver damage. Moreover, IFN-γ might be useful in predicting the clinical outcome of the combination therapy of Peg IFN /RBV [2, 9, and 12]. CXCL-motif-10 is part of a family of α-chemokines that bind CXCR3, which is induced by IFN-γ and CXCL-11 [3, 5, and 15]. After binding receptors of CXCR3 with CXCL-10 act as a chemoattractant makes levels of CXCL-10 are elevated in HCV-infected patients with high levels of liver inflammation and fibrosis [12]. CXCL-10 recruits a pro-inflammatory which considered as anti-viral immune response for chronic hepatitis C (CHC) [3, 6].

Dialysis patients are suffering from chronic renal disease in which evolution, prognosis and treatment options for HCV-related liver disease remain problematic [4]. Several mechanisms have been suggested that adsorption of HCV onto the dialysis membrane, destruction of HCV particles and escape of HCV into the dialysate, makes increase of plasma IFN-α levels during dialysis [10].

The aim of this study was to evaluate the role of CXCL-10 and IFN-γ levels on sustained virological response (SVR) and compared with patients receiving intermittent treatment (RIT). All dialysis patients with chronic HCV under study have the positive results for anti-HCV. Currently study includes (84) blood samples (47) male and (37) female. A 60 blood samples were collected from dialysis patients in Central Health Laboratory from February 2019 to June 2019 in addition to (24) control samples. Their ages between (1-75) years divided into 3 groups. Consent form was obtained from every patient prior to the sample collection which was performed according to standard protocols approved by the local health authority of Mosul city. Patients questionnaire were collected to find out whether they were receiving treatment periodically or sporadically.

II. MATERIALS AND METHODS

Chronic hepatitis C (CHC) patients who treated with PegIFN/RBV combination therapy were enrolled in the study. Sustained virological response (SVR) is definite as undetectable HCV RNA throughout a 24 week post-treatment follow-up period (data not found). RIT is defined as patients receiving intermittent treatment due to that treatment is not available continuously in health centers (taken from questionnaire).

Participants subcutaneously received a course of IFN-α2b (1.5 µg/kg/week) or PegIFN α -2a (180 µg/week) plus Ribavirin (1000 mg/day) therapy for the duration more than 32 weeks according to the availability of drugs in the health institutions. Currently study includes (84) blood samples (47) male and (37) female samples. Ages of patients and healthy control donors were between 1-75 years old which grouped into 3 classes: 1= (1-25 years), 2= (26-50 years), and 3= (51-75 years). Hemodialysis patients were recruited consecutively of CHC. Samples were divided into 3 groups, SVR (38), RIT (22) and health control (24).Positive and negative results for the healthy control group were confirmed and the results were evaluated. The demographic data and clinical history of patients were gathered based on a questionnaire completed.

Samples handling: Venous blood samples (5ml) were collected from each patient under complete aseptic conditions. Sera separated and stored frozen at -20˚C till tests analysis.

Detect HCV antibodies using ELISA: Presence of anti-HCV antibodies in serum was detected using enzyme linked immunosorbent assay (ELISA) commercial kit from (DIALAB- Austria) purchased from according to the manufacturer's instruction for more than (6) months for all patients.

Determine ALP using activity colorimetric assay kit: Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in alkaline buffer. Changes in alkaline phosphatase level and activity are associated with various disease states such in liver.ALP kit purchased according to the manufacturer's instruction from Komabiotech-Chinausing Smart-150 device- USA.

Detect CXCL-10 level using ELISA: Serum concentrations of CXCL-10 were measured in duplicate using a commercial human platinum ELISA kit from (antibodies-online, Germany). The OD_{450}nm were determined using an ELISA reader (Awreness-USA). The chemokine standards were also prepared and the concentration of CXCL-10 (pg. /ml) was determined using the standard curve.

Determine IFN-γ level using ELISA: serum level of IFN-γ for all samples were measured using commercial Human IFN-gamma ELISA Kit from (Komabiotech-China) using (Rayto-RT ELISA– Germany) in OD_{450}nm. The IFN-γ standards were also prepared and the concentration (pg. /ml) was determined using the standard curve.

III. STATISTICAL ANALYSIS

Data analysis was completed using SPSS Inc. Chicago, IL, USA software version 16.0 especially for the calculation of mean values, standard deviations (SD) and standard error (SE) forserological and biochemical parameters. The homogeneity and correlation efficient with *P* value between parameters were used to compare significantly value at $P < 0.01$ and $P < 0.05$. Multivariate logistic regression test was further performed to identify the independent factors of SVR and RIT groups. Microsoft office excel version 2013 was used to explain the values graphically. The differences between the groups were analyzed by Pearson test with overall correlation between parameters.

IV. RESULTS

All SVR and RIT dialysis patients got positive result for HCV antibody test using ELISA. Relying on the drug status groups, the number, mean, standard deviations and standard errors for all parameters were revealed in Table 1. According to the age groups, the number, mean, standard deviations and standard errors for all parameters were revealed in Table 2.

ALP serum level: According to the drug status groups, the ALP serum levels for control samples is in the normal range. Some samples of SVR group shows elevated of ALP levels while most samples of RIT group got high elevation. Correlation coefficient between control, SVR and RIT groups shows significant value at $P < 0.05$ with ALP serum level Fig. 1 (a) numbers in Table (3). The correlation between ALP serum levels and drug status groups were presented significant value $P < 0.01$ with Pearson test Fig. 1 (b). On the other hand, there is no significant value $P > 0.05$ between ALP serum levels with sex groups (Fig. 2) and age groups Fig. 3.

Table 1: Number, mean, standard deviations and standard errors for all parameters with the drug status groups.SVR: sustained virological response, RIT: receiving intermittent treatment, ALP: Alkaline phosphatase, IFN-γ: interferon gamma, CXCL-10: chemokine motif 10.

Parameters		N	Mean	Std. Deviation	Std. Error
ALP (U/L)	Control	24	1.4952E2	±57.26026	11.68820
	SVR	38	2.3700E2	±133.11121	21.59349
	RIT	22	3.8955E2	±175.56839	37.43131
	Total	84	2.5196E2	±157.35497	17.16883
IFN-γ	Control	24	12.1517	±6.75855	1.37958
	SVR	38	52.0958	±43.04492	6.98281
	RIT	22	35.9495	±29.11002	6.20628
	Total	84	36.4544	±36.54989	3.98792
CXCL-10	Control	24	2.5958	±4.24986	0.86750
	SVR	38	1.1763	±3.63276	0.58931
	RIT	22	49.9318	±29.82854	6.35947
	Total	84	14.3512	±26.28672	2.86812

Table 2: Number, mean, standard deviations and standard errors for all parameters with the age groups.

Parameters		N	Mean	Std. Deviation	Std. Error
ALP (U/L)	1-25 y	30	2.6873E2	±162.51210	29.67051
	26-50 y	37	2.4374E2	±162.01202	26.63461
	51-75 y	17	2.4024E2	±143.83872	34.88601
	Total	84	2.5196E2	±157.35497	17.16883
IFN-γ	1-25 y	30	33.7320	±26.71947	4.87828
	26-50 y	37	42.9982	±43.67479	7.18009
	51-75 y	17	27.0161	±33.71258	8.17650
	Total	84	36.4544	±36.54989	3.98792
CXCL-10	1-25 y	30	20.1350	±32.28824	5.89500
	26-50 y	37	8.7416	±18.69767	3.07388
	51-75 y	17	16.3535	±27.72319	6.72386
	Total	84	14.3512	±26.28672	2.86812

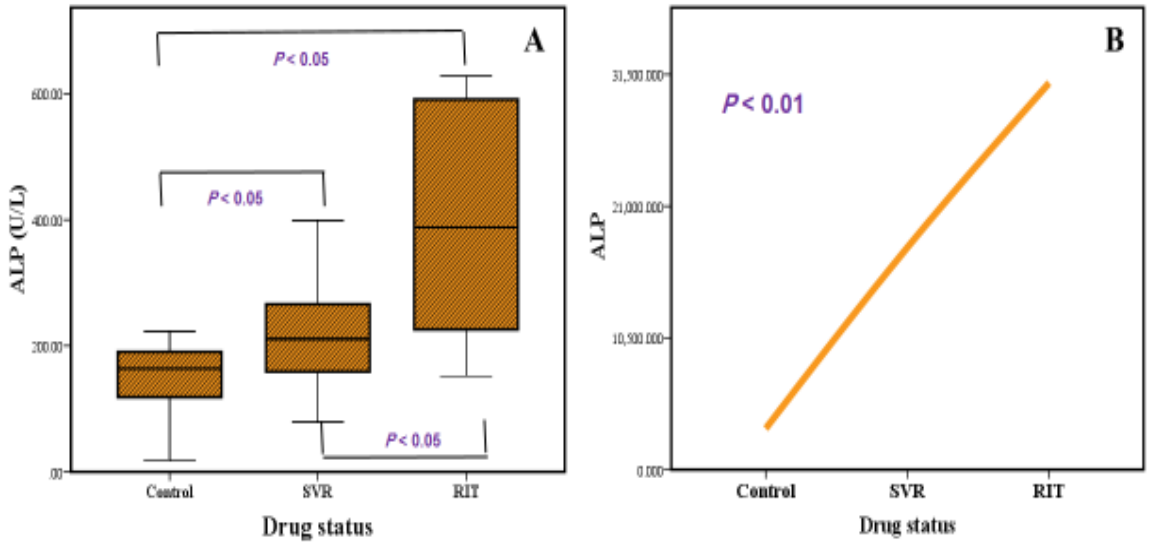

Fig. 1. (a) ALP serum levels of drug status groups and correlations between groups. (b) Variance correlation coefficient of ALP serum level with drug status.

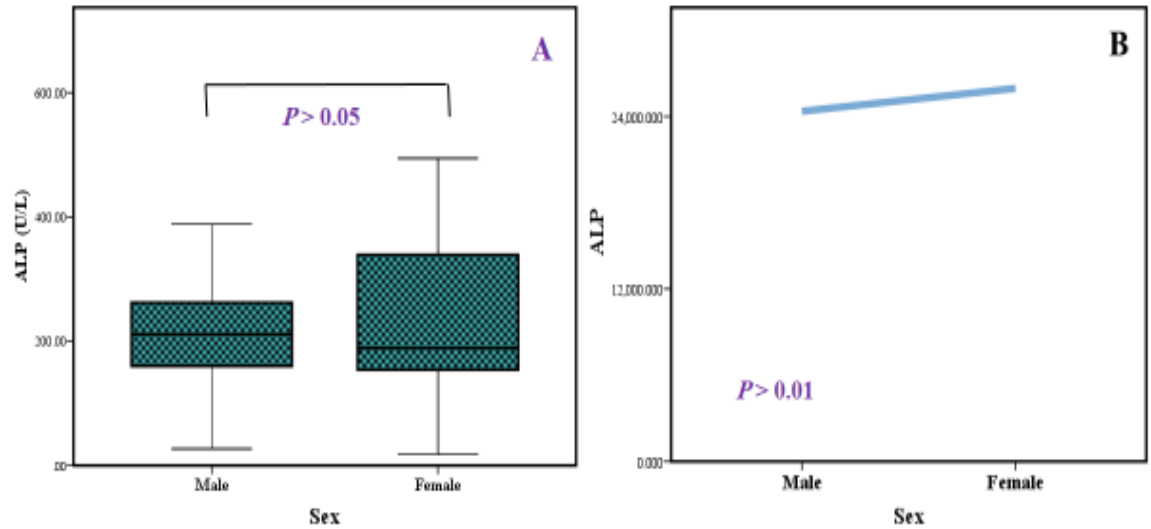

Fig. 2. (a) ALP serum levels with sex groups and correlation coefficient. (b) Variance correlation.

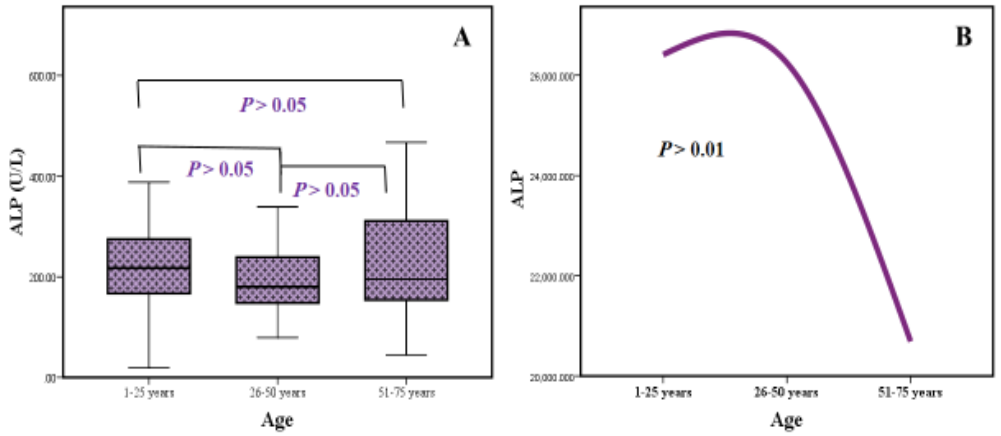

Fig. 3. (a) ALP serum levels with age groups and correlation coefficient. (b) Variance correlation.

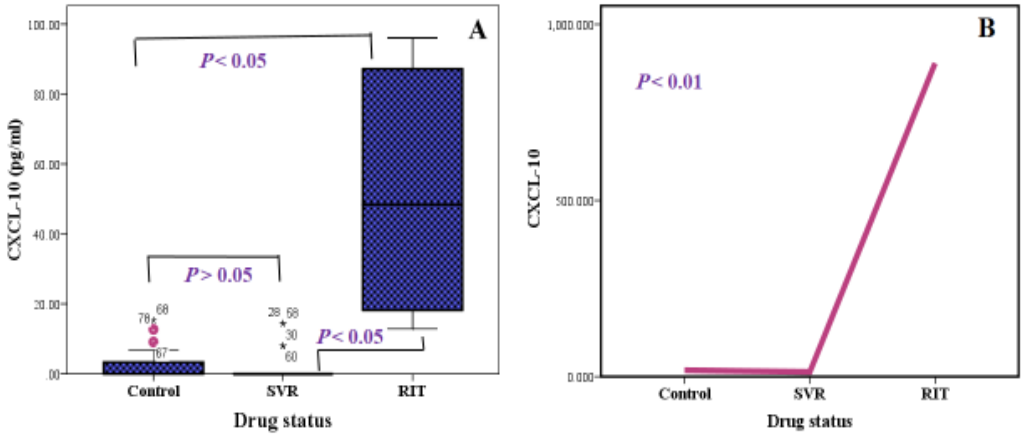

Fig. 4. (a) CXCL-10 serum levels of drug status groups and correlations between groups. (b) Variance correlation coefficient of CXCL-10 serum level with drug status.

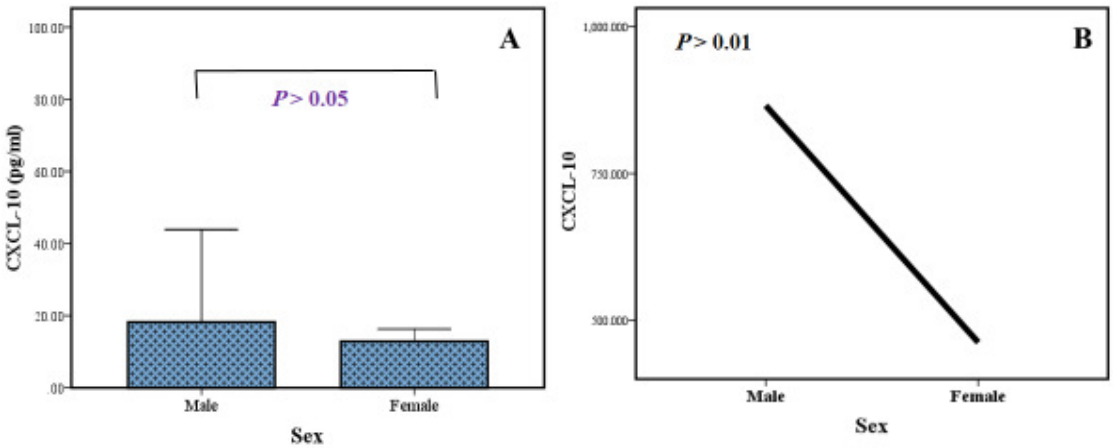

Fig. 5. (a) CXCL-10 serum levels of sex groups and correlations between groups. (b) Variance correlation coefficient of CXCL-10 serum level with sex groups.

CXCL-10 serum level: Both of control and SVR groups presented normal range of CXCL-10 serum levels while it was eminent with RIT group Fig. 4. Correlation between drug status groups and CXCL-10 serum levels indicated high significant value at $P < 0.01$ using Pearson test Fig. 4 (b). Correlation coefficient of CXCL-10 with (control+ RIT) and (SVR+RIT) groups are significant value at $P < 0.05$ while no significant association between (control+ SVR) groups Fig. 4 (a) numbers in Table 3. On the contrary, there is no significant value between CXCL-10 with sex and age groups at value $P > 0.05$ using Pearson test Figs. 5, 6.

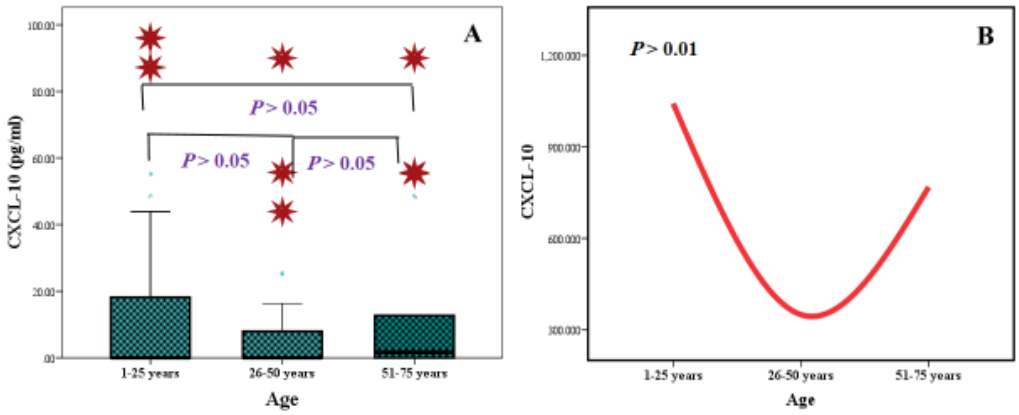

Fig. 6. (a) CXCL-10 serum levels of age groups and correlations between groups. (b) Variance correlation coefficient of CXCL-10 serum level with age groups. ✷ : Extreme samples from other outcome values.

IFN-γ serum level: SVR and RIT groups have elevation of IFN-γ serum levels compared with control samples Fig. 7. There is a significant correlation between IFN-γ serum level with all drug status groups at $P < 0.01$ using Pearson test Fig. 7 (b). The comparisons between groups according IFN-γ serum level were significant value at $P < 0.05$ for (control + SVR) and (control + RIT) groups but on the contrary there was no significant value at $P > 0.05$ between (SVR+RIT) Fig. 7 (a) numbers in Table (3). Moreover, there were no significant correlation of IFN-γ serum level depending on sex and age groups at value $P > 0.05$ using Pearson test Figs. (8 and 9).

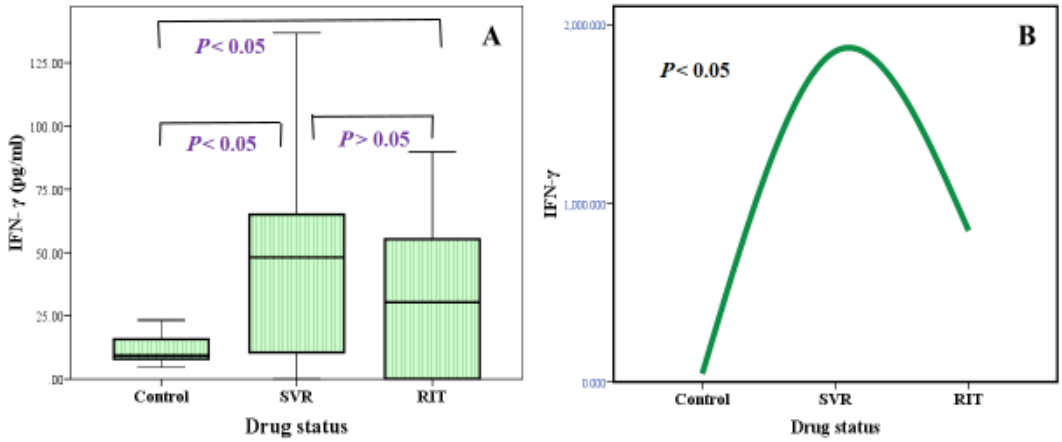

Fig. 7. (a) IFN-γ serum levels of drug status groups and correlations between groups. (b) Variance correlation coefficient of IFN-γ serum level with drug status.

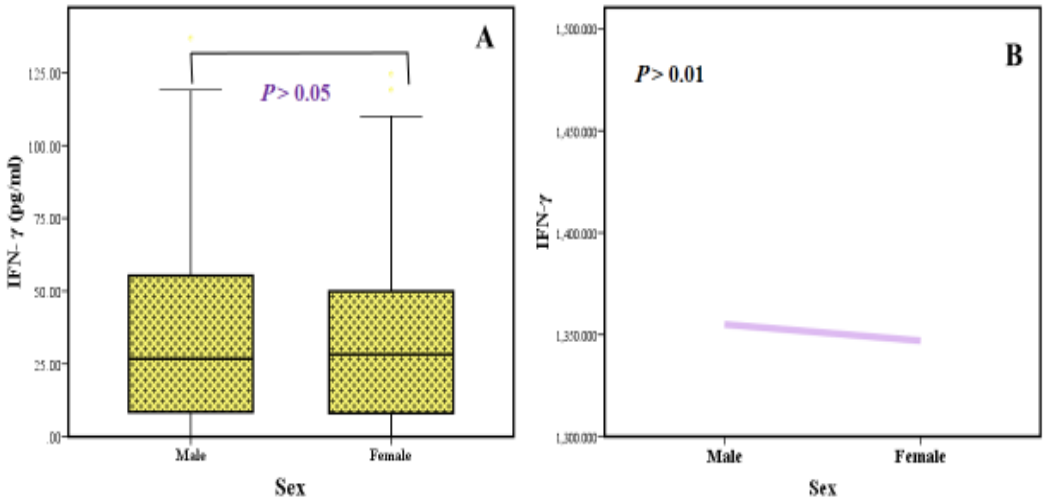

Fig. 8 (a) IFN-γ serum levels of sex groups and correlations between groups. (b) Variance correlation coefficient of IFN-γ serum level with sex groups.

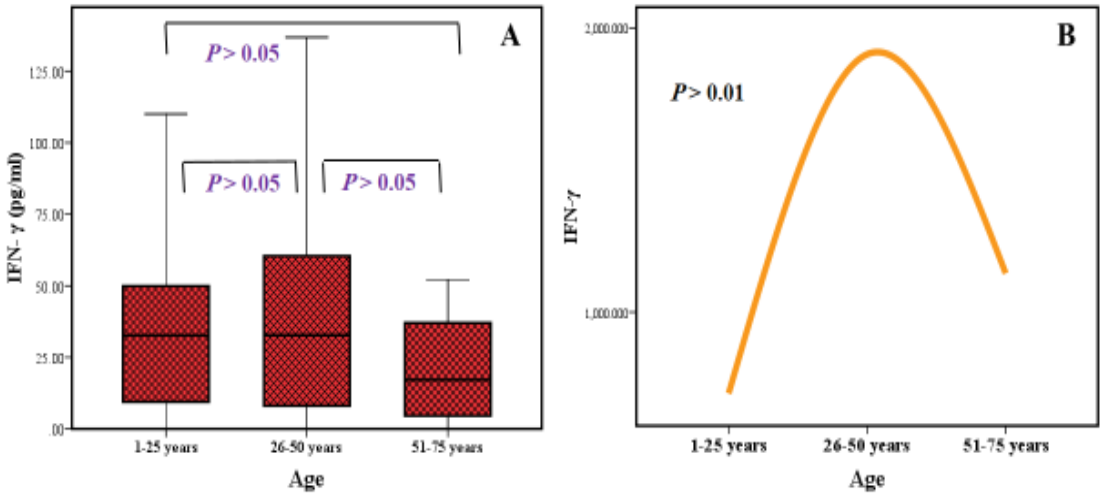

Fig. 9. (a) IFN-γ serum levels of age groups and correlations between groups. (b) Variance correlation coefficient of IFN-γ serum level with age groups.

Table 3: Correlation coefficient and mean differences between drug statuses with study variables.

Dependent Variable	Drug status	Mean Differences	Sig.
ALP	Control SVR	87.48125*	0.002
	Control RIT	240.02670*	0.000
	RIT SVR	152.54545*	0.004
IFN-γ	Control SVR	39.94412*	0.000
	Control RIT	23.79779*	0.003
	RIT SVR	16.14633	0.245
CXCL-10	Control SVR	1.41952	0.454
	Control RIT	47.33598*	0.000
	RIT SVR	48.75550*	0.000

V. DISCUSSION

Roughly 170 million individuals were infected with hepatitis C worldwide [5]. Several studies have revealed the mechanisms by which the immune system is capable of mediating viral clearance. More than 100 different inflammatory cytokines have been identified, which regulate the balance between humoral and cell-mediated immunity. These cytokines are participate in the defense against viral replications and modulating the host immune function [11]. HCV during dialysis is the major problem in many of developing countries [1, 19]. In our country, we are still facing this problem in cumulative the number of dialysis patients with the HCV. In this study, we aimed to find the impact of CXCL-10 serum level on the patients whom have HCV under treatment and compare it with IFN-γ and ALP. Our clinical study conducted of inflammatory cytokines during PegIFN/RBV therapy and the other patients whom took intermittent treatment. We elected two groups of dialysis patients with HCV in this study, SVR sustained virological response whom had taken combination therapy of PegIFN /RBV for more than (6) months. This group presented low viral RNA load in the serum (data not found). CXCL-10 declines simultaneously with the level of viremia and related to viral load [12]. Second group is RIT receiving intermittent treatment whom have discontinuous treatment due to unavailable drugs in the health institutions at all time. All information were collected from patient's questionnaire. Our analytical data revealed several correlations between variable parameters. The drug status groups were presented significant correlation coefficient with ALP, CXCL-10 and IFN- γ at $P < 0.01$using Pearson test. Age and sex group didn't give significant correlation for all parameters under study at $P > 0.05$ value. We exposed that RIT group got high levels for ALP, CXCL-10 and IFN- γ compared with control group. SVR group showed elevation for CXCL-10 and IFN- γ due to these cytokines emerge under infections. SVR group presented normal range for ALP due to hypothesis that most proteins intensities return to the normal range of chronic infections. Correlation between groups under study publicized different points. Significant correlation were found between control, SVR and RIT groups with ALP levels. Moreover, association between (control+ SVR) and (control+ RIT) groups were accessible with IFN- γ levels. Another association registered between (control+ RIT) and (SVR+ RIT) groups with CXCL-10 levels. All significant correlations were at $P < 0.05$ value. Non-significant correlations enrolled between (SVR+ RIT) groups with IFN-γ levels. Furthermore, no correlation found between (control + SVR) groups with CXCL-10 levels. Non-significant correlations were at $P > 0.05$ value.

Our study disclosed that CXCL-10 serum level plays a crucial role for distinguish between CHC patients whom have continuously and discontinuously taken drugs due to elevation of CXCL-10 in RIT patients compared with SVR patients. Our findings agree with other studies that have demonstrated the CXCL-10 levels. A study was reported that the baseline levels of cytokines (IL-2, IL-4, IL-8, and IL-10) and IFN-γ were significantly elevated in the non-SVR group compared with the SVR group. A positive correlation between CXCL-10 levels and fibrosis index was observed suggesting a possible role of CXCL-10 as a non-invasive marker of liver fibrosis [15].Four clinical independent studies have demonstrated baseline levels of CXCL-10 predictive of the failure responding to HCV treatment [5].Furthermore, combination therapy of SVR group for genotype 1b with high viral load still remains only 40–50% [17]. A clinical study publicized that pretreatment serum CXCL-10 level was significantly lower in patients who achieved SVR than in those in non–responder drug NRD [11].

Currently study enrolled high serum levels of IFN- γ form both SVR and RIT groups without a correlation significant compared with control samples. This is related as an immune response against infection. IFN-α and IFN-γ are secreted from natural killer (NK) cells in the liver seems to play an important role as antiviral activity in serum mouse model [13]. Our data agree with other studies found altitude of IFN-γ serum level in CHC patients under combination therapy. Some studies provided an evidence that up-regulation of IFN-γ plays an important role in the poor outcomes of PegIFN/ RBV combination therapy [11, 14].

We have listed that there is a correlation between CXCL-10 and IFN- γ. We found a significant association of CXCL-10 serum level between RIT and SVR though only RIT group showed rise of CXCL-10. Even though, there were elevation of IFN- γ serum level in both SVR and RIT groups, there were no significant correlation between them. One study reported that baseline levels of CXCL-10 which induced by IFN- γ before treatment was higher in patients who did not achieve an SVR after the end of treatment [6]. Finally, we found that there was no relationship between ALP serum level with dialysis patients, although there were significant relationships between groups of drug status. Our previous study showed that cytokines is an important parameters to detect HBV infection and compared with other biochemical parameters.

Our study limitation was not the follow up of CXCL-10 trend during treatment as it would have been useful for the complete understanding of the pathogenic mechanism. Not only that but it was necessary eventually for the need of following this marker as a long term prognosis factor. Different approaches have to be attempted with new ongoing studies showing promising data and good results.

VI. CONCLUSION

HCV infection remains a major health problem around the world that can cause extensive liver-related morbidity and mortality. Currently study, we tried to identify immunological factors that predict the outcomes of antiviral therapy.

CXCL-10 serum level may be used as a marker to detect chronic hepatitis C patients under treatment. IFN-γ serum level has no indication with dialysis patients who take the drug regularly or intermittently and the same thing applies with ALP serum level.

VII. FORWARD STUDY

It will be important for future study with use other cytokines that could be detected the HCV in patient's serum such as CXCL-6, CXCL-8, IFN- β and IFN-α and the relationships between them [7].

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