# Correlations of folic acid, vitamin $B_{12}$, homocysteine, and thrombopoietin to platelet count in HCV infection

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**Introduction**
The platelet count is known to decrease in proportion to the advancement of the stage of liver disease in chronic hepatitis C (CHC) viral infection. The platelet count is currently used as an index for fibrosis staging. The pathophysiology of thrombocytopenia (TCP) in patients with hepatitis C virus (HCV) infection is not completely understood.

**Purpose**
This work aimed to study the correlations of folic acid (FA), vitamin $B_{12}$ (Vit $B_{12}$), homocysteine (Hcy), and thrombopoietin to the platelet count in HCV infection.

**Patients and methods**
Sixty-seven patients (51 men and 16 women) with HCV infection were included in this study. All patients were sero-negative for hepatitis B viral markers. In addition, 20 healthy volunteers, matched for sex and age, were included as a control group. All patients and control individuals were subjected to the following: assessment of medical history, thorough clinical examination, and laboratory investigations including the following: complete blood cell counts, viral hepatitis markers, liver and renal function tests, HCV-RNA by quantitative PCR, serum folate, Vit $B_{12}$, thrombopoietin, and plasma Hcy. Abdominal ultrasonography and ultrasound-guided liver biopsy for histopathologic examinations were carried out for the patients. Patients were divided into two groups of 36 patients with CHC and 31 patients with cirrhosis with HCV liver cirrhosis (LC).

**Results**
The results indicated a significant decrease in the platelet count in CHC and LC patients compared with the healthy control group. There was a highly significant decrease in the FA level in CHC and LC patients compared with the control group; also, a significant decrease in the platelet count was found in LC patients compared with CHC patients. Hcy was significantly increased in CHC and LC patients. There was a nonsignificant decrease in Vit $B_{12}$ in CHC patients, whereas it was significantly increased in LC patients. There was a nonsignificant decrease in thrombopoietin in CHC patients compared with the control group, whereas in LC patients, there was a highly significant decrease. There was a highly significant positive correlation between the platelet count and FA, but an insignificant correlation between the platelet count and Hcy, Vit $B_{12}$, thrombopoietin, and viral load.

**Conclusion**
This study concluded that TCP in HCV-related chronic liver diseases is multifactorial and decreased FA is involved in its pathogenesis as an independent risk factor. Increased Hcy may cause TCP through platelet activation and endothelial dysfunction.

**Keywords:**
folic acid, homocysteine, thrombocytopenia and hepatitis C virus, thrombopoietin, vitamin $B_{12}$

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## Introduction

Hepatitis C virus (HCV) is a leading cause of chronic liver disease (CLD), namely, chronic hepatitis C (CHC), cirrhosis, and hepatocellular carcinoma, as well as the most common indication for liver transplantation in many countries [1].

Thrombocytopenia (TCP) is known to occur in chronically infected HCV patients. The prevalence of TCP ranges from 0.16 to 45.4%, and may interfere with diagnostic procedures, such as liver biopsy, because of the risk of bleeding. It may also exclude patients from effective antiviral treatment. The most widely used definition of TCP is a platelet count of up to $150 \times 10^9$/l [2].

The pathophysiology of TCP in patients with an HCV infection is not completely understood, but is believed to be multifactorial [2]. TCP appears to be related to the



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severity of the liver disease and is more common in patients with cirrhosis [3]. Hypersplenism, formation of antiplatelet antibodies caused by aberration of the immune system (autoimmune TCP), and decreased thrombopoietin production are reported causes [2]. The vascular endothelial dysfunction is also involved in TCP during a chronic HCV infection [4]. In addition, patients with HCV who undergo antiviral treatment with interferon and ribavirin can typically experience TCP as a common side effect [5].

The liver stores large amounts of vitamins, including vitamin $B_{12}$ (Vit $B_{12}$), and lesser amounts of folate. Megaloblastic anemia is a cause of TCP [6]. Halifeoglu *et al.* [7] have reported that plasma folate and Vit $B_{12}$ were decreased in cirrhotic patients. Hyperhomocysteinemia has been reported in CHC and proved to promote steatosis and fibrosis [8]. Folate circulates as 5-methyltetrahydrofolate and serves as a methyl donor to homocysteine (Hcy), which is converted into methionine, in a reaction catalyzed by methionine synthase, a Vit $B_{12}$-dependent enzyme. Hcy is a sulfur-containing amino acid that is formed as an intermediatecompound in methionine metabolism. Folate deficiency disturbs hepatic methionine metabolism and causes impaired Hcy catabolism in the liver and other tissues inhibiting Hcy remethylation, which can lead to liver damage [9]. Thrombopoietin is a glycoprotein hormone produced mainly by the liver and the kidney. It stimulates the production and differentiation of platelets by the bone marrow [10].

This work aimed to study serum folic acid (FA), Vit $B_{12}$, thrombopoietin (TPO), and plasma Hcy levels and determine their correlations to the platelet count in patients with HCV-related CLDs.

## Patients and methods

Sixty-seven patients (51 men and 16 women) with HCV-related CLD were included in this study. They were selected from inpatient and outpatients clinics of the Hepatology Department, National Liver Institute (NLI), Menoufiya University, and internal medicine and tropical medicine departments of Al Zahra University Hospital. They had virologic, biochemical (HCV Ab and PCR for HCV RNA), and histological findings compatible with HCV-related CLD. All patients were sero-negative for hepatitis B viral markers (HBs Ag, HBe Ag, and HBc-Ab). They provided informed consent before participation in the study.

### Exclusion criteria

Patients with CLDs other than HCV (alcoholic, nonalcoholic steatohepatitis, autoimmune hepatitis, biliary disorders, schistosomal patients), hepatocellular carcinoma, or other malignancies, congestive heart failure, diabetes mellitus, renal disease, and hypertension were excluded. None of the patients were receiving antiviral, antimalignancy, or antiplatelet therapy.

In addition, 20 healthy volunteers, matched for sex (15 men and five women) and age ($33.75 \pm 5.75$), were included as a control group. These control individuals had a normal complete blood count, normal liver and renal function tests, and were sero-negative for HCV antibodies and hepatitis B virus (HBV) markers.

The patients and control individuals were subjected to the following:

(1) Assessment of medical history and thorough clinical examination.
(2) Abdominal ultrasonography.
(3) Ultrasound-guided liver biopsy (when possible) was performed for patients using a true-cut needle or a liver biopsy gun for the patients for histopathologic examinations. The specimens were fixed in 10% saline-buffered formalin and processed for routine diagnostic histopathological examination. Assessment of the stage of liver fibrosis (0–6) was carried out using the Ishak scoring system [11]: the stage 0 = no fibrosis, stage 1–2 = portal fibrosis (minimal fibrosis), stage 3–4 = bridging fibrosis (moderate fibrosis), and stage 5–6 = cirrhosis (advanced fibrosis).

### Laboratory investigations

Venous blood samples were withdrawn under aseptic conditions from all the patients and controls for routine evaluation. Serum was separated and blood samples were withdrawn in EDTA, centrifuged, and stored within 15 min from collection to prevent an in-vitro increase in Hcy, because of its release by red blood cells [12]; both serum and plasma samples were stored at $-80^{\circ}C$ until used. The serum folate, serum Vit $B_{12}$, and plasma Hcy levels were measured using a fluorescence polarization immunoassay (Axym analyser; Abbott Laboratories, Rungis, France). The normal reference ranges were as follows: FA 7.0–28.0 ng/ml; Vit $B_{12}$ from 223 to 1132 pg/ml; and Hcy: 5–14 µmol/l. Serum TPO was measured using a commercially available ELISA kit (Quantikine human TPO immunoassay; R&D system Minneapolis, Minnesota, USA), reference value 0–228 pg/ml. Complete blood cell counts were measured using a Sysmix K-21 automatic cell counter (Japan). Liver function tests including alanine aminotransferase, aspartate aminotransferase, serum albumin, and total bilirubin were carried out on COBAS-Intergra 400 (Roche, Germany). Hepatitis markers (HBVs Ag, HBe Ag, anti-HBc-Ab, and HCV antibody) were assessed by enzyme immunoassay (COBAS-Amplicore; Roche). HCV-RNA by quantitative PCR HCV-RNA levels were analyzed by a reverse transcriptase PCR using a commercial kit (Roche Diagnostic, Branchburg, New Jersey, USA) according to the manufacturer's instructions. Alpha fetoprotein was measured by automated chemiluminescence using ACS-180SE (Chiron Diagnostic, Germany).

According to the laboratory, radiological, and histological examinations, the patients were divided into two groups of 36 patients with chronic hepatitis C without cirrhosis (CHC), mean age ± SD ($38.39 \pm 6.83$), and 31 patients with liver cirrhosis with HCV (LC), mean age ± SD ($48.52 \pm 5.603$).

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### Statistical analysis

Data were collected and entered into the computer using the statistical package for social science program for statistical analysis (version 19; SPSS Inc., Chicago, Illinois, USA). Two types of statistics were determined: (a) Descriptive statistics, where qualitative data were expressed as frequency and percent, and quantitative data, shown as mean and SD, and (b) Analytical statistics, where the *t*-test was used for comparison of two groups with quantitative normally distributed data. The Mann–Whitney test was used when the data were not normally distributed. The one-way analysis of variance test was used for comparison between three or more groups with quantitative normally distributed data and the post-hoc test was carried out; Pearson's correlation was used to study the correlation between two variables with normally distributed data. However, Spearman's correlation was used when data were not normally distributed. A *P*-value was considered statistically significant when it was less than 0.05.

## Results

Table 1 shows the descriptive data of the patients; we found that 16 (44.4%) of the CHC patients and seven (22.6%) of the cirrhotic patients had normal spleens.

Table 2 shows that there were significant differences between CHC and controls in all the parameters studied, except for age and alpha fetoprotein serum level.

Table 3 shows that there were significant differences between CHC and LC groups in all the parameters studied except for aspartate aminotransferase, alkaline phosphatase, gamma glutamic transeferase, total bilirubin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH).

Table 4 shows that there were significant differences between the three groups (LC, CHC, control) in all the variables studied (FA, TPO, Vit $B_{12}$, Hcy); these differences were mainly because of significant differences between the three groups (LC, CHC, control) in FA, between LC and control LC and CHC in TPO, between LC and control, LC and CHC in Vit $B_{12}$, and between CHC and control LC and control in Hcy.

Table 5 shows that there were significant positive correlations between FA and platelet count and Hb, and Vit $B_{12}$ and serum albumin; however, significant negative correlations were found between FA and MCV (Fig. 1) and Hcy and MCH in the CHC group. There were insignificant correlations between all of Hcy, Vit $B_{12}$ and TPO, and platelet count.

Table 6 shows that there were highly significant positive correlations between FA and all of Hb, MCH, and platelet count; however, the correlation was significantly negative between FA and MCV. Significant positive correlations were found between TPO and serum albumin and Hcy and Vit $B_{12}$, whereas significant negative correlations were found between Hcy and both TPO and serum albumin in the LC group.

**Table 1 Descriptive data of the patient groups**

| | *N* (%) | |
|---|---|---|
| Studied parameters | Chronic HCV (*n*=36) | Liver cirrhosis (*n*=31) |
| Sex | | |
| Male | 27 (75.0%) | 24 (77.4%) |
| Female | 9 (25.0%) | 7 (22.6%) |
| HCV Ab | | |
| Negative | 0 (0.0%) | 0 (0.0%) |
| Positive | 36 (100.0%) | 31 (100.0%) |
| HBsAg | | |
| Negative | 36 (100.0%) | 31 (100.0%) |
| Positive | 0 (0.0%) | 0 (0.0%) |
| Liver ultrasound | | |
| Normal | 11 (36.6%) | 00 (0.0%) |
| Enlarged | 25 (69.4%) | 27 (87.1%) |
| Shrunken | 0 (0.0%) | 4 (12.9%) |
| Spleen ultrasound | | |
| Normal | 16 (44.4%) | 7 (22.6%) |
| Enlarged | 20 (55.6%) | 24 (77.4%) |

HCV, hepatitis C virus.

Our results indicated a significant decrease in the platelet count in CHC (Table 2) and LC (Table 3) patients compared with the healthy controls. There was a highly significant decrease in the FA level in CHC and LC patients (Fig. 2) compared with the control group; also, a significant decrease was found in LC patients compared with CHC patients (Table 4). Hcy was significantly increased in CHC and LC patients (Fig. 3). There was a nonsignificant decrease in Vit $B_{12}$ in CHC patients, whereas it was significantly increased in LC patients (Fig. 4). There was a nonsignificant decrease in TPO in CHC patients compared with the controls, whereas in LC patients, there was a highly significant decrease (Fig. 5). There was a highly significant positive correlation between the platelet count and FA, but an insignificant correlation between the platelet count and all of Hcy, Vit $B_{12}$, and TPO (Tables 5 and 6).

In CHC patients, there was a significant positive correlation between the platelet count and FA, but an insignificant correlation between the platelet count and all of Hcy, Vit $B_{12}$, TPO, and viral load. In LC patients, there was a significant correlation between the platelet count and FA, whereas no significant correlations were found between the platelet count and all of Hcy, Vit $B_{12}$, TPO, and viral load (Table 7 and Fig. 6).

## Discussion

Egypt has one of the highest prevalence rates of HCV infection in the world [13]. A strong correlation between liver fibrosis and TCP has been found, and the platelet count is currently used as an index for fibrosis staging [14]. The prevalence of TCP within the HCV-infected population is also likely to increase [15].

The pathophysiology of TCP in patients with HCV infection is not completely understood, but is believed to be multifactorial [2]. TCP appears to be related to the severity of the liver disease and is more common in patients with cirrhosis [3]. Splenomegaly appears to

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**Table 2 Comparison of CHC and control groups in terms of the studied parameters**

| | Mean ± SD | | | |
|---|---|---|---|---|
| Studied parameters | Controls ($n=20$) | CHC ($n=36$) | *T* | *P*-value |
| Age (years) | 33.75 ± 5.75 | 38.39 ± 6.83 | 1.569 | 0.073 |
| ALT (U/l) | 23.00 ± 3.43 | 111.50 ± 36.91 | 10.655 | 0.00 |
| AST (U/l) | 23.25 ± 3.48 | 93.81 ± 31.84 | 9.838 | 0.00 |
| Alkaline phosphatase (U/l) | 33.55 ± 6.85 | 52.28 ± 7.27 | 9.427 | 0.00 |
| GGT (U/l) | 20.30 ± 5.22 | 32.83 ± 5.36 | 8.462 | 0.00 |
| Albumin (g/dl) | 4.330 ± 0.25 | 4.08 ± 0.23 | 3.822 | 0.00 |
| Prothrombin time (g/dl) | 7.935 ± 0.20 | 7.31 ± 0.34 | 7.495 | 0.00 |
| Prothrombin concentration (%) | 91.80 ± 4.36 | 75.64 ± 4.43 | 13.157 | 0.00 |
| AFP (ng/ml) | 3.73 ± 1.79 | 4.68 ± 1.73 | 1.929[a] | 0.059 |
| Total bilirubin (mg/dl) | 0.61 ± 0.22 | 2.43 ± 1.17 | 6.857[a] | 0.00 |
| Platelets ($\times 10^3/\mu l$) | 294.45 ± 67.61 | 134.81 ± 33.49 | 11.846 | 0.00 |
| Hb (g/dl) | 13.99 ± 0.97 | 11.98 ± 0.57 | 9.807 | 0.00 |
| MCV (fl) | 80.75 ± 2.69 | 82.94 ± 4.40 | 2.025 | 0.048 |
| MCH (%) | 29.35 ± 1.53 | 28.28 ± 1.47 | 2.582 | 0.013 |

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; GGT, gamma glutamic transeferase; Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.
[a]Mann–Whitney test.

**Table 3 Comparison of CHC and LC groups in the studied variables**

| | Mean ± SD | | | |
|---|---|---|---|---|
| Studied parameters | Chronic HCV ($n=36$) | Liver cirrhosis ($n=31$) | *T* | *P*-value |
| Age (years) | 38.39 ± 6.83 | 48.52 ± 5.60 | 6.57 | 0.00 |
| ALT (U/l) | 111.50 ± 36.91 | 73.68 ± 12.01 | 5.46 | 0.00 |
| AST (U/l) | 93.81 ± 31.84 | 92.84 ± 15.86 | 0.153 | 0.879 |
| Alkaline phosphatase (U/l) | 52.28 ± 7.27 | 53.42 ± 5.47 | 0.72 | 0.476 |
| GGT (U/l) | 32.83 ± 5.36 | 33.32 ± 5.25 | 0.38 | 0.708 |
| Albumin (g/dl) | 4.08 ± 0.23 | 3.32 ± 0.37 | 10.30 | 0.00 |
| Prothrombin time (g/dl) | 7.31 ± 0.34 | 7.14 ± 0.31 | 2.12 | 0.038 |
| Prothrombin concentration (%) | 75.64 ± 4.43 | 61.68 ± 4.48 | 12.81 | 0.00 |
| AFP (ng/ml) | 4.68 ± 1.73 | 15.34 ± 4.89 | 12.23[a] | 0.00 |
| Total bilirubin (mg/dl) | 2.43 ± 1.17 | 2.04 ± 0.52 | 1.69[a] | 0.095 |
| Platelets ($\times 10^3/\mu l$) | 134.81 ± 33.49 | 106.71 ± 29.07 | 3.65 | 0.001 |
| Hb (g/dl) | 11.98 ± 0.57 | 10.89 ± 0.83 | 6.410 | 0.00 |
| MCV (fl) | 82.94 ± 4.40 | 83.10 ± 6.44 | 0.114 | 0.909 |
| MCH (%) | 28.28 ± 1.47 | 27.61 ± 3.12 | 1.143 | 0.257 |

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; GGT, gamma glutamic transeferase; HCV, hepatitis C virus; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.
[a]Mann–Whitney test.

**Table 4 Comparison between the three groups in FA, Hcy, Vit $B_{12}$, and TPO levels**

| | Mean ± SD | | | | | |
|---|---|---|---|---|---|---|
| Studied parameters | Controls ($n=20$) | Chronic HCV ($n=36$) | Liver cirrhosis ($n=31$) | *F* ratio | *P*-value | Post-hoc *P*-value |
| FA (ng/ml) | 7.82 ± 0.79 | 4.66 ± 0.66 | 3.87 ± 0.58 | 225.83 | 0.00 | $P_1=0.00$<br>$P_2=0.00$<br>$P_3=0.00$ |
| TPO (pg/ml) | 128.15 ± 10.12 | 127.42 ± 11.18 | 101.58 ± 18.38 | 34.71 | 0.00 | $P_1=0.58$<br>$P_2=0.00$<br>$P_3=0.00$ |
| Vit $B_{12}$ (pg/ml) | 426.05 ± 15.83 | 419.83 ± 15.93 | 505.13 ± 49.77 | 66.01 | 0.00 | $P_1=0.49$<br>$P_2=0.00$<br>$P_3=0.00$ |
| Hcy (μmol/l) | 4.17 ± 0.84 | 15.08 ± 2.42 | 16.19 ± 2.88 | 181.97 | 0.00 | $P_1=0.00$<br>$P_2=0.00$<br>$P_3=0.58$ |

CHC, chronic hepatitis C; FA, folic acid; HCV, hepatitis C virus; Hcy, homocysteine; $P_1$, between CHC and control, $P_2$, between LC and control; and $P_3$, between CHC and LC groups; Vit $B_{12}$, vitamin $B_{12}$.

be the factor most likely to be responsible for this, although there are several thrombocytopenic cases without splenomegaly, suggesting that other factors may also be responsible. The concept of hypersplenism was never proven without doubt, but was widely accepted because of the lack of an alternative explanation [16]. The

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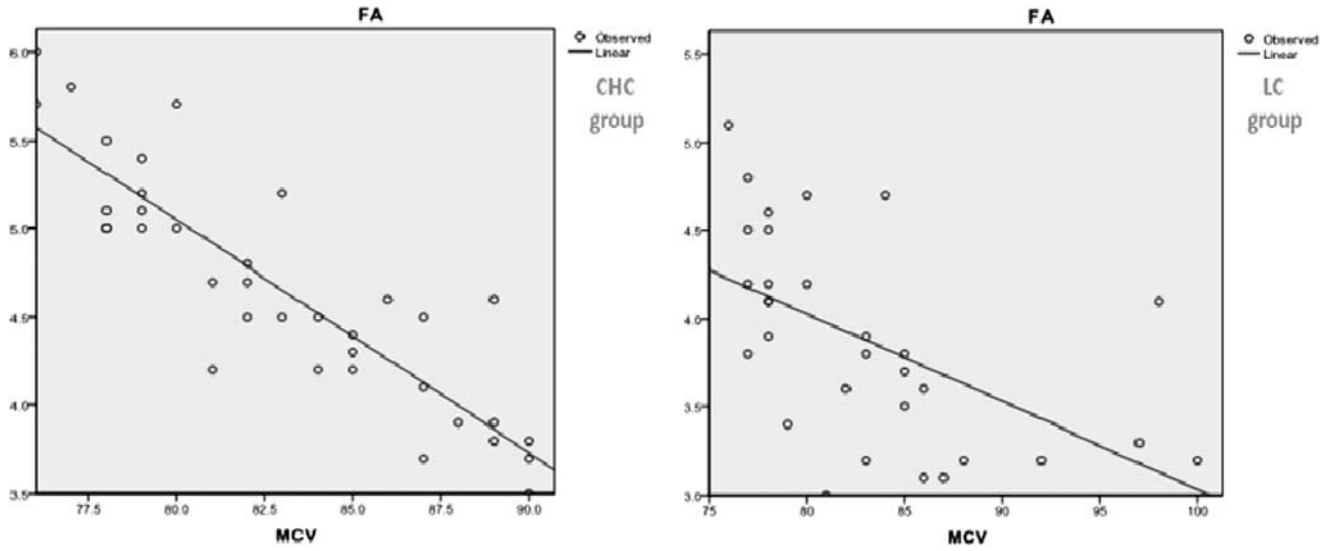
**Table 5 Correlation between the levels of FA, TPO, Hcy, Vit $B_{12}$, and some parameters in the CHC group**

| | FA | | TPO | | Hcy | | Vit $B_{12}$ | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| ALT | −0.007 | 0.966 | 0.069 | 0.690 | 0.206 | 0.228 | 0.128 | 0.456 |
| AST | −0.055 | 0.751 | 0.095 | 0.582 | 0.261 | 0.124 | 0.193 | 0.259 |
| Serum albumin | −0.149 | 0.384 | −0.220 | 0.197 | −0.121 | 0.481 | 0.365 | 0.028 |
| AFP levels | 0.327[a] | 0.051 | 0.174[a] | 0.310 | −0.120[a] | 0.485 | −0.273[a] | 0.107 |
| Hb | 0.918 | 0.000 | 0.177 | 0.302 | −0.307 | 0.069 | −0.142 | 0.408 |
| MCV | −0.884 | 0.000 | −0.214 | 0.210 | 0.147 | 0.394 | 0.034 | 0.845 |
| MCH | 0.130 | 0.448 | −0.025 | 0.886 | −0.427 | 0.009 | 0.100 | 0.562 |
| Platelet count | 0.960 | 0.000 | 0.152 | 0.377 | −0.299 | 0.077 | −0.220 | 0.196 |
| TPO | 0.201 | 0.239 | – | – | 0.080 | 0.642 | 0.188 | 0.273 |
| Hcy | −0.220 | 0.198 | 0.080 | 0.642 | – | – | 0.281 | 0.096 |
| FA | – | – | 0.201 | 0.239 | −0.220 | 0.198 | −0.161 | 0.349 |
| Vit $B_{12}$ | −0.161 | 0.349 | 0.188 | 0.273 | 0.281 | 0.096 | – | – |

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; FA, folic acid; HCV, hepatitis C virus; Hcy, homocysteine; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; Vit $B_{12}$, vitamin $B_{12}$.
[a]Spearman's correlation.

**Figure 1**




Correlation between FA and MCV levels in CHC and LC groups. Significant negative correlation between FA and MCV levels in both groups. CHC, chronic hepatitis C; FA, folic acid; MCV, mean corpuscular volume.

**Table 6 Correlation between the levels of FA, TPO, Hcy, Vit $B_{12}$, and some parameters in the LC group**

| | FA | | TPO | | Hcy | | Vit $B_{12}$ | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| ALT | −0.169 | 0.362 | −0.118 | 0.526 | 0.230 | 0.213 | 0.074 | 0.694 |
| AST | −0.161 | 0.387 | −0.081 | 0.666 | 0.345 | 0.057 | 0.183 | 0.323 |
| Serum albumin | 0.279 | 0.129 | 0.804 | 0.000 | −0.808 | 0.000 | −0.231 | 0.210 |
| AFP levels | −0.103[a] | 0.581 | −0.086[a] | 0.646 | −0.066[a] | 0.724 | −0.178[a] | 0.337 |
| Hb | 0.843 | 0.000 | 0.232 | 0.209 | −0.260 | 0.157 | 0.150 | 0.421 |
| MCV | −0.550 | 0.001 | −0.129 | 0.490 | 0.180 | 0.332 | −0.118 | 0.526 |
| MCH | 0.620 | 0.000 | 0.164 | 0.378 | −0.193 | 0.298 | 0.062 | 0.740 |
| Platelet count | 0.949 | 0.000 | 0.242 | 0.189 | −0.157 | 0.400 | 0.245 | 0.184 |
| TPO | 0.211 | 0.255 | – | – | −0.710 | 0.000 | −0.105 | 0.574 |
| Hcy | −0.101 | 0.588 | −0.710 | 0.000 | – | – | 0.390 | 0.030 |
| FA | – | – | 0.211 | 0.255 | −0.101 | 0.588 | 0.180 | 0.334 |
| Vit $B_{12}$ | 0.180 | 0.334 | −0.105 | 0.574 | 0.390 | 0.030 | – | – |

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FA, folic acid; Hb, hemoglobin; HCV, hepatitis C virus; Hcy, homocysteine; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; Vit $B_{12}$, vitamin $B_{12}$.
[a]Spearman's correlation.

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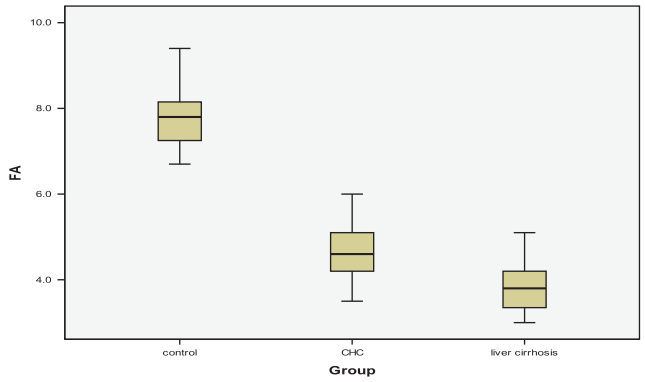
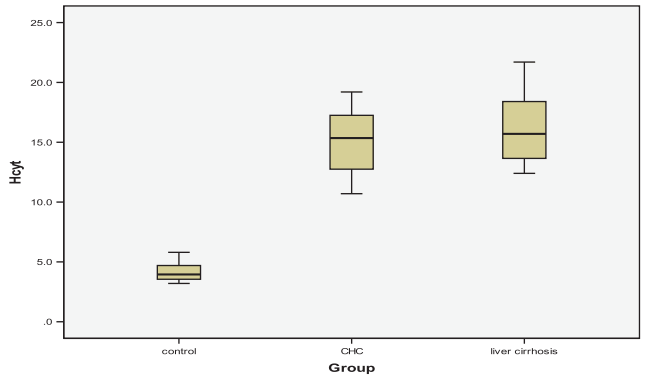
**Figure 2**



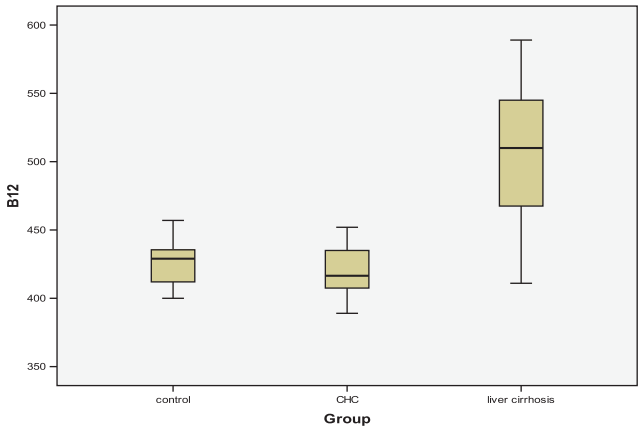

Levels of folic acid in the studied groups.

**Figure 3**



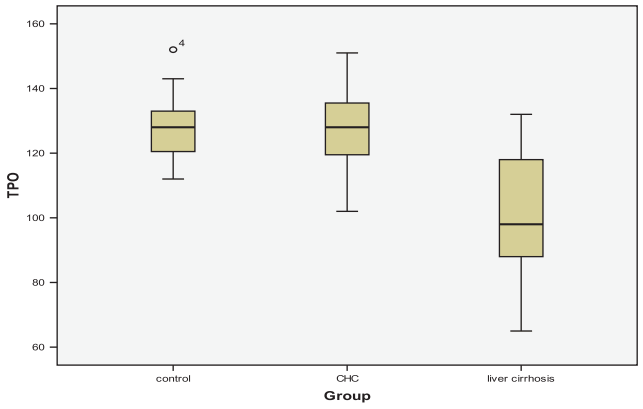

Levels of homocysteine in the studied groups.

**Figure 4**




Levels of vitamin $B_{12}$ in the studied groups.

**Figure 5**




Levels of TPO in the studied groups.

formation of antiplatelet antibodies is caused by an aberration of the immune system (autoimmune TCP) [2].

Fusegawa *et al.* [17] reported that patients with CHC have increased platelet activation and have a higher percentage of platelet microparticles, a marker of platelet activation, which may contribute toward the occurrence of TCP in CHC. However, they did not provide an explanation for the mechanism of platelet activation. The cause of TCP has not been clearly elucidated as yet.

Vascular endothelial dysfunction has also been suggested in TCP during chronic HCV infection by Makoto *et al.* [4], who observed a negative correlation between the platelet count and the vWF antigen value and a negative correlation between the platelet count and thrombomodulin (a marker of endothelial cell damage). Decreased thrombopoietin production is a reported cause [18].

To date, the mechanism of TCP in HCV infection is a matter of debate; thus, this work was designed to study serum FA, Vit $B_{12}$, TPO, and plasma Hcy levels and their correlations to the platelet count in patients with HCV-related CLDs.

The results of this work indicated a highly significant decrease in the platelet count and serum FA level in CHC and LC patients compared with the control group, and also a significant decrease in LC patients compared with CHC patients. These findings are in agreement with those of Halifeoglu *et al.* [7], who observed decreased plasma folate in cirrhotic patients compared with the controls. Also, Sandra Hirsch *et al.* [9] found that serum folate was lower in patients with severe nonalcoholic fatty liver disease than those with minimal damage or without liver damage. Our results showed a highly significant positive correlation between FA and the platelet count, but a significant negative correlation between FA and MCV. There was no significant correlation between FA and both of Hcy and Vit $B_{12}$. This finding is in agreement with that of Gracía-Tevijano *et al.* [19] and Halifeoglu *et al.* [7], and may indicate that decreased FA level is an independent risk factor for TCP in CLDs, especially that related to HCV infection.

The results of the present study showed that plasma Hcy was significantly increased in CHC and LC patients, but there was no significant correlation between Hcy and

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both FA and Vit $B_{12}$, which is in agreement with the result of Adinolfi *et al.* [8], who found that plasma Hcy was significantly higher in patients with grade 3–4 of steatosis than that observed in patients with grade 1–2 steatosis and those without steatosis. Serum levels of both folate and Vit $B_{12}$ did not correlate with plasma levels of Hcy or grade of steatosis. Also, Bosy-Westphal *et al.* [20] observed hyperhomocysteinemia in patients with cirrhosis, and a significant negative association between plasma Hcy and both FA and $B_{12}$ was observed in healthy controls. However, in cirrhosis, only a weak correlation was found between Hcy and FA, which disappeared at advanced stages of liver diseases. They attributed hyperhomocysteinemia in cirrhotic patients to impaired liver function, which leads to impaired transsulfuration and remethylation. Gracía-Tevijano *et al.* [19] observed reduced expression of Hcy-metabolizing genes in both alcoholic and HCV cirrhosis, resulting in hyperhomocysteinemia that was not related to altered plasma levels of FA or Vit $B_{12}$, and indicated that increased Hcy plays a role in vascular and liver damage in cirrhotic patients. Hcy-induced cell toxicity may explain platelet activation and endothelial vascular dysfunction observed by Fusegawa *et al.* [17] and Makoto *et al.* [4], respectively, in the cirrhotic patients, resulting in TCP. This finding may explain how some cirrhotic patients suffer from coronary or cerebrovascular thrombosis while have TCP and coagulopathy.

**Table 7 Correlation between the levels of the platelet count and each of FA, TPO, Hcy, Vit $B_{12}$, and viral load in CHC and LC groups**

| | Platelet count | | | |
|---|---|---|---|---|
| | CHC | | LC | |
| Parameters | *r* | *P* | *r* | *P* |
| FA | 0.960 | <0.001 | 0.949 | <0.001 |
| TPO | 0.152 | 0.377 | 0.242 | 0.189 |
| Hcy | −0.299 | 0.077 | −0.157 | 0.400 |
| Vit $B_{12}$ | −0.220 | 0.196 | 0.245 | 0.184 |
| Viral load | −0.211 | 0.198 | −0.124 | 0.513 |

CHC, chronic hepatitis C; FA, folic acid; Hcy, homocysteine; Vit $B_{12}$, vitamin $B_{12}$.

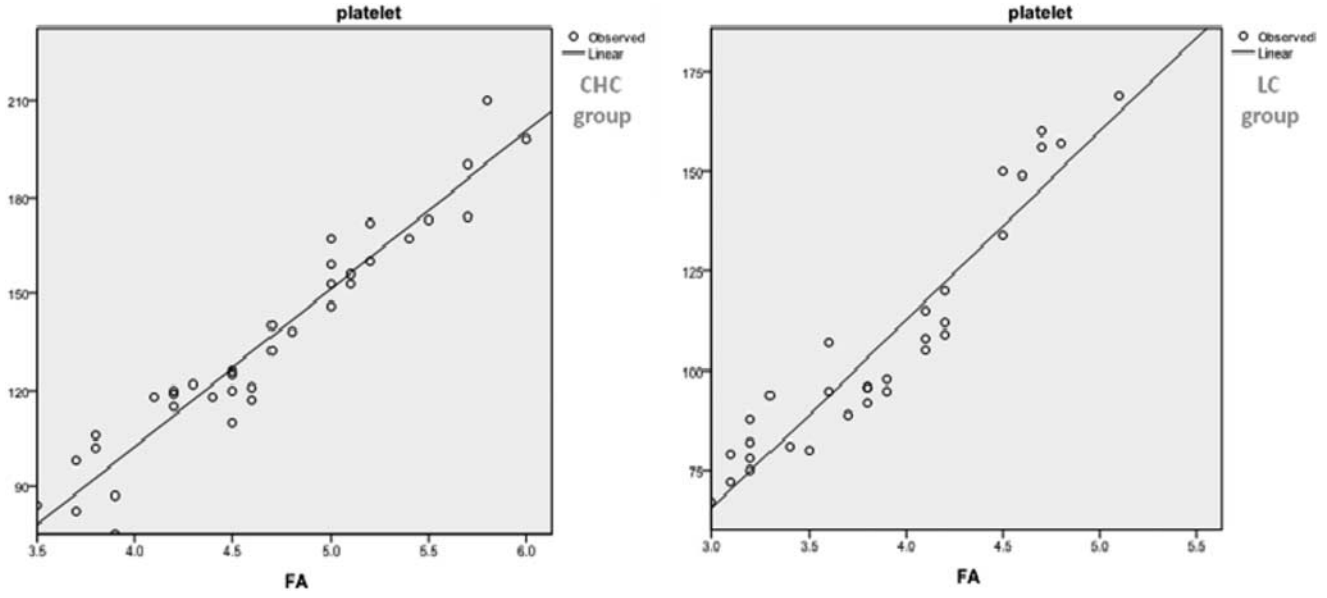
Our results showed a nonsignificant decrease in Vit $B_{12}$ in CHC patients whereas, surprisingly, it was significantly increased in cirrhotic patients.

The results of the present work showed a nonsignificant decrease in TPO in CHC patients, which is not in agreement with the result of Ezzat *et al.* [18], who observed an increased TPO level in CHC patients and suggested that this increased TPO may be a compensatory response to a reduced platelet count by the still-functioning liver. Our showed a highly significant decrease in TPO in cirrhotic patients, but this decrease in TPO was not correlated with the platelet count and significantly positively correlated to serum albumin (synthetic function of the liver). Furthermore, Sanjo *et al.* [21] reported that no differences in serum TPO levels were observed in the cirrhosis and the control group.

## Conclusion

TCP in HCV-related CLDs is multifactorial, and decreased FA is involved in its pathogenesis as an independent risk factor. Increased Hcy may cause TCP through platelet activation and endothelial dysfunction. We can recommend the use of FA to treat TCP in HCV-related CLDs, and advice the patients to include plenty of fresh fruits and

**Figure 6**




Correlation between the platelet count and FA level in CHC and LC groups. Significant positive correlation between the platelet count and FA level in both groups. CHC, chronic hepatitis C; FA, folic acid.

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vegetables in their diet; further research work should be carried out to study TCP in HCV infection.

## Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

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# الملخص العربي

لدى مصر واحد من أعلى معدلات انتشار عدوى التهاب الكبد الوبائي الفيروسي (سي) في العالم.
ومن المعروف أن عدد الصفيحات الدموية تقل بدرجة تتناسب مع مرحلة تقدم المرض في حالات الالتهاب الكبدي الوبائي الفيروسي (سي) المزمنة. وقد لوحظ ارتباط قوي بين تليف الكبد ونقص الصفيحات الدموية (TCP)، ويستخدم حاليا عدد الصفيحات الدموية كمؤشر لدرجة التليف. وتتراوح نسبة انتشار نقص الصفيحات الدموية في مرضى فيروس (سي) المزمن من 0.16٪ إلى 45.4٪ وربما يتعارض مع الإجراءات التشخيصية، مثل عينة من الكبد، وذلك بسبب خطر النزيف. وقد يسبب أيضا استبعاد المرضى من العلاج الفعال بمضاد الفيروسات . لم يتم فهم الية إحداث الخلل الوظيفي المرضي المسبب في نقص الصفيحات الدموية في مرضى الالتهاب الكبدي الفيروسي الوبائي ولكن يعتقد أن تكون متعددة المسببات والعوامل ، ويبدو أن العامل الأكثر مسؤولية في ذلك هو تضخم الطحال ، على الرغم من أن هناك عددا كبيرا من حالات نقص الصفيحات بدون تضخم الطحال، مما يشير إلى أن عوامل أخرى قد تكون مسؤولة أيضا.
تقوم الكبد بتخزين كميات كبيرة من الفيتامينات، بما في ذلك فيتامين ب12 ، وبكميات أقل من حمض الفوليك. ومن المعلوم أن فقر الدم المتميز بتضخم حجم الخلايا هو أحد أسباب نقص الصفيحات الدموية. و قد لوحظ نقص مستوى حمض الفوليك و فيتامين ب12 في دم مرضى التليف الكبدي. وقد لوحظ أيضا زيادة نسبة الهوموسيستايين في دم مرضى التهاب الكبد الوبائي المزمن، وقد ثبت أن هذه الزيادة تساعد على تدهورالتليف والتدهن بالكبد. الثرومبوبويتين هو هرمون بروتين سكري تنتجه أساسا الكبد والكليتان. ويقوم بتحفيز نخاع العظام لانتاج وتمايز الصفيحات الدموية.

**الهدف**
هذا العمل يهدف إلى دراسة مستوى حمض الفوليك ، وفيتامين ب12، والثرومبوبيوتين في مصل الدم ومستوى الحامض الاميني الهوموسيستايين في بلازما الدم ، وايجاد نوع ارتباط عدد الصفيحات الدموية بها في حالات أمراض الكبد المزمنة المتعلقة بفيروس الالتهاب الكبدي (سي).

**المرضى وطرق البحث**
تضمن هذا العمل سبعة وستين مريضا (51 من الذكور والإناث 16) بأمراض الكبد المزمنة ذات الصلة بفيروس الالتهاب الكبدي (سي). وتم التأكد من خلو مصل جميع المرضى من علامات التهاب الكبد الفيروسي(ب). وبالإضافة إلى ذلك، شمل 20 متطوعا من الأصحاءالمتطابقين في الجنس والعمركمجموعة ضوابط.
وقد خضع جميع المرضى والأفراد إلى ما يلي: التاريخ الطبي، الفحص السريري الشامل ، والفحوصات المختبريةالتالية: تعداد كامل لخلايا الدم، وعلامات التهاب الكبد الفيروسي، واختبارات وظائف الكبد والكليتين ، وقياس كمية الحامض النووي لفيروس التهاب الكبد (سي) بواسطة تفاعل سلسلة البلمرة (PCR) الكمي،وقياس مستوى حامض الفوليك وفيتامين ب12، وهرمون الثرومبوبيوتين في المصل ومستوى الحمض الاميني هوموسيستايين في بلازما الدم .
بالإضافة الى عمل موجات فوق الصوتية على البطن وأخذ خزعة من الكبد موضحة بالموجات الصوتية وذلك لاجراء الفحص المرضى للأنسجة . وقد تم تقسيم المرضى إلى موجموعتين : 36 مريضا ومريضة يعانون من الالتهاب الكبدي الوبائي (سي) المزمن ، و 31 مريضا ومريضة مصابين بتليف الكبد المترتب على الإصابة بفيروس التهاب الكبد (سي).

**النتائج**
أظهرت النتائج انخفاض ملحوظ في عدد الصفيحات الدموية في المرضى الذين يعانون الالتهاب الكبدي الوبائي (سي) المزمن وتليف الكبد بالمقارنة مع مجموعة الضوابط الصحية. وقد لوحظ وجود انخفاض كبير جدا في مستوى حمض الفوليك في مصل مرضى الإلتهاب الكبدي الوبائي (سي) المزمن ومرضى التليف الكبدي مقارنة بمستواه في مجموعة الضوابط الصحية وقد كان هذا الإنخفاض أشد في مرضى التليف الكبدي عنه في مرضى الإلتهاب الكبدي المزمن .. وقد لوحظ وجود زيادة كبيرة في مستوى الحمض الأميني هوموسيستايين في مرضى الإلتهاب الكبدي المزمن ومرضى التليف الكبدي . وقد وجد انخفاض غير ملحوظ في مستوى فيتامين ب 12 عند مرضى الإلتهاب الكبدي (سي) المزمن ، في حين لوحظت زيادة كبيرة في مستواه لدى مرضى التليف الكبدي . وأيضا وجد انخفاض غير ملحوظ في مستوى الثرومبوبيوتين في مصل مرضى الإلتهاب الكبدي (سي ) المزمن, في حين وجود انخفاض شديد للغاية في مستواه لدى مرضى التليف الكبدي مقارنة بمستواه في مجموعة الضوابط الصحية .
وقد لوحظ وجود علاقة ذات دلالة إحصائية إيجابية عالية بين عدد الصفيحات الدموية ومستوى حمض الفوليك في مصل المرضى ، في حين لم تلاحظ علاقة بين عدد الصفيحات الدموية ومستوى كل من فيتامين ب12و الثرومبوبيوتين والهوموسيستايين وكمية الحامض النووي للفيروس (سي ) .

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**اختتام**

اختتمت هذه الدراسة بأن نقص عدد الصفيحات الدموية لدى المرضى الذين يعانون من امراض الكبد المزمنة المترتبة على الإصابة بفيروس الإلتهاب الكبدي ( سي ) ذو مسببات وعوامل عديدة .ويعد انخفاض مستوى حمض الفوليك أحد العوامل المسببة له , ويمكن اعتباره عامل خطر مستقل . كما قد يسبب زيادة الحمض الأميني هوموسيستايين نقص صفيحات الدم من خلال تنشيط الصفيحات الدموية وإحداث خلل وظيفي في الخلايا المبطنة للاوعية الدموية . يمكن أن نوصي باستخدام أقراص حمض الفوليك لعلاج نقص الصفيحات الدموية في حالات أمراض الكبد المزمنة المترتبة على الإصابة بفيروس الإلتهاب الكبدي (سي ) . ولأن كلا من الطهي والتجميد يسبب تدمير حمض الفوليك لذلك ينصح المرضى بتناول كمية وفيرة من الفواكه والخضروات الطازجة في نظامهم الغذائي . ونوصي ببذل مزيد من العمل البحثي لدراسة كيفية ومسببات حدوث نقص الصفيحات الدموية في حالات أمراض الكبد المزمنة المترتبة على الإصابة بفيروس التهاب الكبد (سي ) .

**كلمات البحث:**

حمض الفوليك، فيتامين ب12, هوموسيستايين ، ثرومبوبيوتين، نقص صفيحات الدم ، وأمراض التهاب الكبد سي الوبائية المزمنة.

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