Original article

Correlation of Serum Hepatitis C Viral Load and Immunobiochemical biomarkers in Combined Therapy Treated Patients

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ARTICLE INFO	ABSTRACT
	Hepatitis C virus (HCV) infection is a major global disease that frequently causes chronicity and can potentially cause liver failure and is widespread in Egypt. The current study aimed to explore the potential correlation between quantitatively detected virus C load using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assay and some immunological, biochemical, and hematological biomarkers. Results from 600 HCV-positive people ranging between the age of 25 and 55 years old were analyzed using Prism, a computer (Graph Pad software. Version 5, San Diego, CA), and concluded that; there was a significant relation between HCV viral load and immuno-biological biomarkers. Also, the present study referred to the efficacy of Sofosbuvir/Daclatasvir combined therapy where 100% Sustained Virological Response (SVR) in all patients.

Graphical abstract
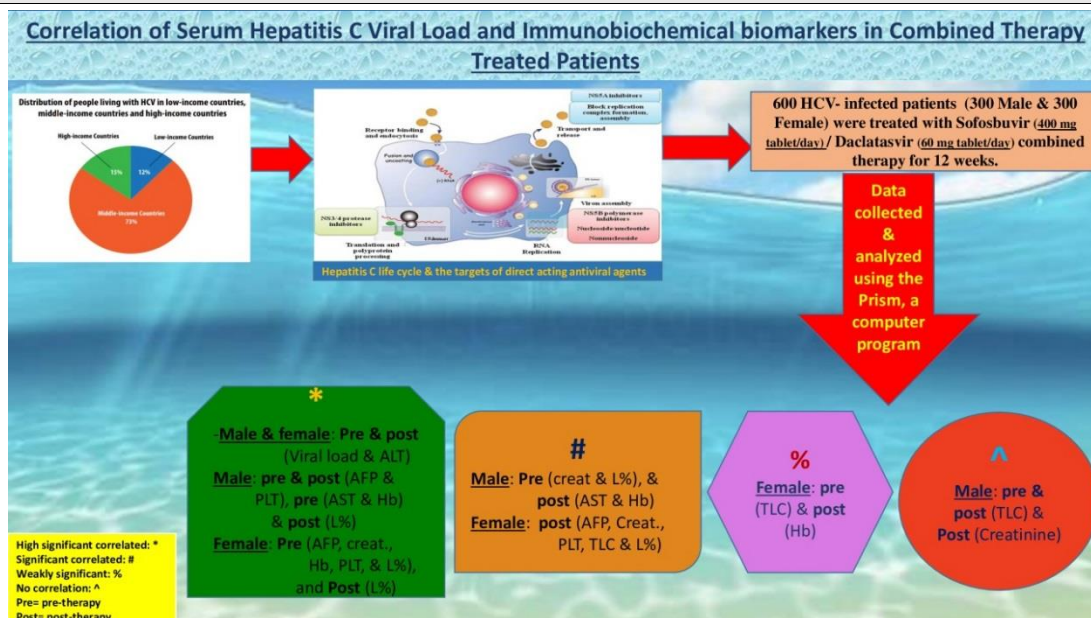
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1. Introduction

Hepatitis C virus, one of the most common chronic diseases in the world, also has the potential to lead to liver failure. Each year, thousands of people die as a result of various HCV infection-related complications, such as liver failure, cirrhosis, hepatocellular carcinoma (HCC), and others [1, 2].

For the first diagnosis of HCV, serological assays that check a patient's serum for anti-HCV antibodies are used. Molecular assays that are both qualitative and quantitative are used to confirm the initial diagnosis [3], as well as to determine viral load and the dominant strain's genotype. Because it provides great specificity and sensitivity, nucleic acid amplification was used in early experiments [4]. Information about the genotype and viral load is also used to determine the best course of treatment [5], [6].

A new family of medications called direct-acting antivirals (DAAs) targets particular phases of the HCV life cycle. DAA drugs have transformed HCV treatment and made it feasible to completely eradicate the disease. They suppress viral replication and production with a brief course of treatment has low adverse effects, and a strong persistent immune response [7].

The US Food and Drug Administration (FDA) approved Gilead's RNA polymerase inhibitor sofosbuvir (Sovaldi) in 2013, and it has the potential to fundamentally alter HCV treatment. As a pan-genotypic HCV inhibitor, sofosbuvir is effective against the majority of HCV genotypes while daclatasvir blocks the function of the NS5A protein, which is necessary for HCV replication and assembly [8]. Direct-acting antivirals stop HCV from replicating by targeting the viral enzyme system that is involved in RNA replication [9].

A correlation analysis examines the relationships between different variables. The degree and direction of the association between two or more variables, or "how things are related," are measured using correlation statistical studies, according to *Dodge* [10], which are used to examine relationships between quantitative variables or categorical variables. A high correlation indicates a significant association between two or more variables, whereas a low correlation indicates a minimal relationship between the variables. A measure of the strength of the linear relationship between two random variables is the simple correlation coefficient. Studies on the association between biomarkers and illness progression enable monitoring of the effectiveness of treatment [11]. To make meaningful judgments about the therapeutic utility of these novel markers, appropriate statistical measurements are required [12].

Aim of work

The current study investigates the potential relationships between several immunobiological biomarkers, such as α-fetoprotein, ALT, AST, creatinine, hemoglobin, platelets, leucocyte count, and lymphocyte percentage, and the quantitatively discovered virus C load using the qRT-PCR technique.

1. Patients & methods:
1. Study design

1.1- Patients

The recruitment period ran from October 1, 2018, till April 30, 2019. Patients were initially identified as HCV-infected utilizing ELISA technology (DIA source, Belgium), physical examination, blood pressure, full blood count, ALT, AST, serum creatinine, and serum AFP. As soon as volunteers were contacted, the study medication was meant to begin. The study excluded participants who were pregnant, had a history of drinking, had hepatitis B virus (HBV), had HIV, or had any other known liver problems. The study enabled a longitudinal, prospective analysis based on viral load measurement of sustained response.

600 ELIZA-HCV-positive patients were confirmed by an RT-PCR target amplification HCV RNA quantification assay.

Blood samples were collected and handled by highly skilled individuals in the laboratories of the Ministry of Health in Cairo, Egypt. The research team worked with the information supplied by Cairo, Egypt's Ministry of Health's Central Laboratories. The protocol was followed and approved by National Organization for Drug Control and Research NODCAR Research Ethics Committee (No. NODCAR/1/26/2021)

1.2-Drugs
Sofosbuvir
Daclatasvir

2. Methods
Test groups:

Patients with hepatitis C who sought care at public hospitals in Egypt were referred to the central laboratories, of Egypt's Ministry of Health. Data was gathered while patients received medical care for 12 weeks. Participants had their serum samples checked for HCV positive, and underwent laboratory evaluation.

The studied test groups were:

Virus C untreated-infected patients Group: Hepatitis C untreated infected patients (female patients n: 300 and male patients n: 300).

SOF/DAC treated-infected patients (after 12 weeks therapy) Group: (female patients n: 300 and male patients n: 300).

One 400 mg tablet of sofosbuvir with one 60 mg tablet of Daclatasvir taken orally once a day. The efficacy of double therapy was followed up by biochemical, immunological, and hematological parameters.

Parameters data included:

Immunological parameters include: ELISA-based HCV and HBV antibodies (DIA source, Belgium), alpha-fetoprotein (Diagnostic Automation, inc AFP-

ELISA Kit, USA, normal< 8.5 ng/ml), and quantitative real-time PCR for measuring HCV RNA levels in serum (Qiagen extraction kit & Abbott real-time HCV test, RT_PCR, USA, negative or undetected<34 IU/ml).

Biochemical parameters include: alanine transferase (ALT) (DiaSys Reagent Diagnostic systems ALT FS (IFCC mod) Germany, ALT in women < 34 U/L and in men < 45 U/L), aspartate transferase (AST) (DiaSys Reagent Diagnostic systems AST FS (IFCC mod.) Germany, and AST in women < 31 U/L and in men < 35 U/L), and serum creatinine (DiaSys Reagent Diagnostic systems Creatinine FS, Germany, normal in female < 1.1 and in male <1.3mg/dL).

Hematological parameters include: blood hemoglobin, platelet count, total leucocyte count, and lymphocyte percentage (BEST-LAB, CBC Diluent, Lyse, Rinse, and EZ cleaner for (Diagon D-Cell 60 Hematology Analyzer [Hungary], Cairo, Egypt.

Data collection:

Ten ml of venous blood was drawn from each patient in stringent aseptic circumstances. Before centrifuging one portion to separate the serum for clinical examination, including ALT, AST, creatinine, and alpha-fetoprotein levels, the other portion was allowed to clot. Quantitative RT-PCR was used to determine the concentrations of HCV RNA in serum. In accordance with the manufacturer's instructions, all assays were performed. Using EDTA blood, hematological parameters were calculated.

Statistics

Prism, a computer, was used to analyze the results (Graph Pad software. Version 5, San Diego, CA).

a-One-Way ANOVA: One-way analysis of variance (ANOVA) was used to calculate statistical differences between groups for multiple comparisons before the Tukey-Kramer test. P values of 0.05 were regarded as the minimal threshold for significance.

b-Pearson's r correlation coefficient: For a measure of linear correlation between two variables. It gauges the degree and nature of the connection between two sets. The correlation coefficient squared, or r^2, is also referred to as the coefficient of determination. The data must fully match the linear model to have an r^2 of 1.0. Any r^2 score below 1.0 indicates that at least some of the data variability cannot be explained by the model. The study's many parameters were correlated with HCV viral load using the statistical method r-squared. If the P value was ≤0.05, it was deemed significant.

Ethical issues: The protocol used in this study was according to National Organization for Drug Control And Research (NODCAR) Ethics Committee, NODCAR-REC, Ethics Application Form (No. NODCAR/1/26/2021).

3. Results

Immunological parameters

Both male and female patient's post-therapeutic serum HCV viral load were highly significantly correlated to pre-therapeutic virus load ($r^2 = 0.005$ at P = < 0.0001), ($r^2 = 0.323$ at P = < 0.0001), respectively (Table 1 & Fig. 1 (A & B)).

Male patients' serum α-fetoprotein was highly significantly correlated to HCV viral load before and after therapy (($r^2 = 0.119$ at P = < 0.0001), ($r^2 = 0.094$ at P = < 0.0001)), respectively. While, the female patient's serum α-fetoprotein was highly significantly positively correlated to HCV viral load before therapy ($r^2 = 0.012$ at P = < 0.0001) and only significantly correlated to HCV viral load after therapy ($r^2 = 0.011$ at P 0.0632) (Table 1 & Fig. 2 (A, B, C & D)).

Table (1): Correlation of HCV viral load and α-fetoprotein across pre- and post-treated HCV infected patients.

	HCV viral load				AFP			
	Male		Female		Male		Female	
	r^2	P	r^2	P	r^2	P	r^2	p
Pre-therapeutic					0.119	= < 0.0001	0.012	= < 0.0001
Post-therapeutic	0.005	=< 0.0001	0.323	= < 0.0001	0.094	= < 0.0001	0.011	= < 0.0632

r^2: correlation coefficient square "coefficient of determination", P ≤ 0.05 as significant and P ≤ 0.001 as highly significant.

HCV viral load:

(A)

Correlation of HCV Viral Load pre- and Post-treatment in Males

r²=0.005
p≤0.0001

[Figure A: Scatter plot. Y-axis: Serum HCV viral load (IU/mL) [Post treatment], scale 0 to 900000. X-axis: Serum HCV viral load in infected pts (IU/mL), scale 0 to 60000000.]

(B)

Correlation of HCV Viral Load pre- and Post-treatment in Females

r²=0.005
p≤0.0001

[Figure B: Scatter plot. Y-axis: Serum HCV viral load (IU/mL) [Post treatment], scale -1000000 to 5000000. X-axis: Serum HCV viral load in infected pts (IU/mL), scale 0 to 25000000.]
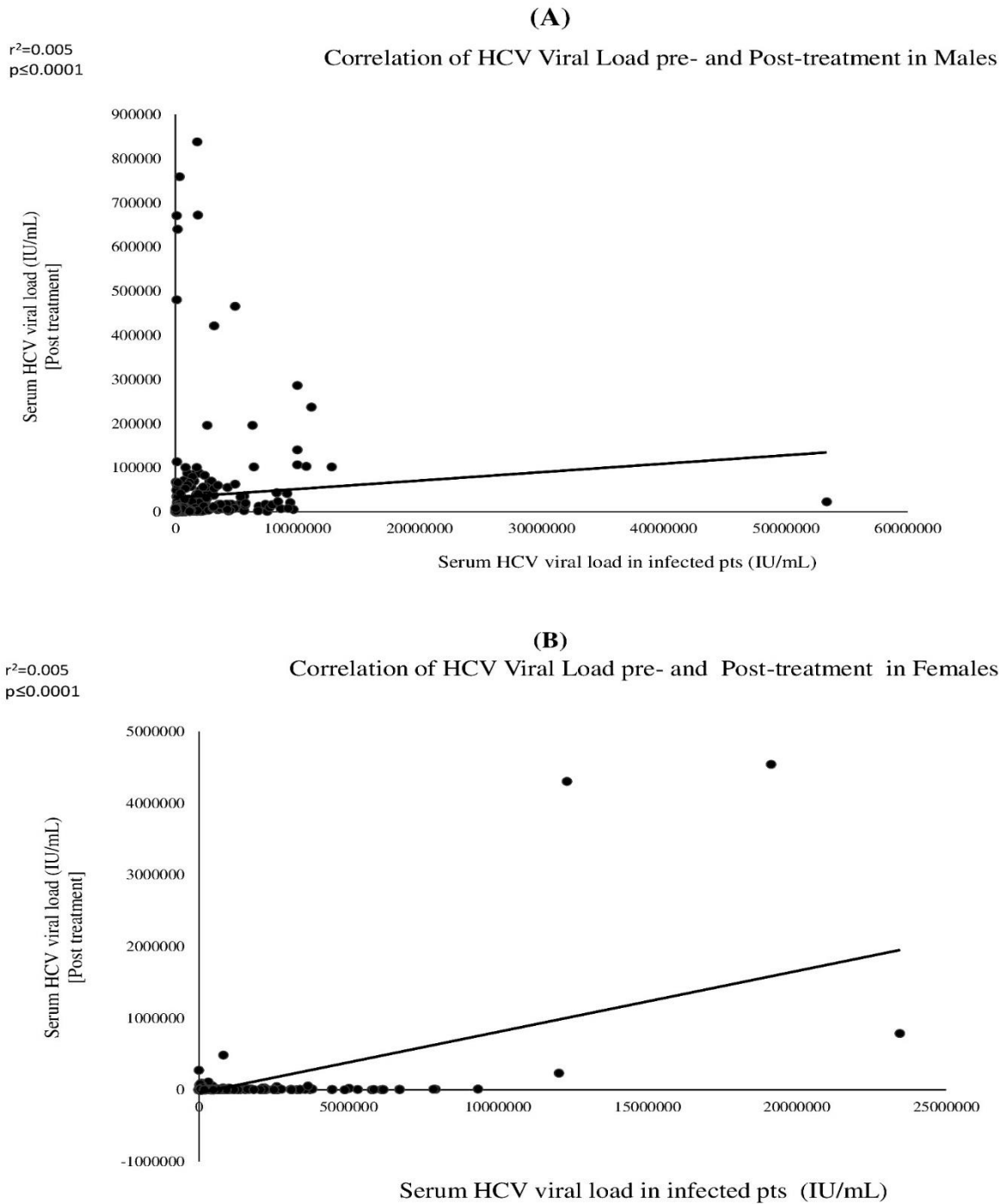
Fig (1): Hepatitis C Viral. Load Correlation with Post-therapeutic serum HCV Viral Load (A: male, B: female). Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05)).
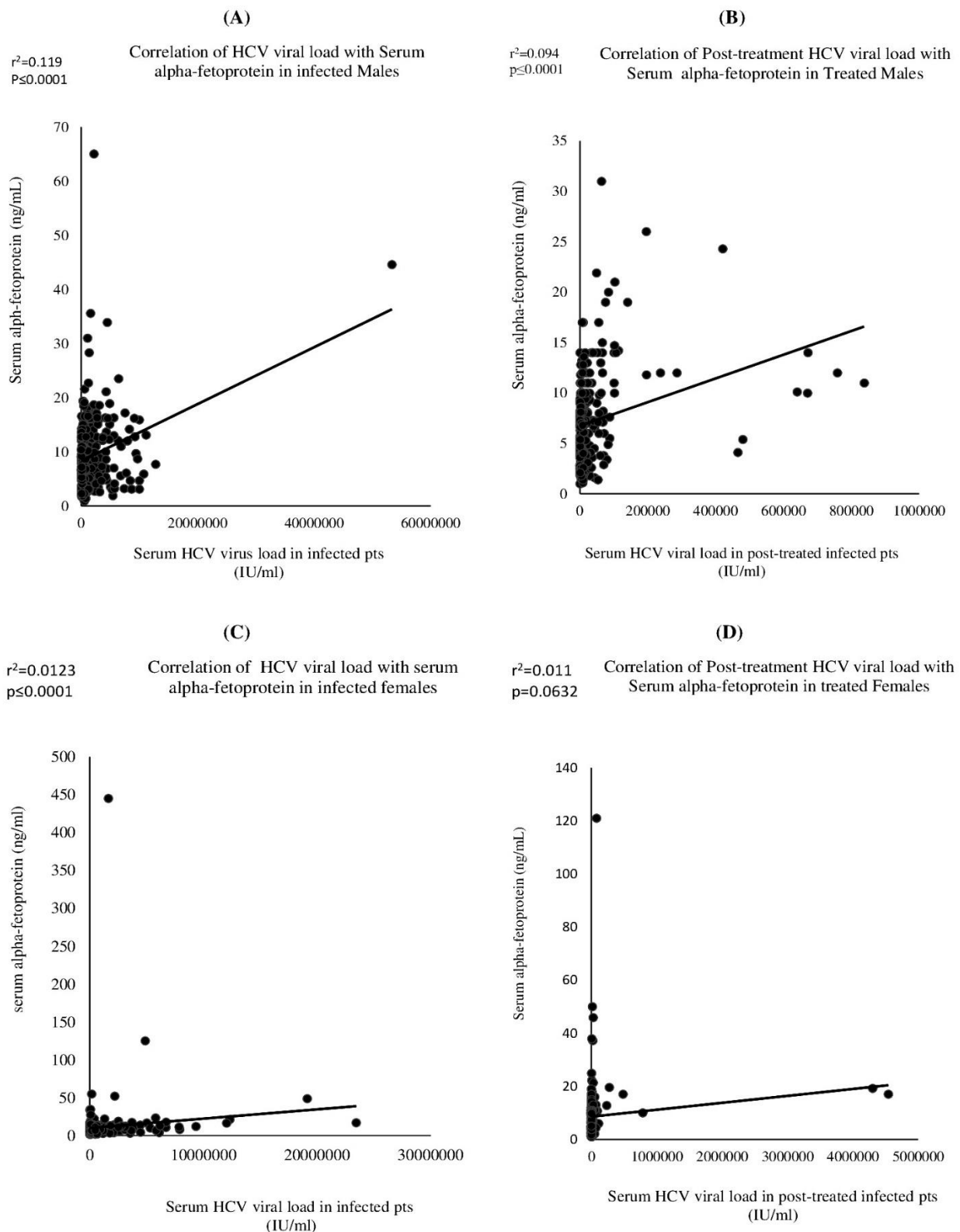
Serum alpha-fetoprotein:

Fig (2): Hepatitis C Viral Load Correlation with alpha-fetoprotein in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).

Biochemical parameters

Both male and female patient's serum ALT results were highly significantly correlated to HCV viral load pre-treatment (r^2 = 0.044 at P = 0.0003), (r^2 = 0.221 at P = < 0.0001), respectively; and also post-therapeutic (r^2 = 0.041 at P = 0.0005), (r^2 = 0.025 at P = 0.0063), respectively (Table 2 & Fig. 3 (A, B, C & D)). Male and female patients' serum AST were highly significantly correlated to HCV viral load before therapy ((r^2 = 0.121 at P = < 0.0001), (r^2 = 0.198 at P = < 0.0001)). While results of post-therapy were significantly correlated in males (r^2 = 0.011 at P =

0.0907) and highly significantly correlated in the female group (r^2 = 0.067 at P = < 0.0001) (Table 2 & Fig. 4 (A, B, C & D)).

Male patient's serum Creatinine (Cr) was significantly correlated to HCV viral load at pre-therapy (r^2 = 0.0197 at P = 0.0149), but post-therapeutic serum Cr was not correlated to post-therapeutic HCV viral load (r^2 = 0.000 at P = 0.9925). While, female patient's serum Cr was highly significantly correlated to HCV viral load at pre-therapy (r^2 = 0.049 at P = < 0.0001); and only significantly correlated to post-therapeutic HCV viral load (r^2 = 0.014 at P = 0.043) (Table 2 & Fig. 5 (A, B, C & D))

Table (2): Correlation of HCV viral load and biochemical parameters across pre- and post-treated HCV infected patients.

		Male		Female	
		r^2	P	r^2	P
ALT	Pre-therapeutic	0.044	0.0003	0.221	=<0.0001
	Post-therapeutic	0.041	0.0005	0.025	0.0063
AST	Pre-therapeutic	0.121	=<0.0001	0.198	=<0.0001
	Post-therapeutic	0.011	0.0907	0.067	=<0.0001
Creat.	Pre-therapeutic	0.0197	0.0149	0.049	=<0.0001
	Post-therapeutic	0.000	0.9925	0.014	0.043

r^2: correlation coefficient square "coefficient of determination", P ≤ 0.05 as significant and P ≤ 0.001 as highly significant.

Hematological parameters:

Male and female patients' blood hemoglobin (HB) were highly significantly correlated to HCV viral load before therapy ((r^2 = 0.127 at P = < 0.0001), (r^2 = 0.239 at P = < 0.0001)), respectively. While, post-therapy blood hemoglobin was significantly correlated to HCV viral load in male patients (r^2 = 0.012 at P = 0.0541), and weakly correlated in female patients (r^2 = 0.004 at P = 0.2518) (Table 3 & Fig. 6 (A, B, C & D)).

Male patient's blood platelet count was highly significantly correlated to HCV viral load at pre- and post-therapy ((r^2 = 0.089 at P = < 0.0001), (r^2 = 0.033 at P = 0.0016)), respectively. Female patient's blood platelets count was highly significantly correlated to HCV viral load pre-therapy (r^2 = 0.049 at P = < 0.0001); while post-therapeutic blood platelets count was significantly correlated to post-therapeutic HCV viral load (r^2 = 0.019 at P = 0.0184) (Table 3 & Fig. 7 (A, B, C & D)).

Male patients' total leukocyte count was significantly not correlated to HCV viral load pre and post-therapy ((r^2 = 0.001 at P = < 0.5256), (r^2 = 0.004 at P = 0.2781)), respectively. Female patient's HCV viral load was weakly significantly correlated to blood total leukocyte count pre-therapy (r^2 = 0.009 at P = 0.0989); and significantly correlated to HCV viral load at post-therapy (r^2 = 0.013 at P = 0.0516) (Table 3 & Fig. 8 (A, B, C & D)). Male patient's blood lymphocyte percent was significantly correlated to HCV viral load pre-therapy (r^2 = 0.025 at P = 0.0061), and highly significant at post-therapy (r^2 = 0.049 at P = < 0.0001). Female patient's blood lymphocyte percent was highly significantly correlated to HCV viral load pre-therapy (r^2 = 0.035 at P = 0.0011); while post-therapeutic blood lymphocyte percent is significantly correlated only to post-therapeutic HCV viral load (r^2 = 0.031 at P = 0.0024) (Table 3 & Fig. 9 (A, B, C & D)).

Table (3): Correlation of HCV viral load and hematological parameters across pre- and post-treated HCV infected patients.

	Male				Female			
	Pre-therapeutic		Post-therapeutic		Pre-therapeutic		Post-therapeutic	
	r^2	P	r^2	P	r^2	P	r^2	P
HB	0.127	=<0.0001	0.012	0.0541	0.239	=<0.0001	0.004	0.2518
PLT.	0.089	=<0.0001	0.033	0.0016	0.049	=<0.0001	0.019	0.0184
WBCs	0.001	0.5256	0.004	0.2781	0.009	0.0989	0.013	0.0516
L%	0.025	0.0061	0.049	=<0.0001	0.035	0.0011	0.031	0.0024

r^2: correlation coefficient square "coefficient of determination", P ≤ 0.05 as significant and P ≤ 0.001 as highly significant.
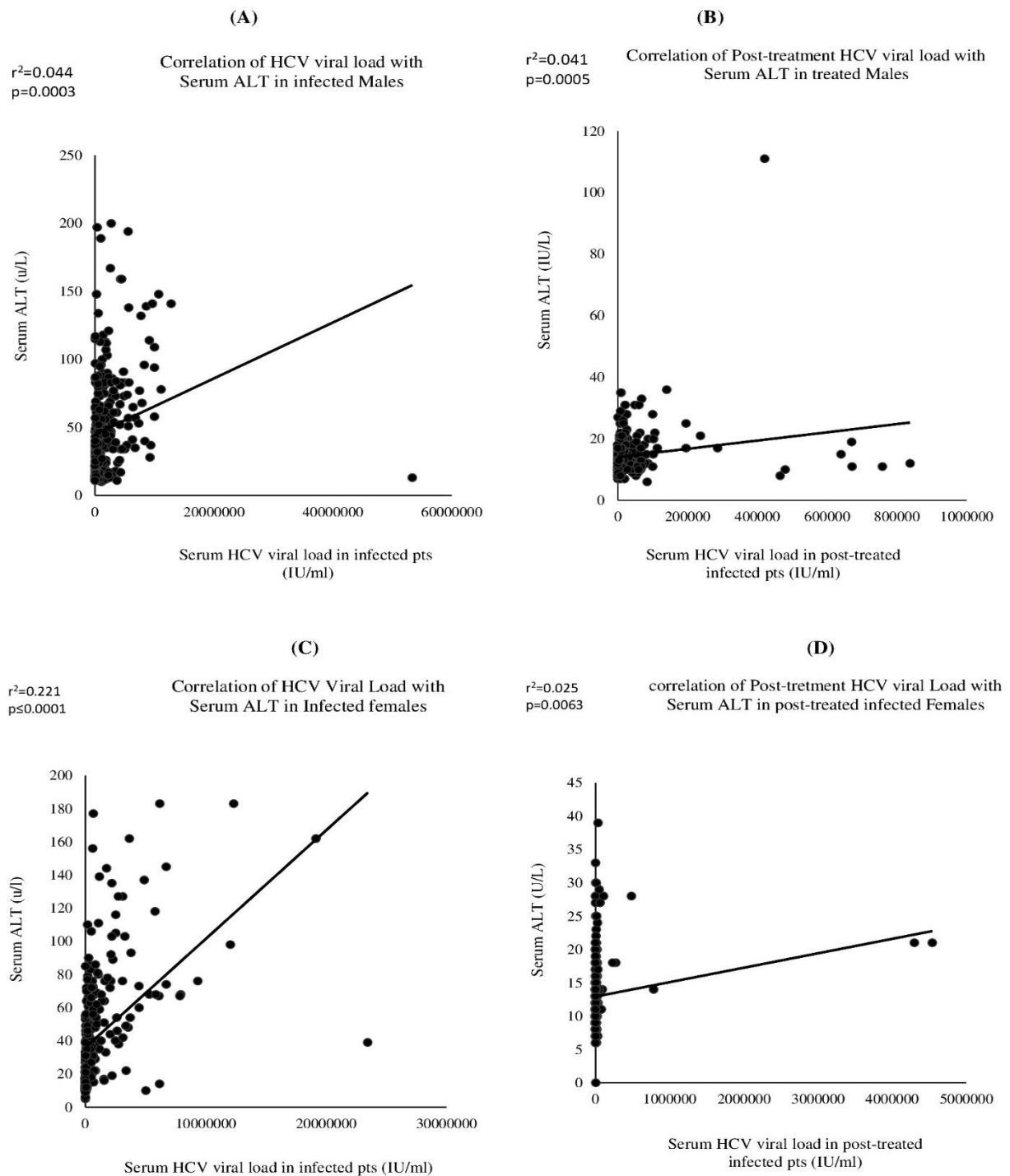
Serum Alanine aminotransferase (ALT):

(A)

r²=0.044
p=0.0003

Correlation of HCV viral load with
Serum ALT in infected Males

(B)

r²=0.041
p=0.0005

Correlation of Post-treatment HCV viral load with
Serum ALT in treated Males

(C)

r²=0.221
p≤0.0001

Correlation of HCV Viral Load with
Serum ALT in Infected females

(D)

r²=0.025
p=0.0063

correlation of Post-tretment HCV viral Load with
Serum ALT in post-treated infected Females

Fig (3): Hepatitis C Viral Load Correlation with ALT in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).

Serum aspartate transferase (AST):

(A)

r²=0.121
p≤0.0001

Correlation of HCV viral load with Serum AST in infected Males

(B)

r²=0.011
p=0.0907

Correlation of Post-treatment HCV viral load with Serum AST in Treated Males

(C)

r²=0.198
p≤0.0001

Correlation of HCV viral load with Serum AST in infected females

(D)

r²=0.067
p≤0.0001

Correlation of Post-treatment HCV viral load with Serum AST in post-treated infected Females

Fig (4): Hepatitis C Viral Load Correlation with AST in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).
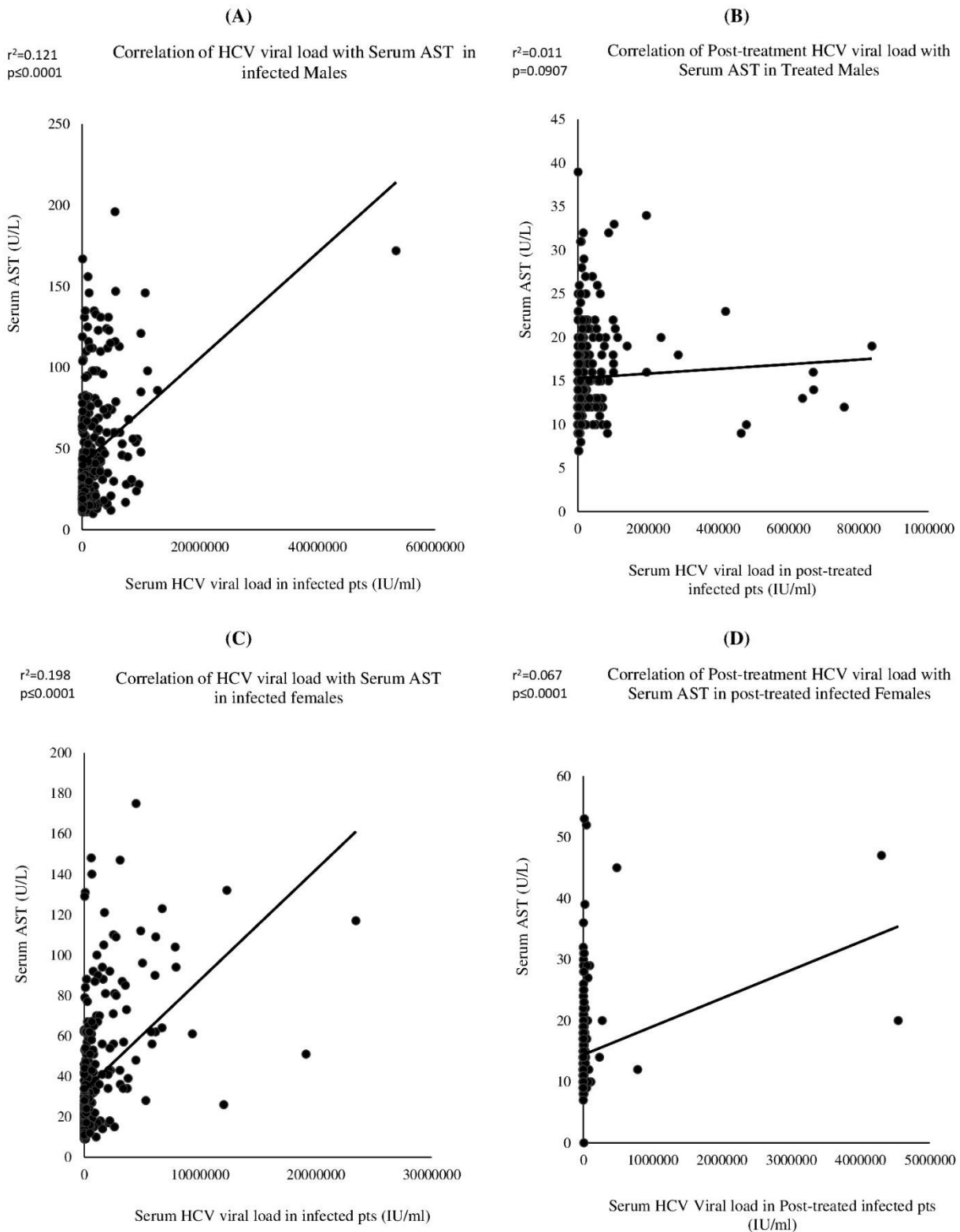
Serum creatinine:

(A)

r²=0.0197
p=0.0149

Correlation of HCV viral load with Serum Creatinine in infected Males

(B)

r²=0.000
p=0.9925

Correlation of Post-treatment HCV Viral load with Serum Creatinine in treated Males

(C)

r²=0.049
p≤0.0001

Correlation of HCV viral load with serum Creatinine in infected Females

(D)

r²=0.014
p=0.043

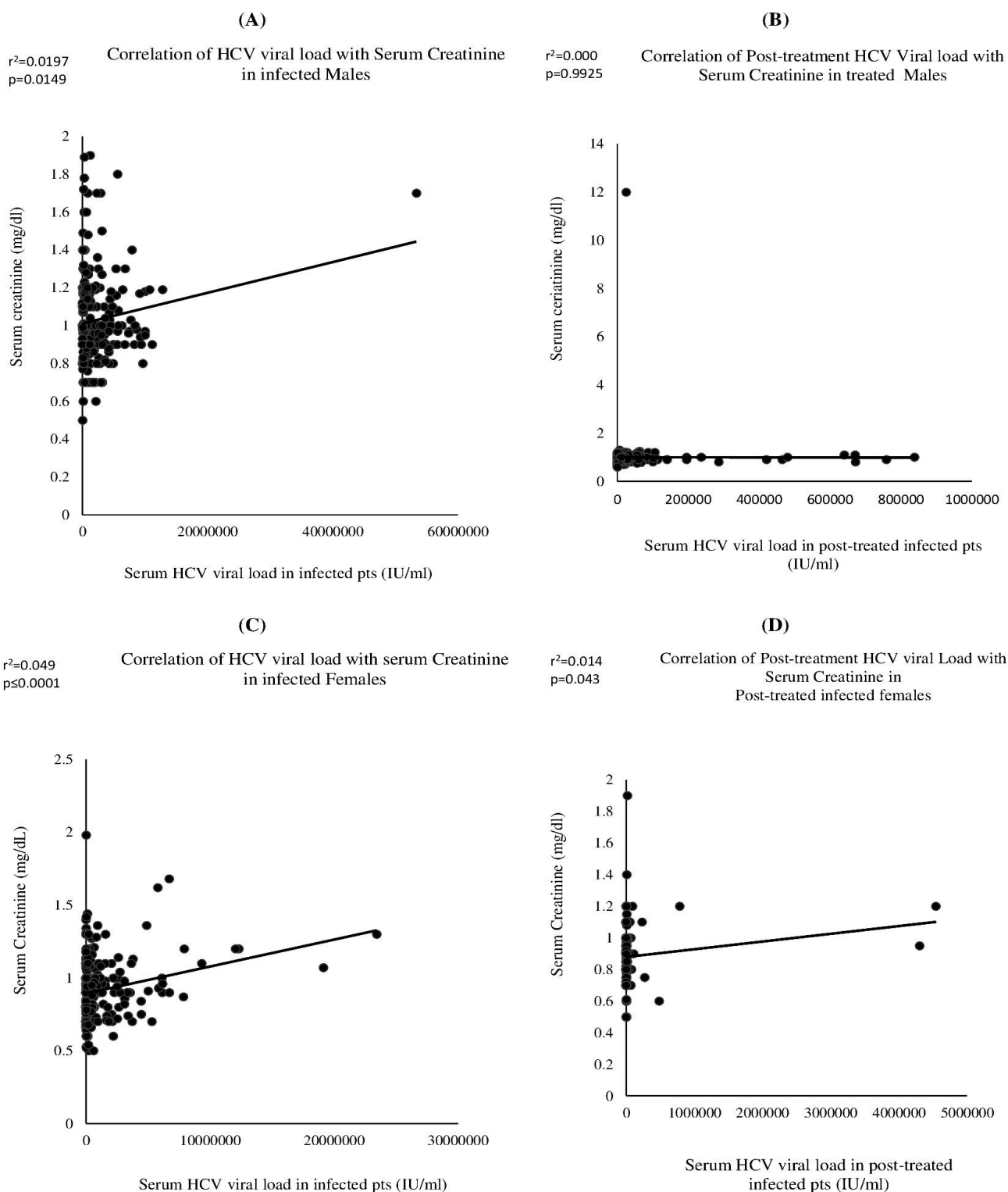Correlation of Post-treatment HCV viral Load with Serum Creatinine in Post-treated infected females

Fig (5): Hepatitis C Viral Load Correlation with S. Creatinine in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).
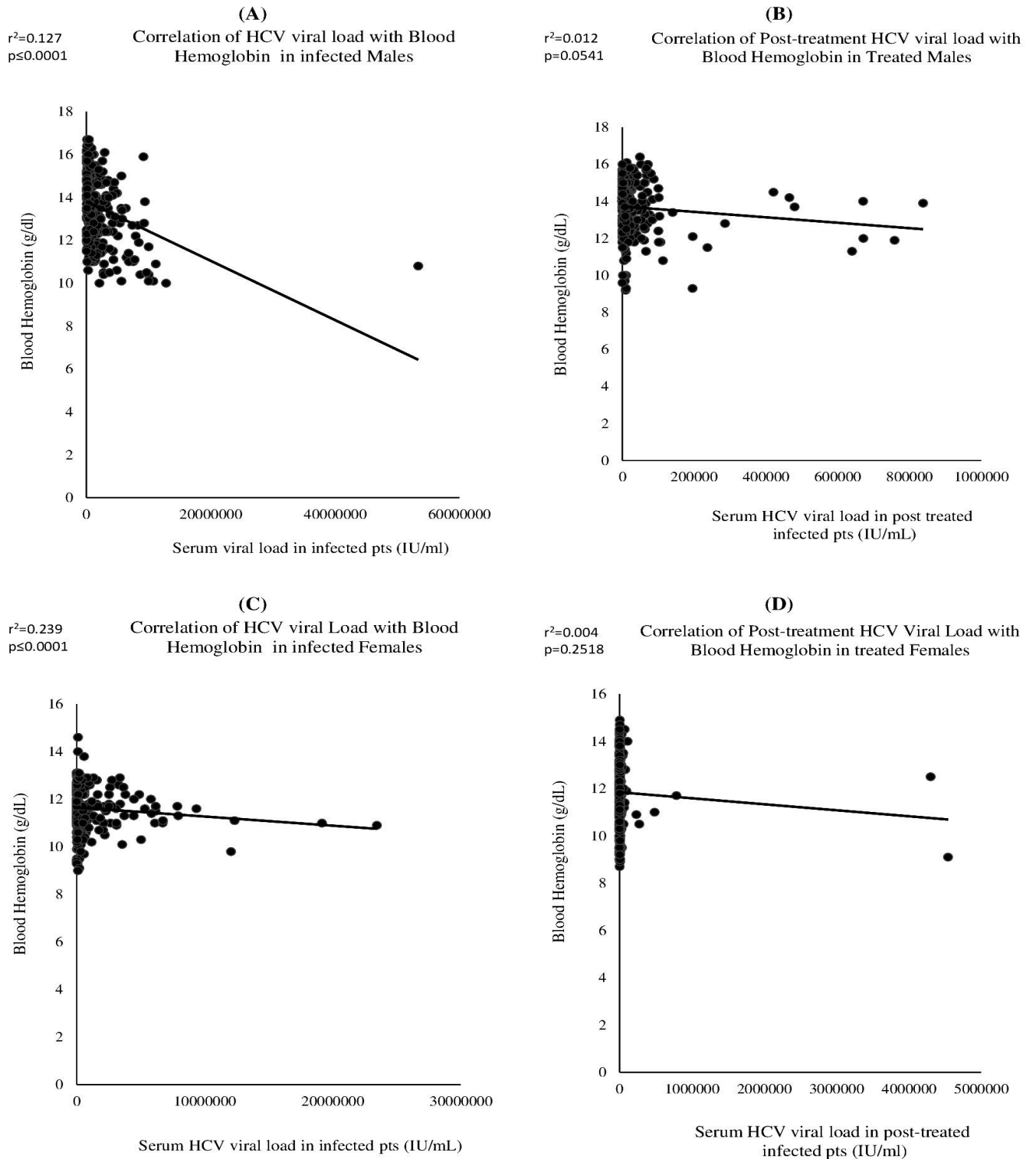
Blood Hemoglobin:

(A)

r²=0.127
p≤0.0001

Correlation of HCV viral load with Blood
Hemoglobin in infected Males

(B)

r²=0.012
p=0.0541

Correlation of Post-treatment HCV viral load with
Blood Hemoglobin in Treated Males

(C)

r²=0.239
p≤0.0001

Correlation of HCV viral Load with Blood
Hemoglobin in infected Females

(D)

r²=0.004
p=0.2518

Correlation of Post-treatment HCV Viral Load with
Blood Hemoglobin in treated Females

Fig (6): Hepatitis C Viral Load Correlation with blood hemoglobin in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).
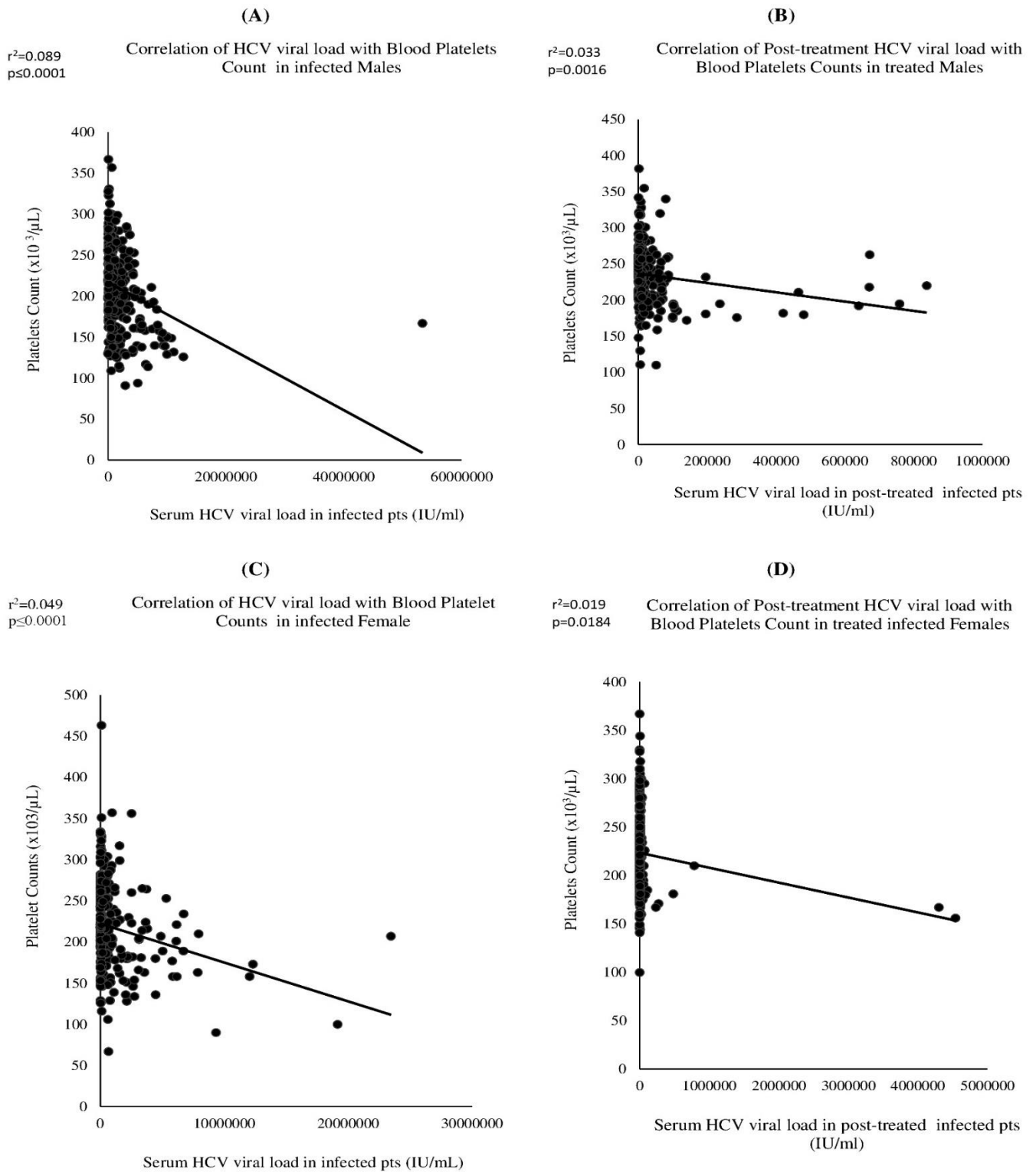
Blood platelets count:

(A)

r²=0.089
p≤0.0001

Correlation of HCV viral load with Blood Platelets Count in infected Males

(B)

r²=0.033
p=0.0016

Correlation of Post-treatment HCV viral load with Blood Platelets Counts in treated Males

(C)

r²=0.049
p≤0.0001

Correlation of HCV viral load with Blood Platelet Counts in infected Female

(D)

r²=0.019
p=0.0184

Correlation of Post-treatment HCV viral load with Blood Platelets Count in treated infected Females

Fig. (7): Hepatitis C Viral Load Correlation with platelets count (PLT) in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).
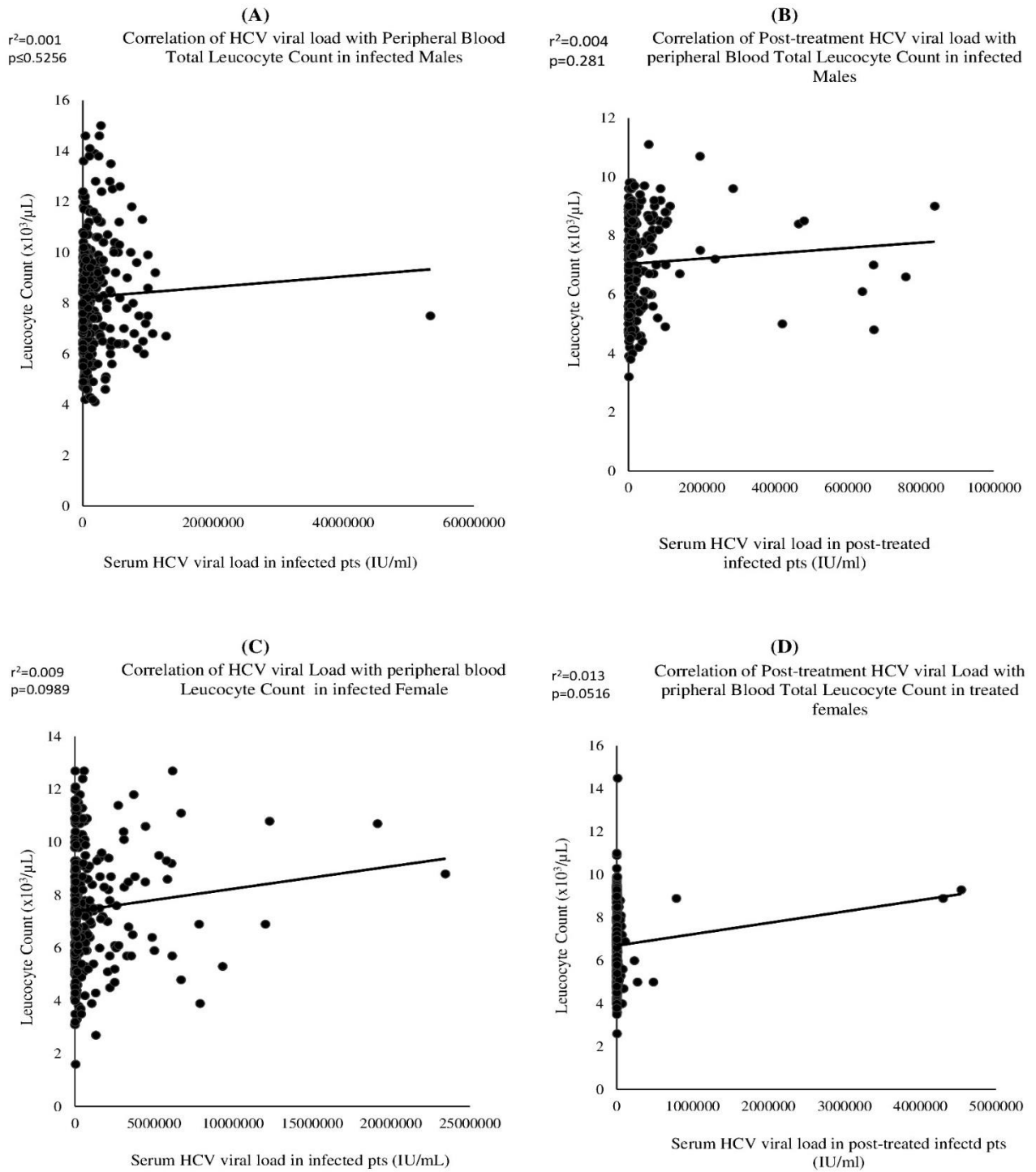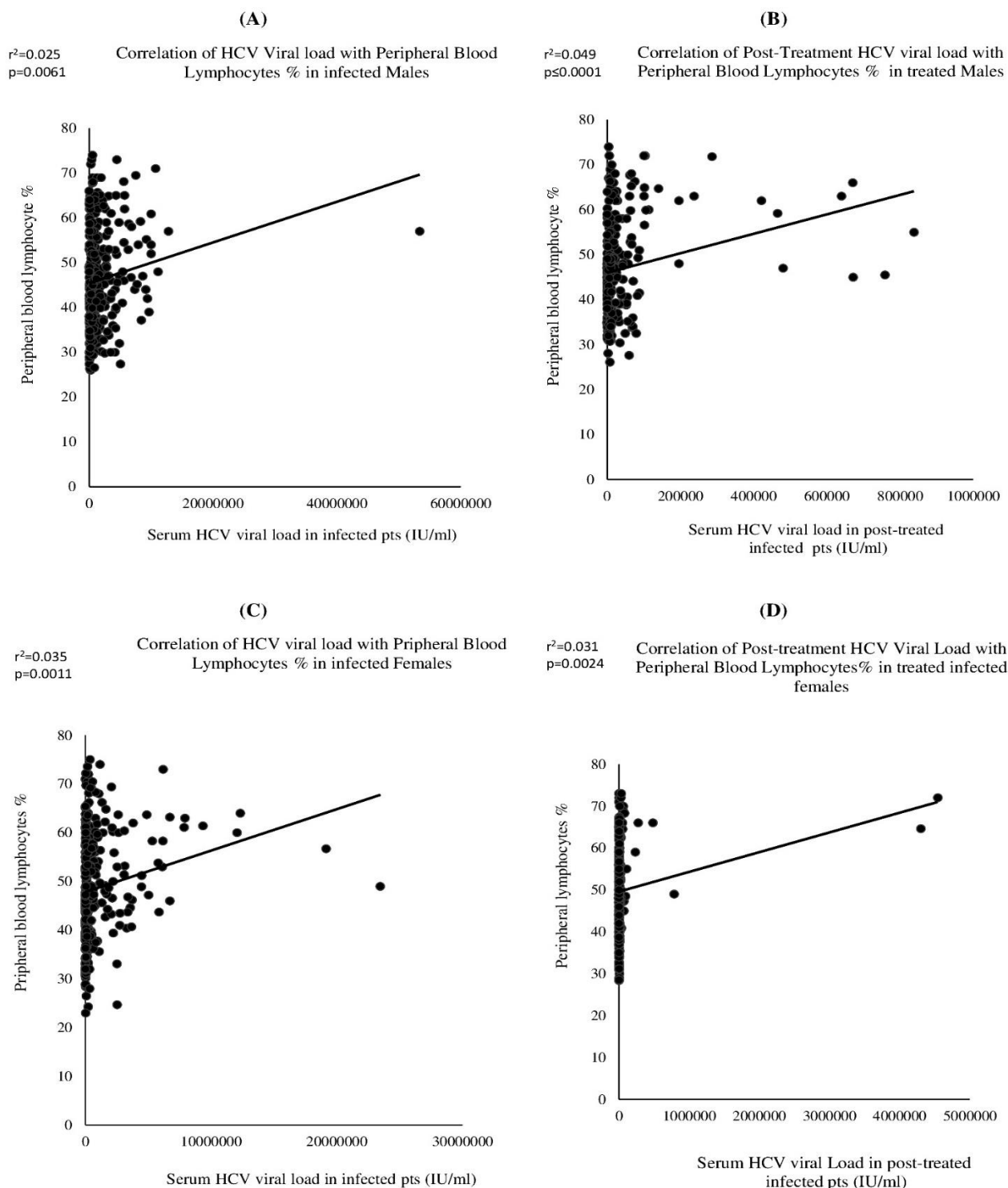
Peripheral blood total leucocyte count:

(A)

r²=0.001
p≤0.5256

Correlation of HCV viral load with Peripheral Blood Total Leucocyte Count in infected Males

Serum HCV viral load in infected pts (IU/ml)

(B)

r²=0.004
p=0.281

Correlation of Post-treatment HCV viral load with peripheral Blood Total Leucocyte Count in infected Males

Serum HCV viral load in post-treated infected pts (IU/ml)

(C)

r²=0.009
p=0.0989

Correlation of HCV viral Load with peripheral blood Leucocyte Count in infected Female

Serum HCV viral load in infected pts (IU/mL)

(D)

r²=0.013
p=0.0516

Correlation of Post-treatment HCV viral Load with pripheral Blood Total Leucocyte Count in treated females

Serum HCV viral load in post-treated infectd pts (IU/ml)

Fig. (8): Hepatitis C Viral Load Correlation with blood leucocyte count (TLC) in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).

Peripheral blood lymphocyte percentage:

(A)

$r^2=0.025$
$p=0.0061$

Correlation of HCV Viral load with Peripheral Blood Lymphocytes % in infected Males

(B)

$r^2=0.049$
$p\leq0.0001$

Correlation of Post-Treatment HCV viral load with Peripheral Blood Lymphocytes % in treated Males

(C)

$r^2=0.035$
$p=0.0011$

Correlation of HCV viral load with Pripheral Blood Lymphocytes % in infected Females

(D)

$r^2=0.031$
$p=0.0024$

Correlation of Post-treatment HCV Viral Load with Peripheral Blood Lymphocytes% in treated infected females

Fig (9): Hepatitis C Viral Load Correlation with blood lymphocytes% (L%) in: (A) HCV-infected male patients, (B) post-therapeutic male patients, (C) HCV-infected female patients, (D) post-therapeutic female patients; Pearson r Correlation (XY Pair, 95% confidence interval, P value (two-tailed), correlation significant (p≤0.05).

1. Discussion

Although HCV viral load in chronic hepatitis is thought to be a reliable indicator of the course of the disease [13], it is not known if rising viral loads correlate

with other circulating biomarkers [14]. The purpose of this study was to assess the circulating

biomarkers in patients with chronic HCV infections that may play a role in the disease's development. Direct-

acting antivirals improve the immune response of individuals with comprised immunity by eradicating HCV [15].

In the current study, evaluation of serum HCV viral load revealed a highly significant correlation between pre-therapeutic and post-therapeutic virus load in both HCV-infected male and female patients. The comparison of HCV viral load measurements before and after therapy is a crucial indicator of the effectiveness of the treatment and the virus elimination. The decision threshold for high vs low viremia has been suggested to be 800 000 IU/mL, according to *Pawlotsky et al*. [16], who noted that the criterion for differentiating between HCV low and high viral loads differed depending on the assay utilized. The best criterion for assessing whether an infected patient has a high or low probability of reaching SVR was subsequently suggested to be a baseline level of 400 000 IU/mL [17]. The ideal pre-treatment viral load cut-off should be utilized to forecast treatment outcomes in naïve patients with chronic hepatitis C [18].

According to *Chua et al.* [19], a combination regimens of DAAs for chronic HCV infection given for 8 or 12 weeks, have high cure rates. Treatment durations that are reduced while maintaining high cure rates may reduce treatment barriers related to affordability and drug adherence. In all patients, plasma HCV RNA levels rapidly decreased during the first two days of treatment and were below the lower limit of quantification by the end of the 6-week treatment period. Also, a novel NNI (novel non-nucleoside inhibitor) with a fixed-combination DAA achieved a virologic cure in 8 of 12 treatment-naive patients with chronic genotype 1 HCV infection without cirrhosis.

In the present study, serum alpha-fetoprotein and HCV viral load in male patients before and after therapy were found to be highly significantly positively correlated. However, the serum alpha-fetoprotein of female patients showed a highly significant correlation with HCV viral load pre-therapy and only a significant connection with HCV viral load post-therapy, back to SVR, and HCV eradication. *Chu et al.* [20] and *Hu et al.* [21] claim that AFP is substantially associated with the female gender. This is explained by *Ruggieri et al.* [22], who found that innate, humoral, and cellular immune responses to viral infections and vaccinations are typically more potent in female participants than in male ones. Sex hormones, in turn, differentially affect the immune responses to viruses, by specific binding to the hormone receptors expressed on the immune cells.

In both pre- and post-treated HCV-infected male and female patients, correlation analyses between HCV viral load and serum AST were highly significant. These results supported those of *Shahid et al.* [33], who found a connection between serum HCV RNA titers and AST. The improvement of ALT and AST is closely correlated with the removal of HCV RNA. When it comes to the prognosis of liver illnesses in HCV patients, men had higher levels of ALT and AST than women [34].

Estrogens have an immune-stimulating effect, while androgens are immune-suppressing [22]. Patients with chronic hepatitis C have reported that women have a higher rate of spontaneous HCV elimination than men [23]. In addition, *Berghöfer et al.* [24] observed that healthy women produce substantially more INF- than men. These results were corroborated by *Leone and Rizzetto* [25], who also noted that there is some debate regarding the connection between virus load, serum α-fetoprotein, HCV genotype, and quasi-species diversity, and the course of liver disease.

In 23.9% of CHC patients, elevated AFP levels were observed [20]. Studies have suggested a connection between elevated blood AFP and elevated alanine aminotransferase (ALT) levels during liver cell regeneration, particularly following severe hepatic necrosis or resection [26], [27]. Even in the absence of HCC, serum AFP levels were shown to be moderately higher in the HCV and recovered groups and to positively correlate with the liver enzymes AST, ALP, and GGT as well as to have a linear positive connection with the degree of cholestasis and hepatic cytolysis. Regardless of the clinical response to therapy, AFP may be a significant outcome measure in hepatitis C patients, even if they have not yet been diagnosed with HCC [28]. On the other hand, reduced clearance of AFP following SVR can be suggested [29].

After combination therapy, a higher platelet count, HCV virus elimination, and a normal AFP was a sign of effective treatment. It was hypothesized that the most significant and frequent reasons of high AFP were necrosis, hepatocellular damage, and inflammation after antiviral treatment because there was a concurrent decrease in AFP and ALT [30].

Serum ALT levels and viral load showed a highly significant relation. Interestingly, there was a significant association between ALT and AFP levels across the board in the research sample. *Zeuzem et al.* [31], discovered a connection between blood HCV RNA levels and the severity of liver damage, as HCV damages the liver through a direct cytopathic effect, abnormal liver function tests, and liver fibrosis. They also found that necroinflammatory activity and serum HCV RNA levels are related. Patients with higher blood ALT levels exhibited a stronger relationship with this factor. The pathogenesis of liver injury may be influenced by the host immunological response, which may reflect viral load [32].

Although AST increases with age in co-infected individuals, ALT decreases with age in noninfected individuals [35]. Hence, eradication could rectify the impairments in hepatic function. Hepatic inflammation brought on by HCV replication may cause the liver to undergo significant stress [36]. Therapy with sofosbuvir and daclatasvir has been demonstrated to lower HCV RNA viral load, improving the AST results of the liver function tests [37].

According to **Babiker et al.** review's **[38]**, HCV infection considerably modifies serum levels of inflammatory indicators, which may change in response to HCV therapy, especially in patients receiving DAA. Interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor (TNF), TNF receptors (TNFR), soluble CD163 (sCD163), and soluble CD14 were among the biomarkers examined **[39]**.

Chemerin is a chemoattractant protein that is thought of as a biomarker that can be used to predict the severity of liver disease in HCV patients, according to **Peschel et al. [40]**. It has a negative correlation with ALT and AST measures of hepatic function and a positive correlation with leukocyte count, both of which are signs of systemic inflammation. Also, it is negatively correlated with severe chronic hepatitis C assessed by the model of end-stage liver disease (MELD) score in HCV-infected patients undergoing DAA treatment. As measured by the fibrosis-4 (FIB-4) score, the aspartate aminotransferase/platelet (AST/PLT) ratio index (APRI) score, and it did not correlate with viral load, advanced liver fibrosis and cirrhosis are significantly correlated with low circulating chemerin levels.

In both male and female HCV patients, this study discovered a relationship between pre-therapeutic HCV viral load and serum creatinine levels. As HCV not only has an effect on the liver but also on the kidney and this is due to the efficacy of HCV in the kidney without treatment. And as a result of the eradication of the HCV virus after treatment, the connection between post-treatment HCV viral load and serum creatinine is less significant in female patients and nonexistent in male patients. These results were corroborated by **Abdelhamid et al. [41]**, who reported that higher HCV viral load, along with female sex, older age, lower hemoglobin, higher international normalized ratio, and higher alanine transaminase, was a significant predictor of low eGFR (estimated glomerular filtration rate) in patients with chronic HCV infection. Serum creatinine and HCV viral load were reported to be significantly correlated in people with CKD (chronic renal disease). The relationship between serum interferons, cytokines, liver functions, and HCV viral load was also shown by **Afify et al. [42]**. The aberrant immunological response in CKD was blamed for these false correlations **[43]**.

According to **Reid et al. [44]**, oxidative stress, decreased hepatic blood flow, poor mitochondrial function, and compromised immunity are all factors that contribute to impaired renal function in older people. Kidney mesangial, endothelial, and tubular cells with HCV RNA and associated proteins may have a direct cytopathic effect **[45], [46]**. Hence, chronic HCV infection was linked to the prevalence of CKD. In particular, active viral responses may cause renal damage and impairment of renal function **[47]**. The risk of developing chronic kidney disease (CKD) considerably increased as HCV viral load rose in a dose-dependent manner **[48]**.

The HCV viral load significantly correlated with blood hemoglobin, platelet count, and lymphocyte percentage, but only weakly with total leucocyte count, according to hematological data from HCV-infected individuals. Similar findings were reported by **Tsai et al. [49]** about predictors for identifying hepatitis C virus infection.

The negative correlation of hemoglobin return to the quantity of proinflammatory monocytes which dramatically decreased by SOF/DCV therapy, this might have happened as a result of reduced monocyte activation and maturation, downregulation of CD16, decreased bone marrow trafficking and/or turnover of this particular subpopulation of myeloid cells **[50]**. In the absence of treatment, HCV infection can cause auto-immune hemolytic anemia **[51]**, which can be treated to cure it **[52]**. Chronic anemia disease is linked to inflammatory, neoplastic, or chronic infection conditions that might be mild or severe **[53]**. **Asaduzzaman et al. [54]** claimed that women are more likely than men to develop anemia.

Hematological alterations were described by **Babitt and Lin [55]** as HCV infection-related kidney dysfunction that resulted in relative erythropoietin insufficiency, higher hepcidin levels, shorter erythrocyte life spans, and altered iron metabolism. Moreover, platelet count and HCV viral load were highly associated in HCV-infected individuals, suggesting that MICS and an aberrant immune response may be present **[56], [41]**. Strict surveillance should be maintained in individuals with low platelet counts and/or high AFP levels even after post-therapeutic eradication of HCV due to the increased risk of HCC in these patients.

Platelets decrease in infected patients by impairment of thermobocytopoiesis by megakaryocyte maturation caused by viruses **[57]** and direct interaction between viruses and PLT in blood circulation **[58]**. The virus may trigger auto-immunogenicity and decrease the production of thrombopoietin. According to **Ong et al. [59]**, these alterations to the bone marrow also contribute to anemia and its main mechanism for inducing neutropenia and thrombocytopenia. Significant thrombocytopenia may be linked to HCV-related liver disease, especially cirrhosis.

Hanberg et al. [60] pointed to the importance of evaluating hematological findings in HCV-infected patients. Their study concluded that the Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were strongly correlated with mortality and hepatic decompensation (HD) and weakly correlated with inflammatory biomarkers. However, most of their association was explained by VACS (veterans aging cohort study) Index 2.0. **Russell et al. [62]** found that longitudinal correlation measurement of lymphocyte-to-monocyte ratio (LMR) during virus infection is reflecting disease symptoms as leucocyte cells are the major target of HCV infection **[62]**.

According to ***Degasperi et al.* [63]**, up to 70% of patients who received antiviral treatment experience a hematologic reaction, including lymphoproliferative disorders.

Finally, it should be taken into consideration that it is unrealistic in correlation statistical studies for assessment of added usefulness of biomarkers to assume that all risk factor levels will remain constant during the follow-up whereas, their trajectories are not known and not measurable at baseline when risk prediction is made **[64, 12]**.

2. Conclusion

The current study emphasized the clinical efficacy of DAA therapy, specifically Sofosbuvir and Daclatasvir combined therapy in 12 weeks courses of treatment, highlighting that this combined therapy is quite effective in curing HCV viral infection and was linked to improvements in many of the evaluated parameters. Additionally, the study supported the significance of correlation associations between HCV RNA viral load and various study parameters, and up to 70% of patients who receive antiviral treatment experience a hematologic reaction, including lymphoproliferative disorders.

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