AIM OF THE WORK

The aim of the present work was to evaluate the plasma levels of *MIR17HG* protein, encoded by the *miR-17~92 cluster host gene*, and the hepatic expression of its target PTEN in patients with chronic HCV infection in relation to hepatic steatosis, inflammation and fibrosis.

MATERIAL

Subjects:

The present study included 30 treatment-naïve patients with chronic HCV infection who were referred to the Hepatobiliary Unit, Department of Internal Medicine, Faculty of Medicine, University of Alexandria. They were 19 males and 11 females and their ages ranged between 20 to 56 years (mean ± SD = 42.27 ± 9.03 years). The diagnosis of chronic HCV infection was based on the following criteria: (1) positive test for HCV antibody; (2) detectable serum HCV RNA and (3) histopathological findings in liver biopsy consistent with the diagnosis of HCV etiology. The diagnosis of cirrhosis was determined by clinical, biochemical and ultrasonographic evidences and histopathological examination when indicated. According to the METAVIR stage, 18 patients were F1-F3 (CHC) and 12 patients were F4 (cirrhosis).

Also, 15 age- and sex-matched healthy subjects with no evidence of liver disease were included as control group. They were 8 males and 7 females and their ages ranged between 25 to 51 years (mean ± SD = 38.20 ± 8.84 years).

Exclusion criteria:

Patients with chronic HCV infection were excluded from the study if they had seropositivity for HBV infection; history of alcohol consumption; other known causes of chronic liver disease; concomitant schistosomiasis; bleeding diathesis; chronic diseases such as diabetes mellitus, connective tissue diseases or other autoimmune diseases; other infections; any kind of malignancy; and cardiac, respiratory or renal disease. Also, patients who have received previous antiviral therapy were excluded from the study.

The study was conducted in accordance with the provisions of the 1975 Declaration of Helsinki and Good Clinical Practice guidelines. An informed consent was obtained from all subjects included in the study.

METHODS

All patients with chronic HCV infection were evaluated as regards:

I. Clinical evaluation focusing on:

The apparent duration and possible risk factors of HCV infection, symptoms and signs of chronic liver disease [right hypochondrial pain, jaundice, ascites, hepatic encephalopathy, previous gastrointestinal (GI) bleeding and bleeding diathesis] and liver and spleen sizes.

II. Abdominal ultrasonography for assessment of:

Liver size and echopattern (normal, bright or coarse) and the presence of cirrhosis, ascites and splenomegaly.[325]

III. Laboratory investigations:

Blood samples were collected from patients with chronic HCV infection and from healthy subjects. The following tests were performed:

a. ***Complete blood picture.***[326]

b. ***Liver Test Profile:*** Serum aspartate and alanine aminotransferases (AST and ALT), serum albumin, serum bilirubin, serum gamma glutamyl transferase (GGT) [327] and prothrombin time (PT).[326]

c. ***Viral Testing:***

i. HCV antibodies, hepatitis B surface antigen and hepatitis B core antibody using enzyme-linked immunosorbant assay (ELISA).[328]

ii. HCV RNA levels in serum using RT-PCR assay.[329]

IV. Measurement of plasma *microRNA-17 host gene* protein levels:

Quantitative determination of plasma levels of *MIR17HG* protein in patients with chronic HCV infection and healthy subjects was performed using an in vitro human *MIR17HG* protein ELISA kit (Cusabio, Wuhan, Hubei Province, China) according to the manufacturer's instruction.

Sample collection and storage:

Plasma was collected using a plasma separator tube (PST) and samples were centrifuged for 15 minutes at 1000 ×g. Plasma was removed and aliquots were stored at -20°C. Repeated freeze-thaw cycles were avoided.

Principle of the test:

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for *MIR17HG* protein has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any *MIR17HG* protein present is bound by the

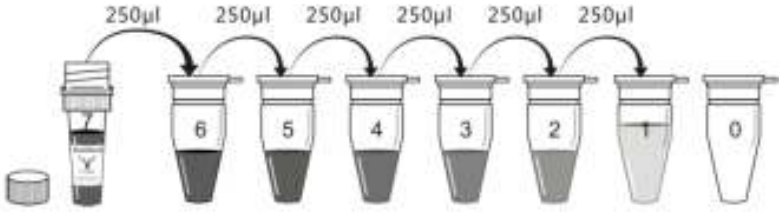
immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody (Ab) specific for *MIR17HG* protein is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of *MIR17HG* bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents:

Reagents	Quantitiy
Assay plate (12×8 coated Microwells)	1 (96 wells)
Standard (Freeze dried)	2
Biotin-antibody (100 × concentrate)	1×120 µl
HRP-avidin (100 × concentrate)	1×120 µl
Biotin-antibody Diluent	1×10 ml
HRP-avidin Diluent	1×10 ml
Sample Diluent	1×20 ml
Wash Buffer (25 × concentrate)	1×20 ml
Tetramethylbenzidine (TMB) Substrate	1×10 ml
Stop Solution	1×10 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

Standard:

- The standard vial was centrifuged at 6000-10000 rpm for 30 seconds.
- The standard was reconstituted with 1.0 ml of Sample Diluent. Other diluents were not substituted. This reconstitution produced a stock solution of 2000 pg/ml.
- The standard was mixed to ensure complete reconstitution and was allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.
- 250 µl of Sample Diluent was pipetted into each tube (S0-S6) and the stock solution was used to produce a 2-fold dilution series (below).
- Each tube was mixed thoroughly before the next transfer. The undiluted Standard served as the high standard (2000 pg/ml). Sample Diluent served as the zero standard (0 pg/ml).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/ml	2000	1000	500	250	125	62.5	31.25	0

***Assay procedure*:**[330]

All reagents and samples were brought to room temperature before use. Samples were centrifuged again after thawing before the assay. All samples and standards were assayed in duplicate. The assay was performed according to the manual instructions:

1. 100µl of standard and sample was added per well, covered with the adhesive strip provided and incubated for 2 hours at 37°C.
2. The liquid of each well was removed but not washed.
3. 100µl of Biotin Ab (1x) was added to each well, covered with a new adhesive strip and incubated for 1 hour at 37°C.
4. Each well was aspirated and washed, repeating the process two times for a total of three washes. Washing was done by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and letting it stand for 2 minutes, After the last wash, any remaining wash Buffer was removed by aspirating or decanting. The plate was invertd and blottted against clean paper towels.
5. 100µl of HRP-avidin (1x) was added to each well. The microtiter plate was covered with a new adhesive strip and incubated for 1 hour at 37°C.
6. The aspiration/wash process was repeated for five times as in step 4.
7. 90µl of TMB Substrate was added to each well and incubated for 15-30 minutes at 37°C away from light.
8. 50µl of Stop Solution was added to each well and the plate was gently tapped to ensure thorough mixing.
9. The optical density of each well was determined within 5 minutes, using a microplate reader set to 450 nm.
10. The duplicate readings were averaged for each standard and sample and the average zero standard optical density was subtracted. A standard curve was created by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. The data were linearized by plotting the log of the *MIR17HG* protein concentrations versus the log of the optical density and the best fit line was determined by regression analysis.

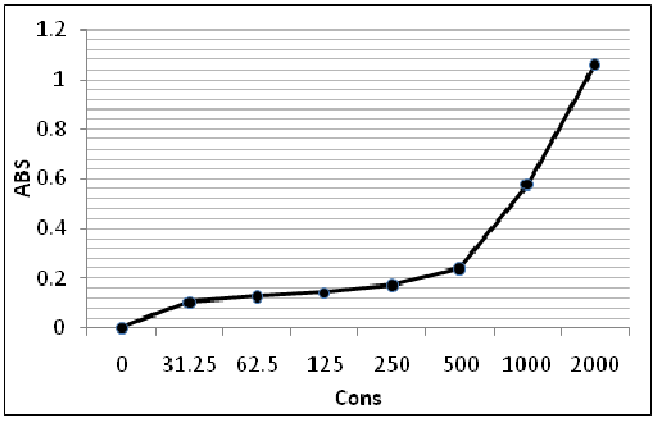


Figure 13: The standard curve of MIR17HG protein by ELISA.

V. Histopathological examination:

Core liver biopsies obtained from patients with chronic HCV infection were fixed in 10% formalin, embedded in paraffin, sectioned (5μm) and stained with hematoxylin-eosin and trichrome stains for histopathological diagnosis and for assessment of histological activity grade (A0-A3) and fibrosis stage (F0-F4) according to the METAVIR scoring system.[76] In addition, the grade of steatosis was determined by estimating the approximate amount of parenchyma involved as follows: 0 = absent; 1 = mild, less than one third; 2 = moderate, one third to two thirds and 3 = marked, more than two thirds.[331]

Immunohistochemistry for PTEN and NF-κB:

Immunohistochemical staining of formalin-fixed paraffin-embedded tissue sections was performed applying the streptavidin-biotin-peroxidase method. The UltraVision LP detection system (Thermo Fisher Scientific, Fremont, CA, USA) was used. The tissue sections (5μm-thick) were placed on positively-charged glass slides, dewaxed in xylene, rehydrated in graded alcohols and then placed in a humidified chamber, with 3% hydrogen peroxide for 20 minutes to inhibit endogenous peroxidase activity. Antigen retrieval was performed by placing the slides in citrate buffer (0.01 mol/l, pH 6.0) in a 700W microwave oven for 8 minutes. Slides were allowed to cool to room temperature, and then an ultra V block was applied for 3–5 minutes to block nonspecific background staining. Then, tissue sections were incubated with primary antibodies at 4°C overnight in a humid chamber.

The following primary Abs were applied:

- *Anti-PTEN / MMAC1 Ab-4 (Clone 17.A)* - mouse monoclonal Ab (Thermo Fisher Scientific, Fremont, CA, USA) at a dilution 1:100.
- *Anti-NF-κB / p65 Ab* - rabbit polyclonal Ab (Thermo Fisher Scientific, Fremont, CA, USA) at a dilution 1:100.

Slides were then incubated with biotinylated goat anti-polyvalent (linking reagent) for 20 minutes at room temperature, followed by peroxidase-conjugated streptavidin, again for 20 minutes at room temperature. Tissue sections were washed with phosphate buffered saline for 5 minutes after each step. A brown color reaction was developed by using DAB (3-3′ diaminobenzidine tetrahydrochloride). The slides were finally dehydrated, counterstained with hematoxylin and mounted. Negative control sections (where the primary antibody has been omitted), were included in each run.

***Evaluation of PTEN immunohistochemical staining*:**

Positive PTEN immunostaining was scored as follows: score 0: negative staining; score 1: weakly positive staining; score 2: moderately positive staining; and score 3: strongly positive staining.[332]

***Evaluation of NF-κB immunostaining*:**

Positive NF-κB expression was defined as nuclear staining, which could be easily identified at low-power magnification (<=100x). Based on intensity of intranuclear staining, the level of NF-κB intranuclear expression was categorized into four grades: score 0: negative staining; score 1: weakly positive staining; score 2: moderately positive

staining; and score 3: strongly positive staining. The expression of NF-κB was further divided into: high expression (score 2 or 3) and low expression (score 0 or 1). Only high expression of NF-κB with nuclear translocation in cells was considered as constitutive NF-κB activation.[333]

Statistical Analysis

Data were collected and entered into the personal computer. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS version 20.0) software. The data were expressed as Mean ± Standard deviation (SD) or proportions.

- Comparison between two means was performed using Student's *t* test for normally distributed quantitative variables or by the non-parametric Mann-Whitney *U*-test for abnormally distributed quantitative variables.
- The Kruskal Wallis test was used for comparison between means of three groups.
- Comparison between proportions was determined by the Chi square (χ^2) test.
- Correlations between variables were analyzed by using Pearson's correlation coefficient or Spearman's rank test as appropriate.
- Statistical significance was assessed at $P < 0.05$. All calculated P values were two-tailed.
- The sensitivity and specificity of plasma *MIR17HG* protein levels in discriminating patients with chronic HCV infection from healthy subjects, patients with CHC from patients with cirrhosis and patients with early fibrosis (METAVIR F1 or F2) from patients with advanced fibrosis (METAVIR F3 or F4) were assessed by plotting a receiver-operating characteristic (ROC) curve and determining its cut-off value.

RESULTS

I. Clinical evaluation:

The clinical characteristics of patients with chronic HCV infection are shown in tables I and II. The apparent duration of disease ranged between 1-36 months (mean ± SD = 11.30 ± 10.34 months). The possible risk factors for HCV infection were previous surgery in 6 (20.0%) patients, dental procedure in 5 (16.7%) patients, familial contact in 3 (10.0%) patients and undefined in 16 (53.3%) patients. The presenting symptoms were right hypochondrial pain in 16 (53.3%) patients while none of the patients presented with jaundice, ascites, or previous GI bleeding.

II. Abdominal ultrasonography:

Ultrasonographic examination in patients with chronic HCV infection showed that the liver was enlarged in 6 (20.0%) patients, normal in 18 (60.0%) patients and shrunken in 6 (20.0%) patients. The liver echopattern was bright in 6 (20.0%) patients, normal in 14 (46.7%) patients and coarse in 10 (33.3%) patients. Splenomegaly was found in the 12 (40.0%) patients with cirrhosis (Tables I and II).

III. Laboratory evaluation:

The laboratory data of patients with chronic HCV infection and healthy subjects are presented in tables III and IV respectively. Statistical comparisons between the two groups are shown in table V.

1. Complete blood picture:

The hemoglobin (Hb) concentration, total leukocyte count and platelet count ranged between 9.4-15.7 g/dl, 3.2-9.7 $x10^3$/cmm and 86.9-315.0 $x10^3$/cmm respectively in patients with chronic HCV infection (Table III), while in healthy subjects, they ranged between 12.5-16.0 g/dl, 4.5-9.5 $x10^3$/cmm and 150-420 $x10^3$/cmm respectively (Table IV). The mean Hb concentration and total leukocyte count were not significantly different between patients with chronic HCV infection and healthy subjects (13.49 ± 1.43 g/dl vs 14.27 ± 1.06 g/dl, $P = 0.068$ and 5.94 ± 1.94 $x10^3$/cmm vs 6.89 ± 1.5 $x10^3$/cmm, $P = 0.105$ respectively). The mean platelet count showed a significant decrease in patients with chronic HCV infection compared with that in healthy subjects (187.46 ± 64.83 $x10^3$/cmm vs 282.60 ± 76.68 $x10^3$/cmm, $P < 0.001$) (Table V).

2. Liver test profile:

a. Serum liver enzymes:

The serum levels of AST, ALT and GGT in patients with chronic HCV infection ranged between 22-183 U/L, 27-223 U/L and 13-88 U/L respectively (Table III) while normal values ranged between 17-23 U/L, 18-24 U/L and 19-28 U/L respectively (Table IV). The mean serum levels of AST, ALT and GGT were significantly higher in patients with chronic HCV infection than in healthy subjects (61.53 ± 37.96 U/L vs 20.4 ± 1.80 U/L, P < 0.001; 76.63 ± 43.88 U/L vs 20.47 ± 1.96 U/L, P < 0.001 and 36.63 ± 16.76 U/L vs 23.00 ± 2.51 U/L, P = 0.003 respectively) (Table V).

b. Serum albumin:

The serum albumin concentration ranged between 3.0-4.4 g/dl in patients with chronic HCV infection (Table III), while it ranged between 3.5-4.2 g/dl in healthy subjects (Table IV). The mean serum albumin concentration was not significantly different between patients with chronic HCV infection and healthy subjects (3.83 ± 0.40 g/dl vs 3.86 ± 0.21 g/dl, P = 0.785) (Table V).

c. Serum bilirubin:

The serum bilirubin levels ranged between 0.3-1.8 mg/dl in patients with chronic HCV infection (Table III) while the normal values ranged between 0.3-1.0 mg/dl (Table IV). The mean serum bilirubin level was not significantly different between patients with chronic HCV infection and healthy subjects (0.90 ± 0.45 mg/dl vs 0.72 ± 0.19 mg/dl, P = 0.146) (Table V).

d. Prothrombin time:

The PT ranged between 11.0-15.6 seconds in patients with chronic HCV infection (Table III), while normal values ranged between 10.0-13.0 seconds (Table IV). The mean PT showed a significant increase in patients with chronic HCV infection compared with that in healthy subjects (12.73 ± 1.08 seconds vs 11.70 ± 0.86 seconds, *P* = 0.002) (Table V).

3. Serum HCV-RNA Level:

The serum HCV-RNA level in patients with chronic HCV infection ranged between 5.02-7400.00 $x10^3$ IU/ml (mean ± SD = 1333.52 ± 1752.40 $x10^3$ IU/ml) (Tables III and V).

IV. Plasma microRNA-17 host gene protein levels:

The plasma *MIR17HG* protein levels ranged between 19.0-588.0 pg/ml in patients with chronic HCV infection, while the normal values ranged between 17.2- 26.2 pg/ml. Patients with chronic HCV infection showed a significant increase in the mean plasma *MIR17HG* protein levels compared with healthy subjects (139.69 ± 172.92 pg/ml vs 21.36 ± 2.89 pg/ml; *P* = 0.012) (Table VI, Figure 14).

By plotting ROC curve, the sensitivity and specificity of the plasma *MIR17HG* protein levels in discriminating patients with chronic HCV infection from healthy subjects were 83.3% and 100% respectively [Area under the curve (AUC) = 0.957] at a cut-off level of 26.6 pg/ml (Figure 15).

V. Histopathological examination:

The histopathological findings and immunohistochemical analysis of liver biopsies in patients with chronic HCV infection were shown in tables VII - X.

Histopathological features consistent with chronic HCV infection were observed in all liver specimens including the presence of lymphoid aggregation in portal tracts, variable degrees of steatosis and mild bile duct injury. According to METAVIR scoring system, 4 (13.3%) patients showed mild activity (A1), 15 (50.0%) patients showed moderate activity (A2) and 11 (36.7%) patients showed severe activity (A3). As regards fibrosis stage, 3 (10.0%) patients showed portal fibrosis without septa (F1), 11 (36.7%) patients showed portal fibrosis with rare septa (F2) and 4 (13.3%) patients showed

numerous septa without cirrhosis (F3) and 12 (40.0%) patients showed cirrhosis (F4). Steatosis was absent in 12 (40.0%) patients, mild in 8 (26.7%) patients, moderate in 7 (23.3%) patients and marked in 3 (10.0%) patients (Tables VII and VIII).

Immunostaining of phosphatase and tensin homolog (PTEN):

Positive immunostaining for PTEN was detectable in the hepatocytes in all patients with chronic HCV infection mainly as cytoplasmic staining and also as nuclear staining in some patients. Out of the 30 patients, 7 (23.3%) patients showed weakly positive staining, 11 (36.7%) patients showed moderately positive staining and 12 (40.0%) patients showed strongly positive staining (Tables IX and X, Figure 16-19).

Immunostaining of nuclear factor-kappa B (NF-κB):

Positive nuclear immunostaining for NF-κB was detectable in 27 (90%) patients with chronic HCV infection; 5 (16.7%) patients showed weakly positive staining, 16 (53.3%) patients showed moderately positive staining and 6 (20.0%) patients showed strongly positive staining (Tables IX and X, Figures 16,20,21).

VI. Statistical correlations:

Statistical correlations between plasma *MIR17HG* protein levels, intrahepatic expression of PTEN and NF-κB and other parameters in patients with chronic HCV infection are presented in Table XI and showed the following results:

- No statistically significant correlations were found between plasma *MIR17HG* protein levels, intrahepatic expression of PTEN and NF-κB on one hand and age, apparent duration of HCV infection and serum HCV RNA levels on the other hand ($P > 0.05$).
- The plasma *MIR17HG* protein levels showed positive correlations with serum levels of AST ($r = 0.445$, $P = 0.014$) and ALT ($r = 0.385$, $P = 0.036$) (Figure 22), METAVIR histological activity grade ($r = 0.549$, $P = 0.002$) and fibrosis stage ($r = 0.886$, $P < 0.001$) (Figure 23) and steatosis grade ($r = 0.565$, $P = 0.001$; Figure 24).
- There was no significant correlation between plasma *MIR17HG* protein levels and serum GGT levels ($r = 0.036$, $P = 0.850$).
- The plasma *MIR17HG* protein levels were inversely correlated with intrahepatic PTEN expression ($r = -0.536$, $P = 0.002$; Figure 25) and positively correlated with intrahepatic NF-κB expression ($r = 0.449$, $P = 0.013$; Figure 26).
- The intrahepatic PTEN expression showed significant inverse correlations with serum GGT levels ($r = -0.454$, $P = 0.012$), METAVIR histological activity grade ($r = -0.459$, $P = 0.011$; Figure 27) and fibrosis stage ($r = -0.530$, $P = 0.003$; Figure 28) and steatosis grade ($r = -0.591$, $P = 0.001$; Figure 29).
- There were no significant correlations between intrahepatic PTEN expression and the serum levels of AST ($r = -0.269$, $P = 0.150$) and ALT ($r = -0.232$, $P = 0.216$).
- The intrahepatic NF-κB expression showed positive correlations with serum levels of AST ($r = 0.469$, $P = 0.009$; Figure 30) and ALT ($r = 0.489$, $P = 0.006$; Figure 31), METAVIR histological activity grade ($r = 0.408$, $P = 0.025$; Figure 32) and fibrosis stage ($r = 0.509$, $P = 0.004$; Figure 33) and steatosis grade ($r = 0.477$, $P = 0.008$; Figure 34).

- There was no significant correlation between intrahepatic NF-κB expression and serum GGT levels (r = -0.021, *P* = 0.913).
- The intrahepatic PTEN expression showed an inverse correlation with intrahepatic NF-κB expression (r = -0.489, *P* = 0.006; Figure 35).

Statistical comparisons between patients with chronic hepatitis C and patients with cirrhosis:

Plasma microRNA-17 host gene protein levels:

The plasma *MIR17HG* protein levels ranged between 19.0-85.4 pg/ml in patients with CHC and between 77.8-588.0 pg/ml in patients with cirrhosis. The mean plasma *MIR17HG* protein levels were significantly higher in patients with CHC and patients with cirrhosis than in healthy subjects (34.48 ± 14.95 pg/ml and 297.50 ± 182.21 pg/ml vs 21.36 ± 2.89 pg/ml; *P* = 0.001 and *P* < 0.001 respectively) and in patients with cirrhosis than in patients with CHC (*P* < 0.001) (Table XII, Figure 36).

By plotting ROC curve, the sensitivity and specificity of the plasma *MIR17HG* protein levels in discriminating patients with cirrhosis from patients with CHC were 100% and 88.9% respectively at a cut-off level of 45.5 pg/ml (AUC = 0.995) (Figure 37) and in discriminating patients with early fibrosis (METAVIR F1 or F2) from patients with advanced fibrosis (METAVIR F3 or F4) were 93.8% and 92.9% respectively at a cut-off level of 40.3 pg/ml (AUC = 0.978) (Figure 38).

Immunostaining of phosphatase and tensin homolog (PTEN):

In patients with CHC, the immunostaining of PTEN was weakly positive in one (5.6%) patient, moderately positive in 7 (38.9%) patients and strongly positive in 10 (55.6%) patients. In patients with cirrhosis, the immunostaining of PTEN was weakly positive in 6 (50.0%) patients, moderately positive in 4 (33.3%) patients and strongly positive in 2 (16.7%) patients. Patients with cirrhosis showed a significant decrease in intrahepatic PTEN expression compared with patients with CHC (*P* = 0.012) (Table XIII, Figures 39).

Immunostaining of nuclear factor-kappa B (NF-κB):

The immunostaining of NF-κB in patients with CHC was negative in 2 (11.1%) patients, weakly positive in 5 (27.8%) patients, moderately positive in 9 (50.0%) patients and strongly positive in 2 (11.1%) patients. In patients with cirrhosis, the immunostaining of NF-κB was negative in one (8.3%) patient, moderately positive in 7 (58.3%) patients and strongly positive in 4 (33.3%) patients. There was no statistically significant difference between patients with CHC and patients with cirrhosis as regards intrahepatic NF-κB expression (*P* = 0.154) (Table XIII, Figure 40).

Table I: Clinical and ultrasonographic data of patients with chronic hepatitis C virus (HCV) infection.

Patient No.	Age (years)	Sex	Apparent duration of HCV infection (months)	Possible Risk factors of HCV infection	RHP	Jaundice	Ascites	HE	Previous GI bleeding	Liver size	Liver echopattern	Enlarged spleen
1	40	M	2	Previous surgery	-	-	-	-	-	Normal	Coarse	+
2	31	M	7	-	-	-	-	-	-	Enlarged	Normal	-
3	54	M	10	-	-	-	-	-	-	Normal	Bright	+
4	56	M	6	Dental procedure	+	-	-	-	-	Normal	Coarse	+
5	25	M	1	-	-	-	-	-	-	Normal	Bright	-
6	49	M	9	Familial contact	-	-	-	-	-	Shrunken	Coarse	+
7	38	M	3	-	+	-	-	-	-	Normal	Bright	-
8	45	M	4	Dental procedure	+	-	-	-	-	Shrunken	Coarse	+
9	20	M	2	-	+	-	-	-	-	Normal	Normal	-
10	43	M	9	Previous surgery	+	-	-	-	-	Normal	Coarse	+
11	47	M	5	Dental procedure	+	-	-	-	-	Normal	Normal	-
12	38	M	2	-	-	-	-	-	-	Normal	Normal	-
13	37	M	36	-	-	-	-	-	-	Enlarged	Normal	-
14	56	M	24	Previous surgery	-	-	-	-	-	Normal	Bright	+
15	47	F	12	-	+	-	-	-	-	Normal	Normal	-
16	47	F	7	Dental procedure	+	-	-	-	-	Enlarged	Normal	-
17	50	F	10	Previous surgery	+	-	-	-	-	Normal	Normal	-

Table I (Continued): Clinical and ultrasonographic data of patients with chronic hepatitis C virus (HCV) infection.

Patient No.	Age (years)	Sex	Apparent duration of HCV infection (months)	Possible Risk factors of HCV infection	RHP	Jaundice	Ascites	HE	Previous GI bleeding	Liver size	Liver echopattern	Enlarged spleen
18	38	M	3	-	-	-	-	-	-	Shrunken	Coarse	+
19	36	M	12	-	-	-	-	-	-	Shrunken	Coarse	+
20	40	F	18	Dental procedure	-	-	-	-	-	Normal	Normal	-
21	23	F	6	Previous surgery	-	-	-	-	-	Normal	Normal	-
22	51	F	24	Familial contact	+	-	-	-	-	Normal	Normal	-
23	42	M	36	-	-	-	-	-	-	Shrunken	Coarse	+
24	40	F	6	-	+	-	-	-	-	Enlarged	Bright	-
25	45	F	12	Previous surgery	+	-	-	-	-	Enlarged	Bright	-
26	54	F	18	-	+	-	-	-	-	Normal	Normal	-
27	44	M	12	-	-	-	-	-	-	Shrunken	Coarse	+
28	44	F	3	-	+	-	-	-	-	Normal	Normal	-
29	46	F	36	Familial contact	+	-	-	-	-	Normal	Normal	-
30	42	M	4	-	+	-	-	-	-	Enlarged	Coarse	+

+ = Present; - = Absent; M = Male; F = Female; RHP = Right hypochondrial pain; HE = Hepatic encephalopathy; GI = Gastrointestinal.

Table II: Mean ± SD and frequencies of clinical and ultrasonographic data of patients with chronic hepatitis C virus (HCV) infection.

Parameters	Patients with HCV infection (n = 30)
Age (years): - Range - Mean ± SD	 20 - 56 42.27 ± 9.03
Sex: - Male (%) - Female (%)	 19 (63.3) 11 (36.7)
Apparent duration of HCV infection (months): - Range - Mean ± SD	 1 - 36 11.30 ± 10.34
Possible risk factors: - Previous surgery (%) - Dental procedure (%) - Familial contact (%) - Undefined (%)	 6 (20.0) 5 (16.7) 3 (10.0) 16 (53.3)
Right hypochondrial pain (%)	16 (53.3)
Jaundice (%)	0 (0.0)
Ascites (%)	0 (0.0)
Previous GI bleeding (%)	0 (0.0)
Liver size: - Enlarged (%) - Normal (%) - Shrunken (%)	 6 (20.0) 18 (60.0) 6 (20.0)
Liver echopattern: - Bright (%) - Normal (%) - Coarse (%)	 6 (20.0) 14 (46.7) 10 (33.3)
Splenomegaly (%)	12 (40.0)

Table III: Laboratory and virological data of patients with chronic hepatitis C virus (HCV) infection.

Patient No.	Hb (g/dl)	TLC ($x10^3$/cmm)	Platelet count ($x10^3$/cmm)	Serum AST (U/L)	Serum ALT (U/L)	Serum GGT (U/L)	Serum Albumin (g/dl)	Serum Bilirubin (mg/dl)	PT (seconds)	Serum HCV-RNA ($x10^3$ IU/ml)
1	12.5	3.2	153	35	30	38	4.0	1.4	12.5	190
2	14.1	8.2	209	22	43	30	4.1	0.3	11.0	166
3	12.1	4.5	157	76	114	35	3.1	1.1	12.0	1700
4	13.7	8.5	246	49	81	80	4.0	1.0	15.0	327.66
5	12.5	4.6	210	29	27	19	3.7	0.9	13.3	594
6	12.1	3.5	102	92	97	36	3.0	1.8	12.0	1700
7	14.2	6.5	251	79	95	20	4.3	0.6	12.5	1585.46
8	15.0	5.2	94	183	184	36	3.0	1.8	13.0	2000
9	15.7	4.1	315	43	59	42	4.0	0.7	12.0	189
10	9.4	8.5	154	144	223	40	4.2	0.4	14.0	1300
11	15.5	4.2	141	38	61	17	3.7	1.2	12.0	125
12	15.0	6.0	216	50	70	16	4.3	0.4	11.8	69.6
13	14.0	4.8	156	49	46	31	4.0	0.5	12.0	2200
14	13.5	7.1	210	62	87	42	4.3	1.2	15.6	7400
15	14.7	8.4	286	23	34	28	3.7	0.3	12.0	41.5
16	12.1	5.7	191	33	35	40	4.4	0.8	12.6	5300
17	13.1	3.2	218	55	75	66	3.8	1.0	12.7	4300

Table III (Continued): Laboratory and virological data of patients with chronic hepatitis C virus (HCV) infection.

Patient No.	Hb (g/dl)	TLC ($x10^3$/cmm)	Platelet count ($x10^3$/cmm)	Serum AST (U/L)	Serum ALT (U/L)	Serum GGT (U/L)	Serum Albumin (g/dl)	Serum Bilirubin (mg/dl)	PT (seconds)	Serum HCV RNA ($x10^3$ IU/ml)
18	14.3	7.9	211	61	82	40	4.3	0.7	11.0	3200
19	13.3	4.9	93	37	40	33	3.9	1.3	14.0	267
20	15.3	5.5	171	68	98	13	3.6	0.5	12.0	2563
21	12.0	4.3	201	33	45	88	4.0	0.6	12.8	7.7
22	10.8	4.1	120	45	74	32	4.0	1.1	12.0	1000
23	14.0	8.2	111	71	73	41	3.0	1.8	14.0	1300
24	13.5	9.5	272	143	92	50	3.6	1.5	11.6	59.3
25	15.0	9.7	302	44	51	36	4.0	0.8	12.5	174.4
26	13.0	6.2	260	38	40	23	4.0	0.4	13.0	5.021
27	14.1	3.4	86.9	55	82	34	3.6	0.9	14.3	890
28	12.4	6.1	217	40	37	28	3.7	0.6	12.8	151
29	14.4	5.9	120	46	95	29	3.6	0.6	13.0	1110
30	13.4	6.3	150	103	129	36	4.0	0.76	13.0	90
Range	9.4 - 15.7	3.2 - 9.7	86.9 - 315.0	22 - 183	27 - 223	13 - 88	3.0 - 4.4	0.3 - 1.8	11.0 - 15.6	5.02 -7400
Mean ± SD	13.49 ± 1.43	5.94 ± 1.94	187.46 ± 64.83	61.53 ± 37.96	76.63 ± 43.88	36.63 ± 16.76	3.83 ± 0.40	0.90 ± 0.45	12.73 ± 1.08	1333.52 ± 1752.40

Hb = Hemoglobin concentration; TLC = Total leukocyte count; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyl transpeptidase; PT = Prothrombin time.

Table IV: Laboratory data of healthy subjects.

Subject No.	Hb (g/dl)	TLC ($x10^3$/cmm)	Platelet count ($x10^3$/cmm)	Serum AST (U/L)	Serum ALT (U/L)	Serum GGT (U/L)	Serum albumin (g/dl)	Serum bilirubin (mg/dl)	PT (seconds)
1	14.5	7.0	150	20	22	25	4.0	0.7	12.0
2	13.1	6.5	314	17	20	24	3.8	0.3	11.7
3	15.7	8.5	340	19	19	23	4.0	0.8	12.0
4	14.7	6.0	235	22	18	21	3.7	0.9	11.0
5	16.0	7.4	245	20	19	26	4.0	0.6	11.5
6	13.5	9.4	240	21	18	24	3.5	0.8	12.7
7	15.6	6.5	420	22	21	21	3.9	0.5	13.0
8	14.0	4.5	325	19	22	20	3.8	1.0	12.0
9	13.7	5.5	250	23	18	22	4.0	0.8	11.0
10	15.0	6.0	255	22	24	19	4.2	0.9	10.0
11	12.5	8.0	311	19	23	23	3.6	0.9	11.0
12	13.5	9.5	400	23	20	21	3.5	0.5	12.5
13	13.0	7.5	350	20	22	26	3.9	0.8	12.0
14	14.3	6.0	215	21	19	28	4.0	0.7	10.5
15	15.0	5.0	189	18	22	22	4.0	0.6	12.6
Range	12.5 – 16.0	4.5 - 9.5	150 - 420	17 - 23	18 - 24	19 - 28	3.5 - 4.2	0.3 – 1.0	10.0 – 13.0
Mean ± SD	14.27 ± 1.06	6.89 ± 1.50	282.60 ± 76.68	20.40 ± 1.80	20.47 ± 1.96	23.00 ± 2.51	3.86 ± 0.21	0.72 ± 0.19	11.70 ± 0.86

Hb = Hemoglobin concentration; TLC = Total leukocyte count; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyl transpeptidase; PT = Prothrombin time.

Table V: Statistical comparisons between patients with chronic hepatitis C virus (HCV) infection and healthy subjects as regards laboratory data.

Parameters	Patients with chronic HCV infection (n = 30)	Healthy Subjects (n =15)	*t test*	*P* value
Hb (g/dl)	13.49 ± 1.43	14.27 ± 1.06	1.873	0.068
TLC ($x10^3$/cmm)	5.94 ± 1.94	6.89 ± 1.50	1.657	0.105
Platelet count ($x10^3$/cmm)	187.46 ± 64.83	282.60 ± 76.68	4.365	< 0.001
Serum AST (U/L)	61.53 ± 37.96	20.40 ± 1.80	4.170	< 0.001
Serum ALT (U/L)	76.63 ± 43.88	20.47 ± 1.96	4.926	< 0.001
Serum GGT (U/L)	36.63 ± 16.76	23.00 ± 2.51	3.116	0.003
Serum albumin (g/dl)	3.83 ± 0.40	3.86 ± 0.21	0.274	0.785
Serum bilirubin (mg/dl)	0.90 ± 0.45	0.72 ± 0.19	1.482	0.146
Prothrombin time (seconds)	12.73 ± 1.08	11.70 ± 0.86	3.222	0.002
Serum HCV RNA ($x10^3$ IU/ml)	1333.52 ± 1752.40	-	-	-

Hb = Hemoglobin concentration; TLC = Total leukocyte count; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyl transpeptidase.

Table VI: Plasma *microRNA-17 host gene* (*MIR17HG*) protein levels (pg/ml) in patients with chronic hepatitis C virus (HCV) infection and healthy subjects.

Patient No.	Patients with chronic HCV infection (n = 30)	Healthy Subjects (n =15)
1	549.6	17.2
2	30.2	24.2
3	175.0	21.4
4	103.0	23.0
5	36.4	19.0
6	77.8	19.6
7	44.8	26.2
8	420.0	17.8
9	24.6	24.5
10	243.6	21.9
11	46.2	23.6
12	28.0	20.3
13	32.8	18.6
14	237.0	24.7
15	25.2	18.4
16	34.4	-
17	33.0	-
18	123.0	-
19	225.0	-
20	19.0	-
21	22.2	-
22	85.4	-
23	588.0	-
24	34.0	-
25	31.2	-
26	22.0	-
27	540.0	-
28	27.0	-
29	44.2	-
30	288.0	-
Range	19.0 - 588.0	17.2 - 26.2
Mean ± SD	139.69 ± 172.92	21.36 ± 2.89
	$t = 2.635$, $P = 0.012$	

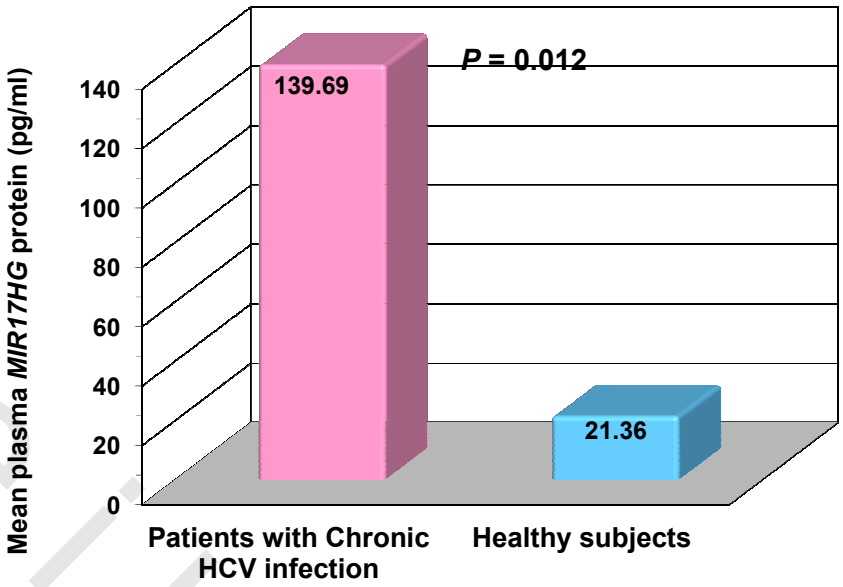


Figure 14: Statistical comparisons between patients with chronic hepatitis C virus infection and healthy subjects as regards plasma *microRNA-17 host gene* (*MIR17HG*) protein levels ($P = 0.012$).

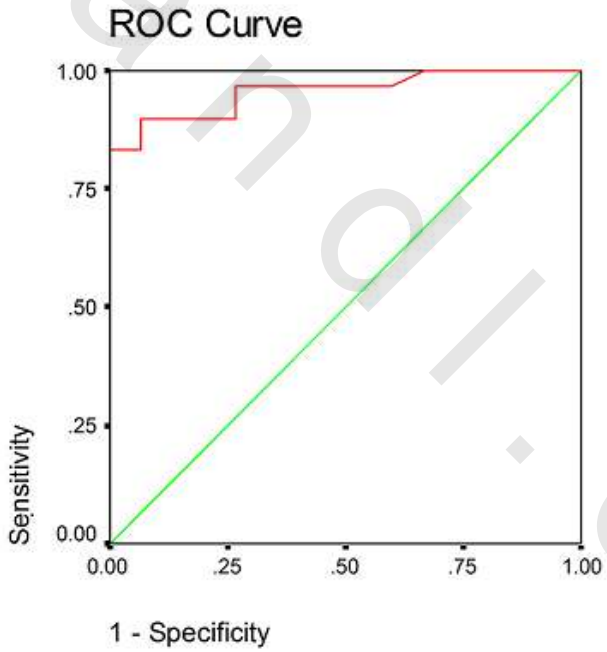


Figure 15: Receiver operating characteristic curve (ROC) shows that the sensitivity and specificity of the plasma *microRNA-17 host gene* (*MIR17HG*) protein levels in discriminating patients with chronic HCV infection from healthy subjects were 83.3% and 100% respectively at a cut-off level of 26.6 pg/ml (Area under the curve = 0.957).

Table VII: Histopathological findings in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.

Patient No.	METAVIR		Steatosis grade
	Histologic activity grade (A)	Fibrosis Stage (F)	
1	A3	F4	2
2	A2	F2	0
3	A1	F4	1
4	A3	F4	2
5	A2	F2	0
6	A3	F4	0
7	A2	F2	0
8	A3	F4	1
9	A1	F1	0
10	A2	F4	3
11	A2	F3	1
12	A3	F2	0
13	A2	F2	1
14	A2	F4	2
15	A2	F2	0
16	A2	F2	0
17	A2	F3	2
18	A3	F4	1
19	A3	F4	2
20	A2	F2	0
21	A1	F1	0
22	A3	F3	1
23	A3	F4	1
24	A2	F2	3
25	A2	F1	2
26	A2	F2	1
27	A3	F4	3
28	A1	F2	0
29	A3	F3	0
30	A2	F4	2

0 = Absent; 1 = Mild; 2 = Moderate; 3 = Marked.

Table VIII: Frequencies of histopathological findings in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.

Parameters	Patients with chronic HCV infection (n = 30)
METAVIR:	
Histologic activity grade :	
– A1 (%)	4 (13.3)
– A2 (%)	15 (50.0)
– A3 (%)	11 (36.7)
Fibrosis stage:	
– F1 (%)	3 (10.0)
– F2 (%)	11 (36.7)
– F3 (%)	4 (13.3)
– F4 (%)	12 (40.0)
Steatosis Grade	
– 0 (%)	12 (40.0)
– 1 (%)	8 (26.7)
– 2 (%)	7 (23.3)
– 3 (%)	3 (10.0)

Table IX: Immunohistochemical staining score of phosphatase and tensin homolog (PTEN) and nuclear factor-kappa B (NF-κB) in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.

Patient No.	PTEN staining score	NF-κB staining score
1	1	2
2	3	1
3	3	0
4	1	2
5	3	1
6	2	2
7	3	2
8	3	2
9	2	0
10	2	3
11	2	3
12	3	2
13	3	2
14	1	3
15	3	1
16	3	2
17	2	2
18	2	2
19	1	2
20	2	3
21	3	1
22	2	2
23	1	2
24	1	2
25	2	1
26	3	2
27	1	3
28	3	0
29	2	2
30	2	3

0 = negative staining; 1= weakly positive staining; 2 = moderately positive staining; 3 = strongly positive staining.

Table X: Frequencies of immunostaining score of phosphatase and tensin homolog (PTEN) and nuclear factor-kappa B (NF-κB) in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.

Parameters	Chronic HCV infection (n = 30)
PTEN staining score:	
– 0 (%)	0 (0.0)
– 1 (%)	7 (23.3)
– 2 (%)	11 (36.7)
– 3 (%)	12 (40.0)
NF-κB staining score:	
– 0 (%)	3 (10.0)
– 1 (%)	5 (16.7)
– 2 (%)	16 (53.3)
– 3 (%)	6 (20.0)

0 = negative staining; 1= weakly positive staining; 2 = moderately positive staining; 3 = strongly positive staining.

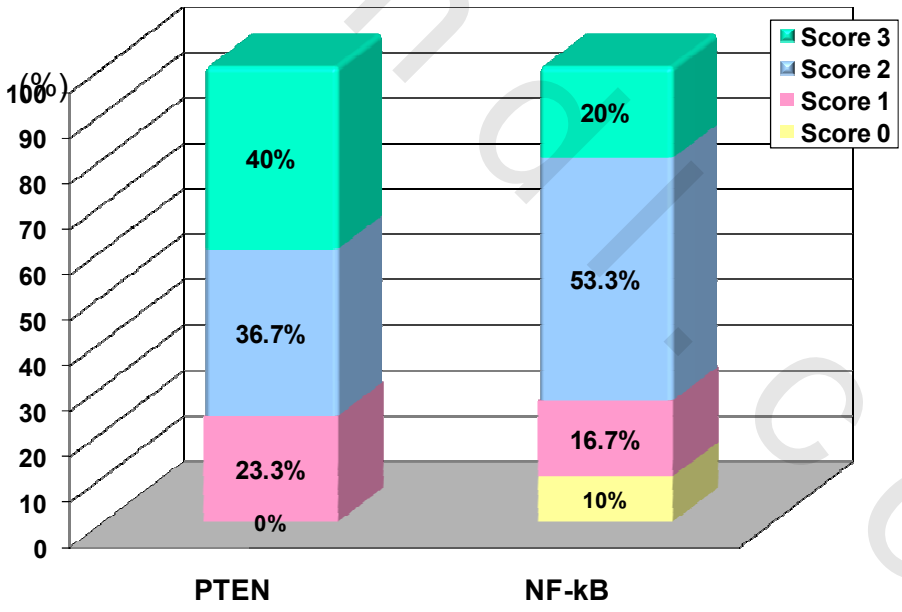


Figure 16: Distribution of immunostaining score of phosphatase and tensin homolog (PTEN) and nuclear factor-kappa B (NF-κB) in liver biopsies of patients with chronic hepatitis C virus (HCV) infection.

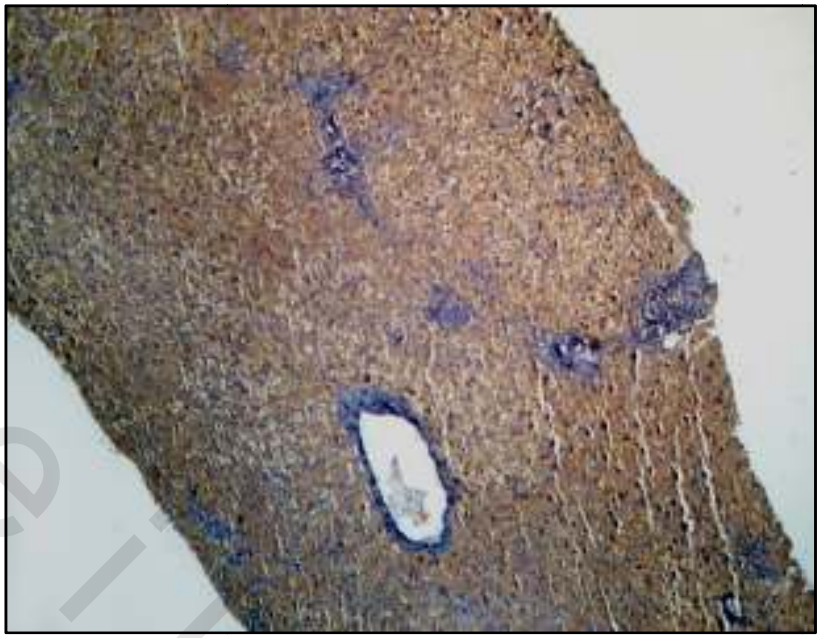


Figure 17: Immunostaining of PTEN in chronic hepatitis C, METAVIR stage F1, with no steatosis. Strong brown positive staining of hepatocytes is seen (staining score 3) (anti-PTEN× 200).

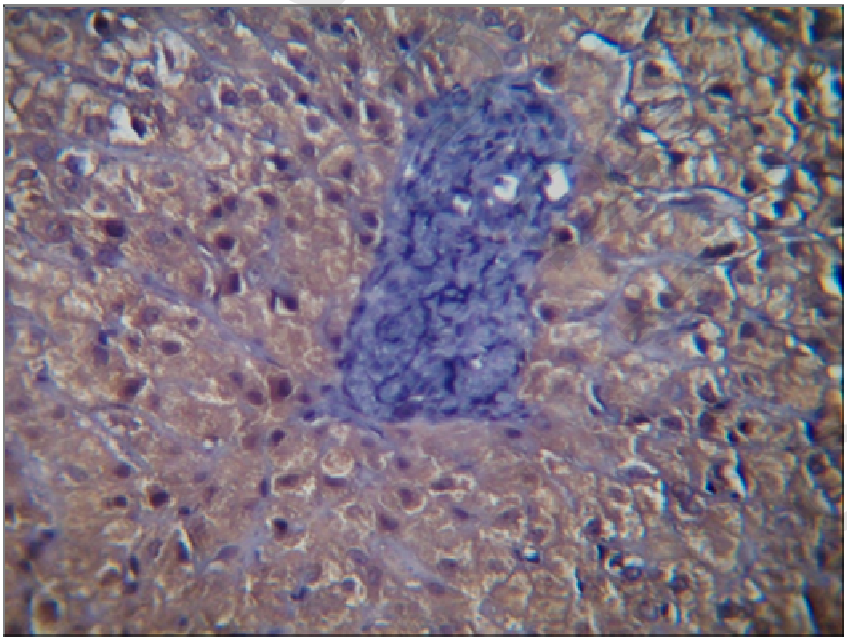


Figure 18: Immunostaining of PTEN in chronic hepatitis C. High power view demonstrating brown cytoplasmic as well as nuclear expression of the stain (anti-PTEN × 400).

Figure 19: Immunostaining of PTEN in hepatitis C virus-related cirrhosis, (METAVIR stage F4) and moderate steatosis, demonstrating weak PTEN expression (staining score 1) (anti-PTEN× 200).

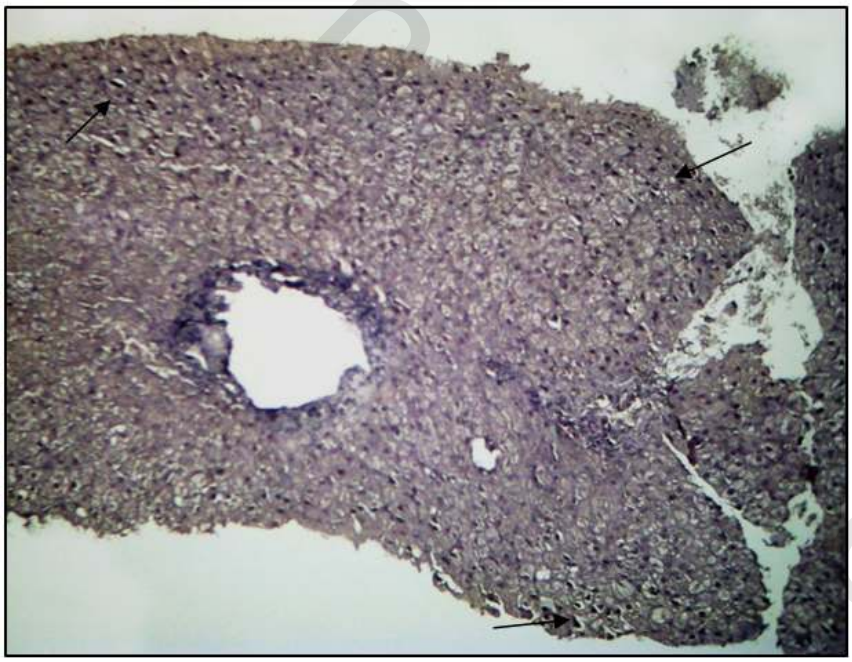


Figure 20: Immunostaining of nuclear factor-kappa B (NF-κB) in chronic hepatitis C. Brown positive nuclear staining is seen in some hepatocytes (arrows) (anti-NF-κB × 200).

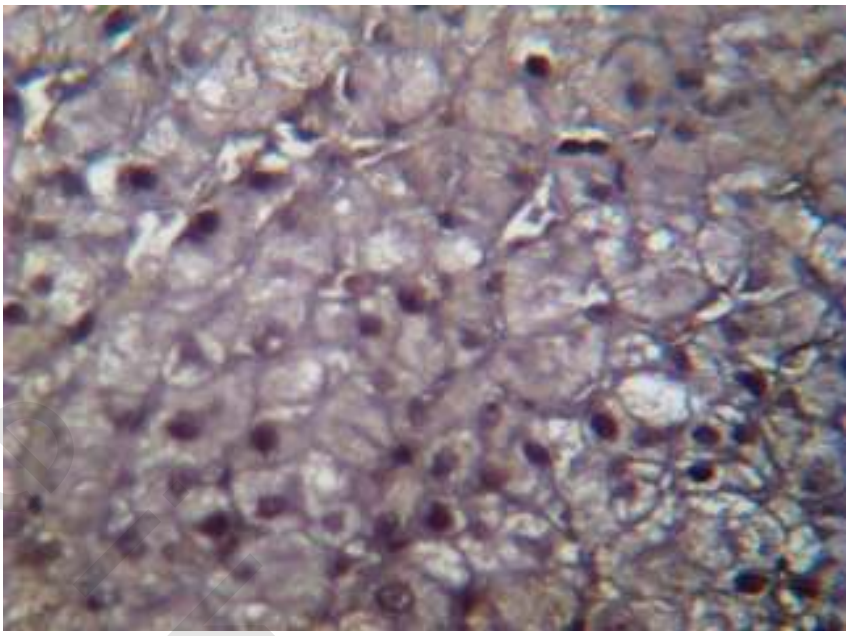


Figure 21: Immunostaining of nuclear factor-kappa B (NF-κB) in chronic hepatitis C. High power view demonstrating brown positive nuclear staining of hepatocytes (anti- NF-κB × 400).

Table XI: Statistical correlations ("r" value) between plasma *microRNA-17 host gene* (*MIR17HG*) protein levels, intrahepatic expression of phosphatase and tensin homolog (**PTEN**) and nuclear factor-kappa B (NF-κB) and other parameters in patients with chronic hepatitis C virus (HCV) infection (n = 30).

Parameters	**Plasma *MIR17HG* protein (pg/ml)**		PTEN staining score		**NF-κB staining score**	
	r value	*P* value	r value*	*P* value	r value*	*P* value
Age (years)	0.124	0.515	-0.147	0.437	0.232	0.218
Apparent duration of HCV infection (months)	0.119	0.530	-0.221	0.240	0.248	0.186
Serum AST (U/L)	0.445	0.014	-0.269	0.150	0.469	0.009
Serum ALT (U/L)	0.385	0.036	-0.232	0.216	0.489	0.006
Serum GGT (U/L)	0.302	0.105	-0.454	0.012	-0.021	0.913
Serum HCV RNA ($x10^3$ IU/ml)	0.036	0.850	-0.118	0.536	0.294	0.115
METAVIR:						
- Histological activity grade*	0.549	0.002	-0.459	0.011	0.408	0.025
- Fibrosis stage*	0.886	< 0.001	-0.530	0.003	0.509	0.004
Steatosis grade*	0.565	0.001	-0.591	0.001	0.477	0.008
PTEN staining score*	-0.536	0.002	-	-	-	-
NF-κB staining score*	0.449	0.013	-0.489	0.006	-	-

AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyl transpeptidase.
* Spearman rho correlation.

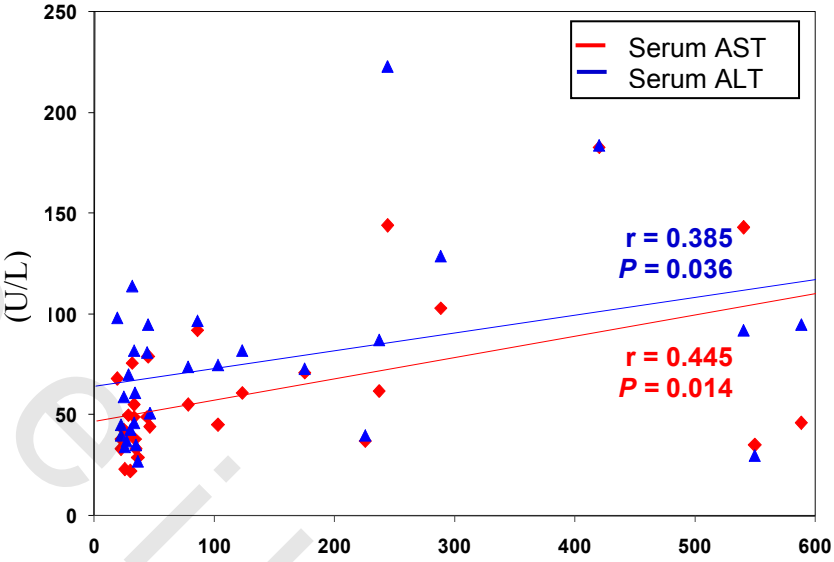


Figure 22: Statistical correlations between plasma *microRNA-17 host gene* (*MIR17HG*) protein levels and serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in patients with chronic hepatitis C virus (HCV) infection ($P = 0.014$ and $P = 0.036$ respectively).

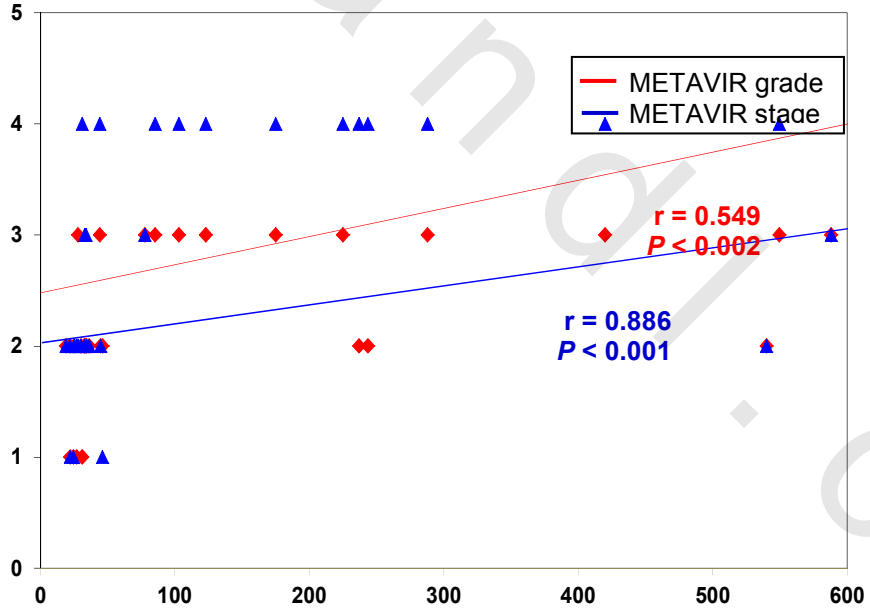


Figure 23: Statistical correlations between plasma microRNA-17 host gene (MIR17HG) protein levels and METAVIR histological activity grade and fibrosis stage in patients with chronic hepatitis C virus (HCV) infection ($P < 0.002$ and $P < 0.001$ respectively).

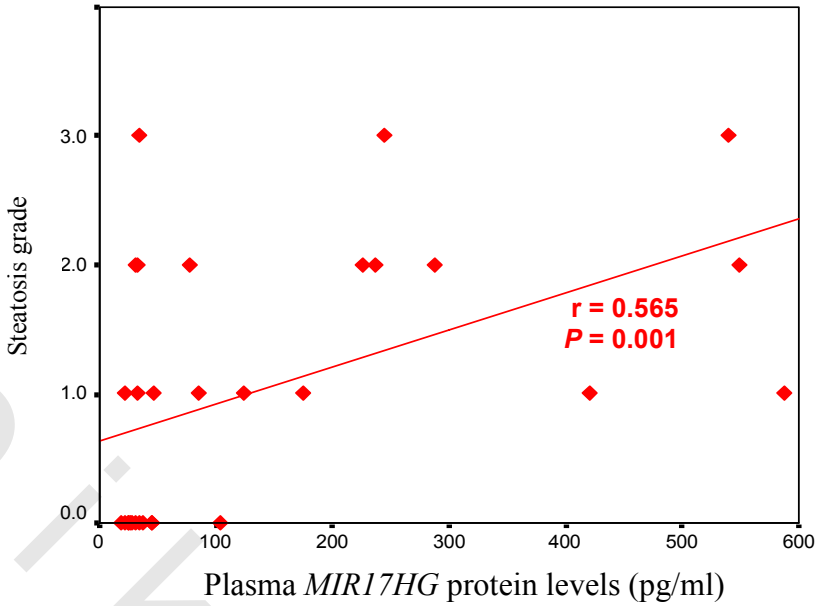


Figure 24: Statistical correlation between plasma *microRNA-17 host gene* (*MIR17HG*) protein levels and steatosis grade in patients with chronic hepatitis C virus (HCV) infection (P = 0.001).

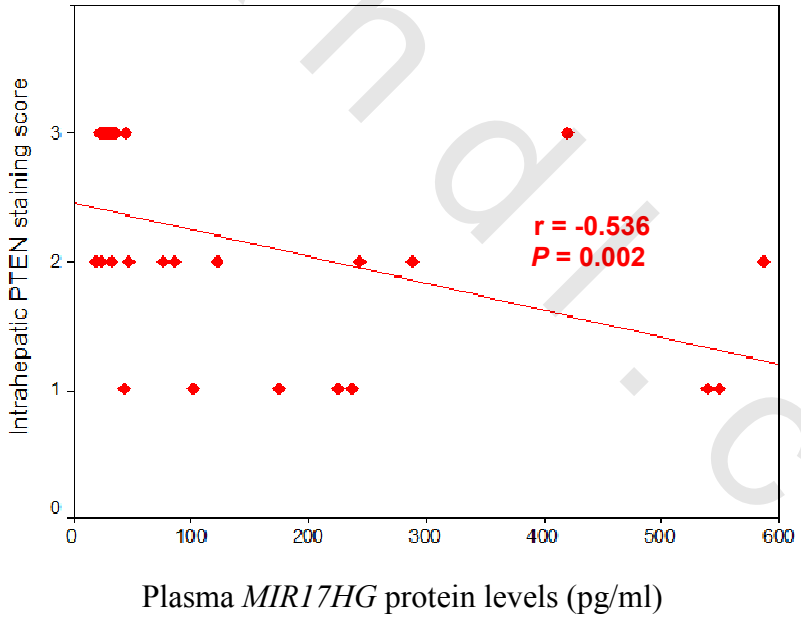


Figure 25: Statistical correlation between plasma *microRNA-17 host gene* (*MIR17HG*) protein levels and intrahepatic phosphatase and tensin homolog (PTEN) expression in patients with chronic hepatitis C virus (HCV) infection (P = 0.002).

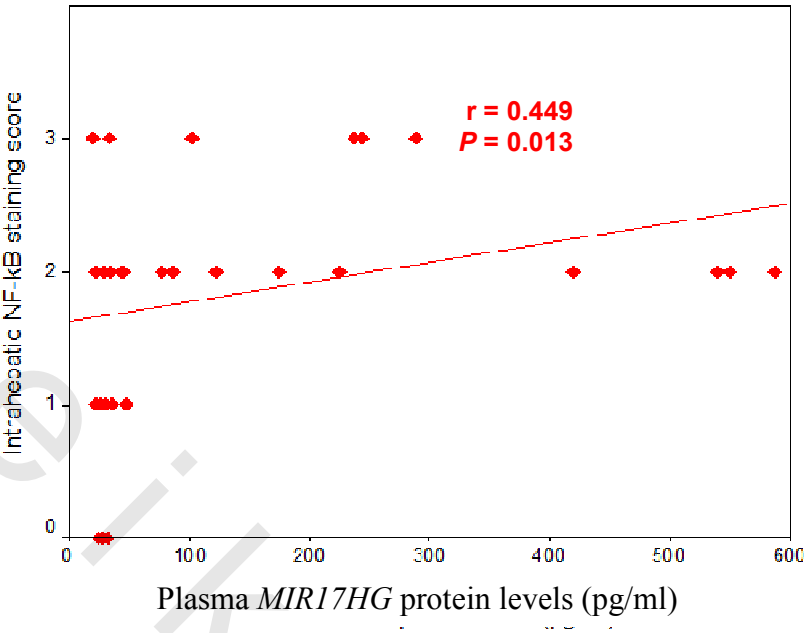


Figure 26: Statistical correlation between plasma *microRNA-17 host gene* (*MIR17HG*) protein levels and intrahepatic nuclear factor-kappa B (NF-κB) expression in patients with chronic hepatitis C virus (HCV) infection (P = 0.013).

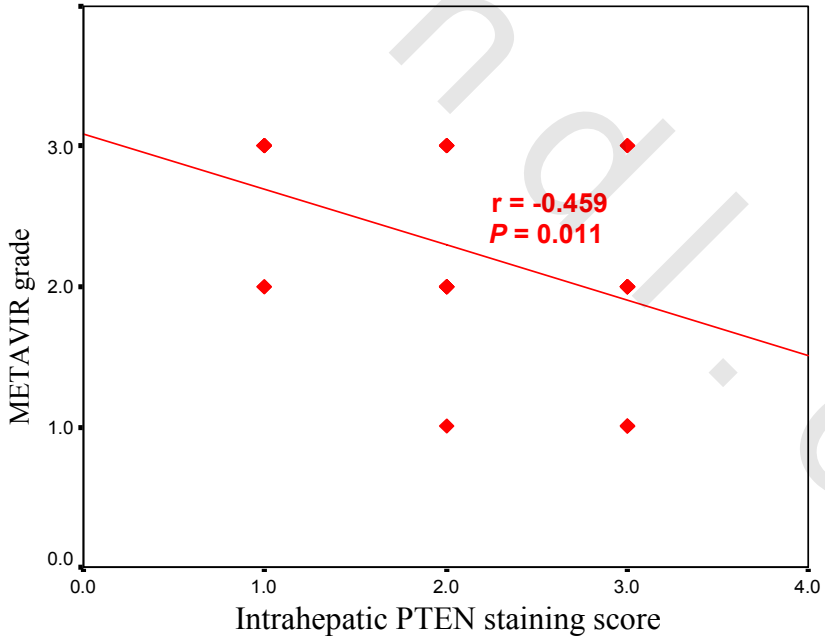


Figure 27: Statistical correlation between intrahepatic phosphatase and tensin homolog (PTEN) expression and METAVIR histological activity grade in patients with chronic hepatitis C virus (HCV) infection (P = 0.011).

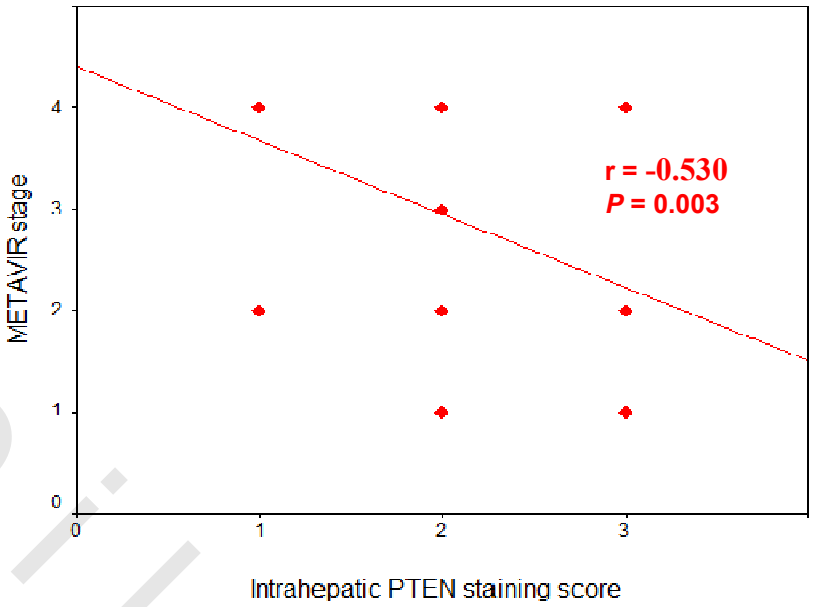


Figure 28: Statistical correlation between intrahepatic phosphatase and tensin homolog (PTEN) expression and METAVIR fibrosis stage in patients with chronic hepatitis C virus (HCV) infection (P = 0.003).

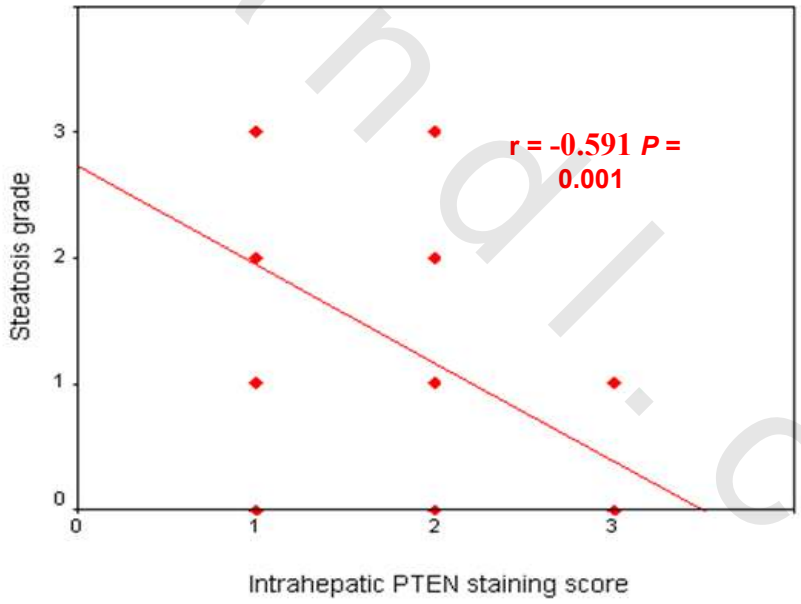


Figure 29: Statistical correlation between intrahepatic phosphatase and tensin homolog (PTEN) expression and steatosis grade in patients with chronic hepatitis C virus (HCV) infection (P = 0.001).

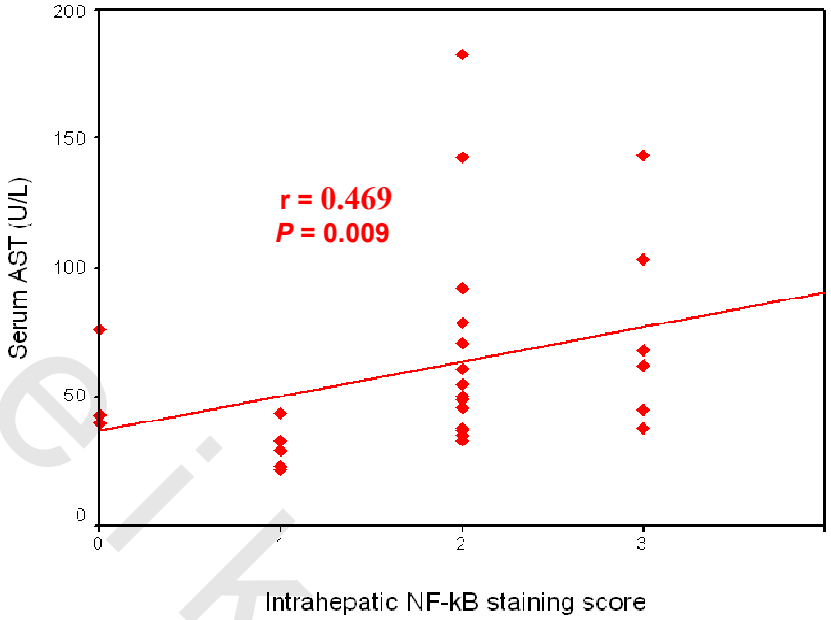


Figure 30: Statistical correlation between intrahepatic nuclear factor-kappa B (NF-κB) expression and serum levels of aspartate aminotransferase (AST) in patients with chronic hepatitis C virus (HCV) infection (P = 0.009).

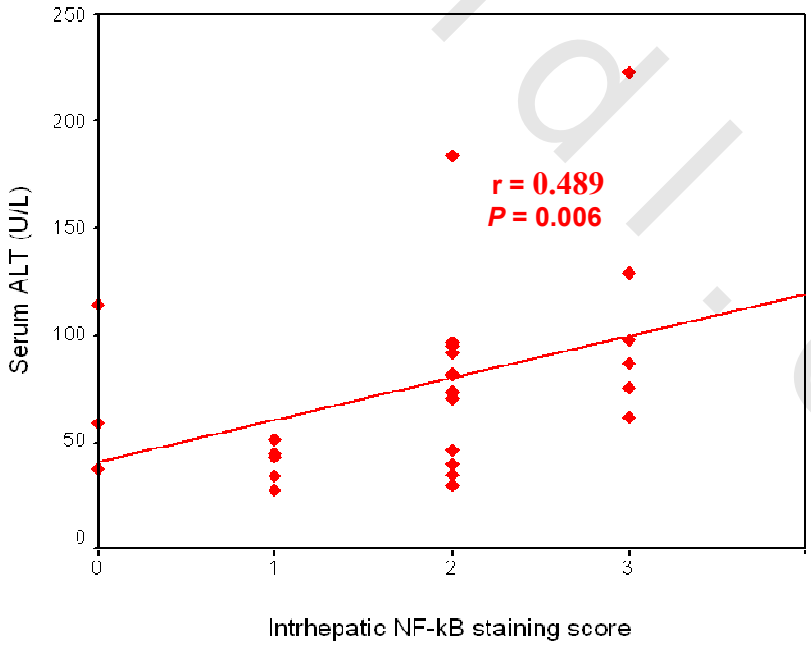


Figure 31: Statistical correlation between intrahepatic nuclear factor-kappa B (NF-κB) expression and serum levels of alanine aminotransferase (ALT) in patients with chronic hepatitis C virus (HCV) infection (P = 0.006).

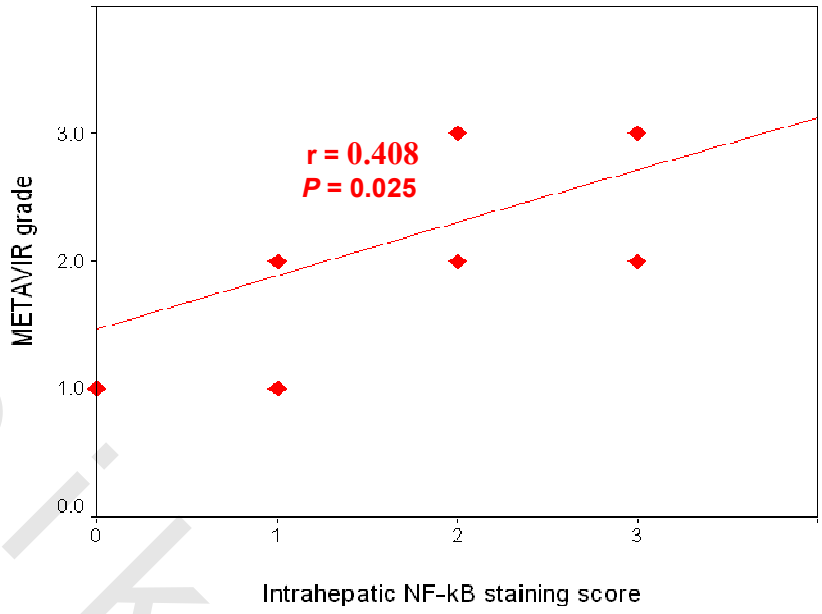


Figure 32: Statistical correlation between intrahepatic nuclear factor-kappa B (NF-κB) expression and METAVIR histological activity grade in patients with chronic hepatitis C virus (HCV) infection (P = 0.025).

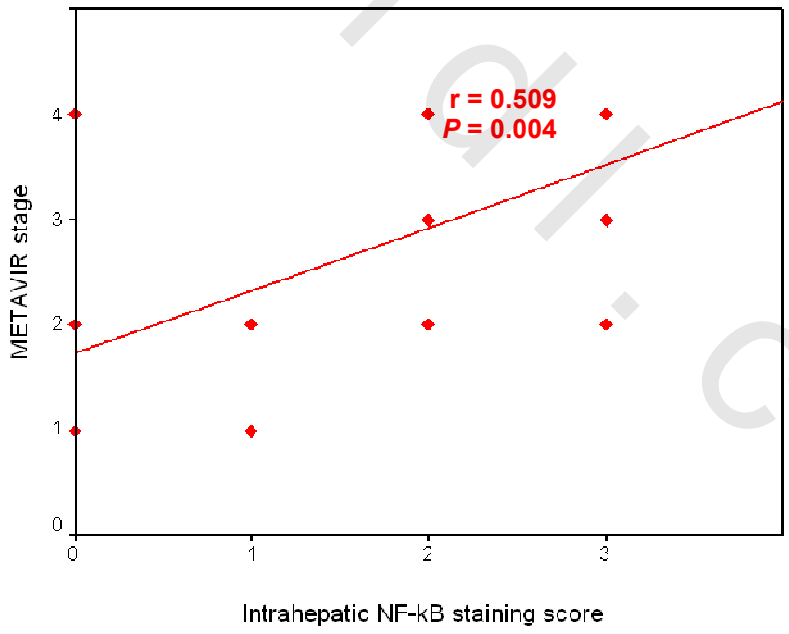


Figure 33: Statistical correlation between intrahepatic nuclear factor-kappa B (NF-κB) expression and METAVIR fibrosis stage in patients with chronic hepatitis C virus (HCV) infection (P = 0.004).

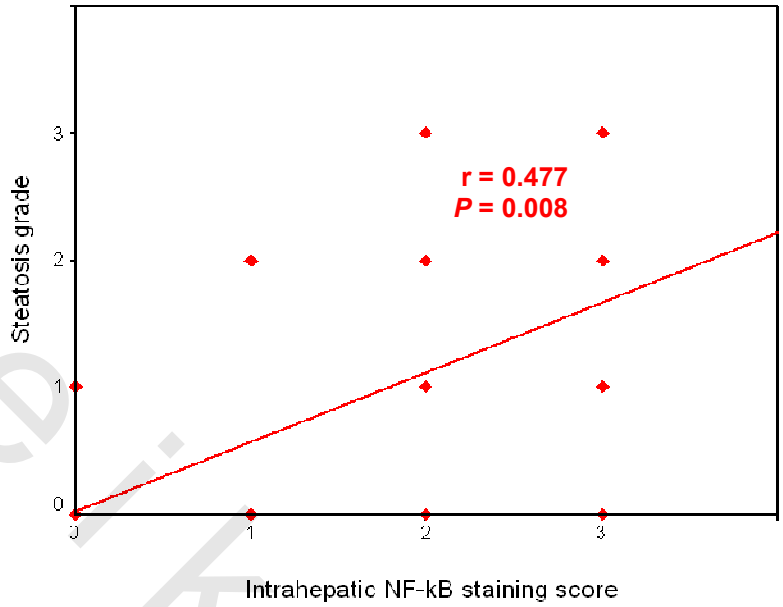


Figure 34: Statistical correlation between intrahepatic nuclear factor-kappa B (NF-κB) expression and steatosis grade in patients with chronic hepatitis C virus (HCV) infection (*P* = 0.008).

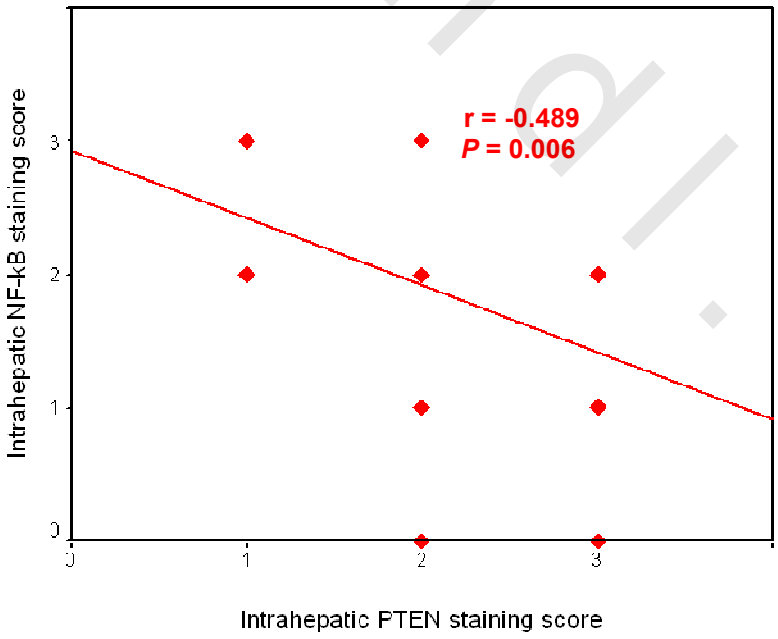


Figure 35: Statistical correlation between intrahepatic expression of phosphatase and tensin homolog (PTEN) and nuclear factor-kappa B (NF-κB) in patients with chronic hepatitis C virus (HCV) infection (*P* = 0.006).

Table XII: Statistical comparisons between patients with chronic hepatitis C (CHC) infection and healthy subjects as regards plasma *microRNA-17 host gene* (*MIR17HG*) protein levels.

Parameters	Patients with CHC (n = 18)	Patients with cirrhosis (n =12)	Healthy Subjects (n =15)	χ^{2*}	*P* value
Plasma *MIR17HG* protein levels (pg/ml): - Range	19.0 - 85.4	77.8 - 588.0	17.2 - 26.2		
- Mean ± SD	34.48 ± 14.95 [ac]	297.50 ± 182.21 [b]	21.36 ± 2.89	35.095	< 0.001

*Kruskal Wallis test.

Mann-Whitney *U* test: a = Significant difference from healthy subjects ($Z = 3.162$, $P = 0.001$); b = Significant difference from healthy subjects ($Z = 4.705$, $P < 0.001$); c = Significant difference from patients with cirrhosis ($Z = 4.530$, $P < 0.001$).

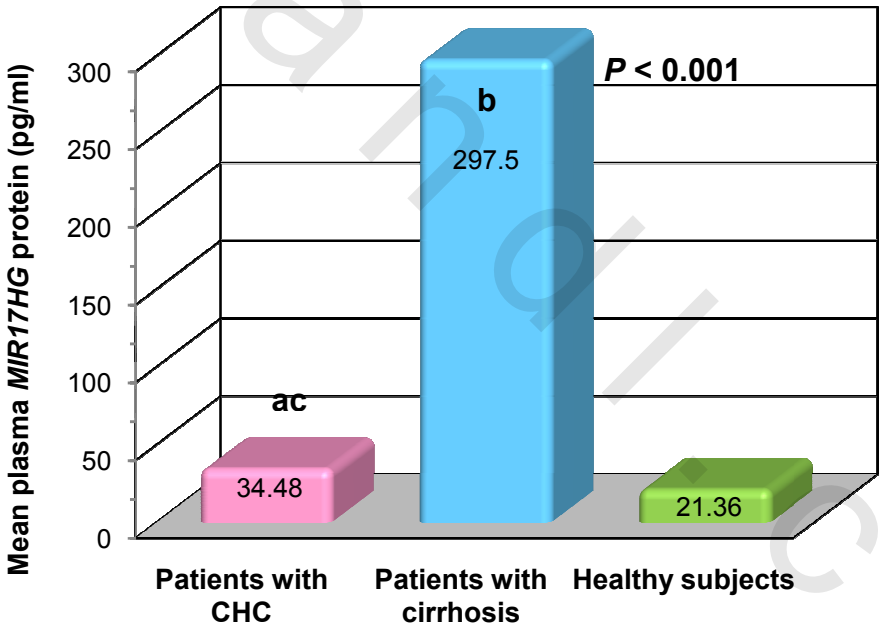


Figure 36: Statistical comparisons between patients with chronic hepatitis C (CHC), patients with cirrhosis and healthy subjects as regards plasma *microRNA-17 host gene* (*MIR17HG*) protein levels ($P < 0.001$). a = Significant difference from healthy subjects ($P = 0.001$); b = Significant difference from healthy subjects ($P < 0.001$); c = Significant difference from patients with cirrhosis ($P < 0.001$); Mann-Whitney *U* test.

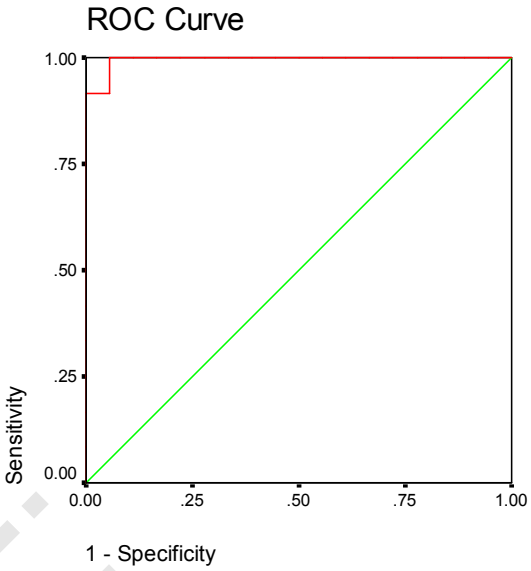


Figure 37: Receiver operating characteristic curve (ROC) shows that the sensitivity and specificity of the plasma *microRNA-17 host gene* (*MIR17HG*) protein levels in discriminating patients with chronic hepatitis C from patients with cirrhosis were 100% and 88.9% respectively at a cut-off level of 45.5 pg/ml (Area under the curve = 0.995).

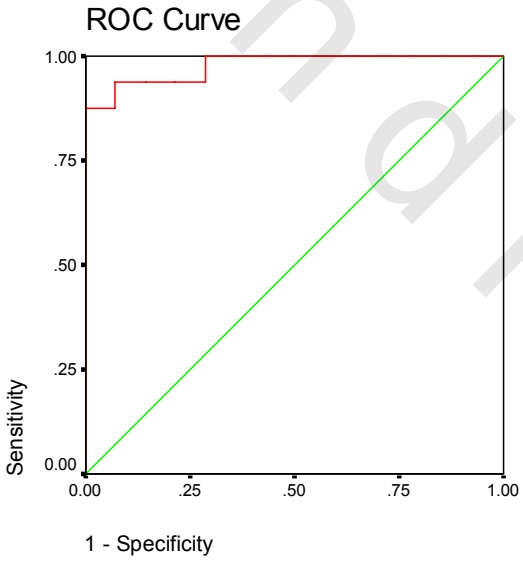


Figure 38: Receiver operating characteristic curve (ROC) shows that the sensitivity and specificity of the plasma *microRNA-17 host gene* (*MIR17HG*) protein levels in discriminating patients with early fibrosis (METAVIR F1 or F2) from patients with advanced fibrosis (METAVIR F3 or F4) were 93.8% and 92.9% respectively at a cut-off level of 40.3 pg/ml (Area under the curve = 0.982).

Table XIII: Statistical comparisons between patients with chronic hepatitis C (CHC) and patients with cirrhosis as regards intrahepatic immunostaining score of phosphatase and tensin homolog (PTEN) and nuclear factor-kappa B (NF-κB).

Parameters	**Patients with CHC** (n = 18)	**Patients with cirrhosis** (n = 12)	χ^2	*P* value
PTEN staining score:				
– 0 (%)	0 (0.0)	0 (0.0)		
– 1 (%)	1 (5.6)	6 (50.0)	8.878	0.012
– 2 (%)	7 (38.9)	4 (33.3)		
– 3 (%)	10 (55.6)	2 (16.7)		
NF-κB staining score:				
– 0 (%)	2 (11.1)	1 (8.3)		
– 1 (%)	5 (27.8)	0 (0.0)	5.260	0.154
– 2 (%)	9 (50.0)	7 (58.3)		
– 3 (%)	2 (11.1)	4 (33.3)		

0 = negative staining; 1= weakly positive staining; 2 = moderately positive staining; 3 = strongly positive staining.

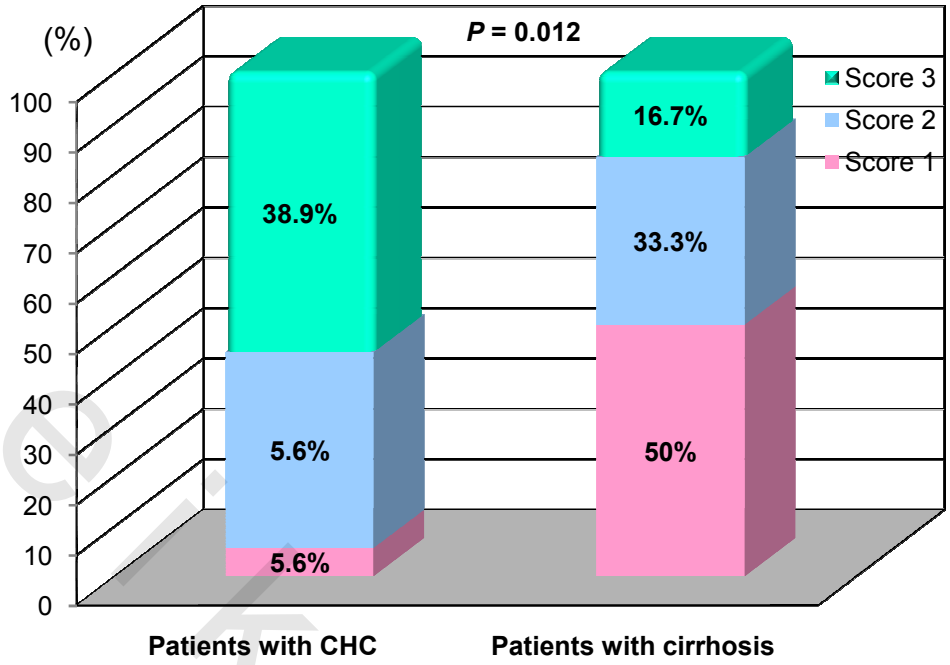


Figure 39: Statistical comparison between patients with chronic hepatitis C (CHC) and patients with cirrhosis as regards intrahepatic immunostaining score of phosphatase and tensin homolog (PTEN) ($P = 0.012$).

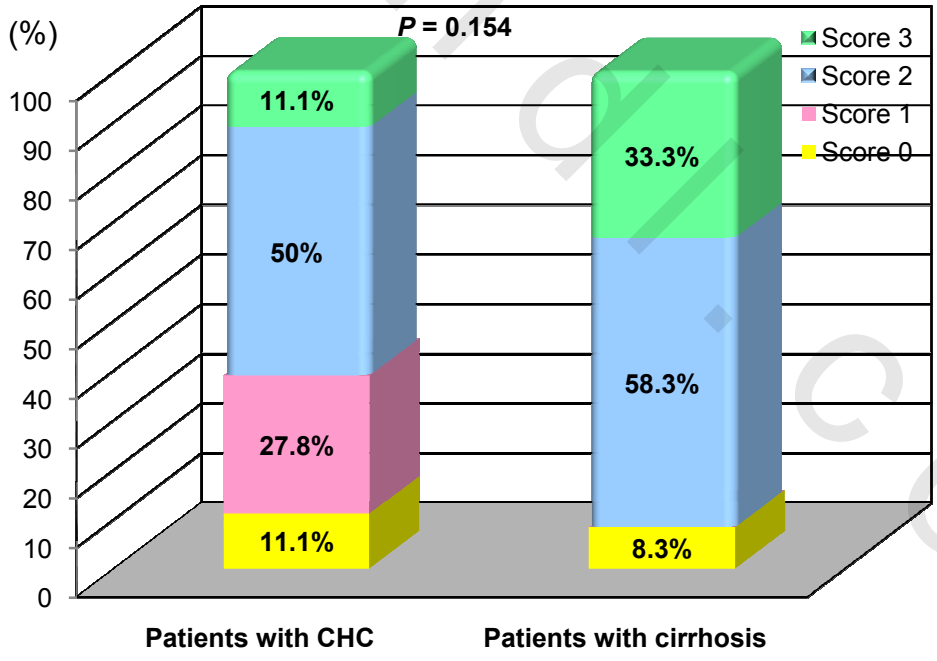


Figure 40: Statistical comparison between patients with chronic hepatitis C (CHC) and patients with cirrhosis as regards intrahepatic immunostaining score of nuclear factor-kappa B (NF-κB) ($P = 0.154$).