Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2024; Vol 23: Issue 1, https://carcinogenesis.com/

# Hepatitis A Virus Seropositivity as a Predictor of Thrombocytopenia and Disease Progression in Chronic Liver Disease

**Mahmoud Mohamed Gamal Eldeen Saleh[1], Sahar Mohamed Hassany[2], Awny Ali Abdelrahman[3], Noha Salah Fatim Abdallah[3], Mohammed Abu-Elmaaty Mohammed[4]**

[1]Department of Clinical Pathology, Faculty of Medicine, Aswan University, Egypt.

[2]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Assuit University, Egypt.

[3]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Aswan University, Egypt.

[4]Department of Internal Medicine, and Hematology, Faculty of Medicine, Assiut University, Egypt.

***Corresponding author:** Mahmoud Mohamed Gamal Eldeen Saleh

E-mail address: mahmoudgamal81@aswu.edu.eg

## ABSTRACT

**Background**: Thrombocytopenia represents a frequent complication in chronic liver disease, yet reliable non-invasive predictive biomarkers remain inadequately characterized. The tissue inhibitor of metalloproteinase-1 (TIMP-1) and matrix metalloproteinase-2 (MMP-2) participate in hepatic fibrogenesis and may serve as indicators of disease progression.

**Aim**: To evaluate hepatitis A virus seropositivity, serum TIMP-1, MMP-2, and their ratio as predictive biomarkers for thrombocytopenia in chronic liver disease patients.

**Methods**: This cross-sectional study enrolled 148 chronic liver disease patients. Participants underwent assessment of anti-HAV IgG status, serum TIMP-1, MMP-2 levels, and complete hematological profiles. Thrombocytopenia was defined as platelet count below 150 × $10^9$/L. Receiver operating characteristic analysis determined optimal cut-off values.

**Results**: HAV IgG-positive patients demonstrated significantly higher thrombocytopenia prevalence (65.0% vs. 42.6%, p=0.025). Serum TIMP-1 was markedly elevated in HAV-positive thrombocytopenic patients (180.46 ± 68.41 ng/mL) compared to HAV-negative patients without thrombocytopenia (80.90 ± 44.05 ng/mL, p<0.001). The TIMP-1/MMP-2 ratio showed excellent discriminative capacity for thrombocytopenia in HAV-positive patients (AUC=0.805, sensitivity 84.6%, specificity 71.4% at cut-off ≥1.15). Multivariate analysis confirmed HAV IgG positivity (OR=2.28, p=0.031), elevated TIMP-1 (OR=1.021 per ng/mL, p<0.001), and reduced MMP-2 (OR=0.988 per ng/mL, p<0.001) as independent thrombocytopenia predictors.

**Conclusion**: HAV seropositivity, serum TIMP-1, MMP-2, and particularly the TIMP-1/MMP-2 ratio represent valuable non-invasive biomarkers for predicting thrombocytopenia in chronic liver disease, potentially reducing dependence on invasive diagnostic procedures.

***Keywords:*** *Chronic liver disease; Thrombocytopenia; TIMP-1; MMP-2; Hepatitis A virus; Biomarkers; Liver fibrosis*

**How to Cite:** Mahmoud Mohamed Gamal Eldeen Saleh, Sahar Mohamed Hassany, Awny Ali Abdelrahman, Noha Salah Fatim Abdallah, Mohammed Abu-Elmaaty Mohammed, (2024) Hepatitis A Virus Seropositivity as a Predictor of Thrombocytopenia and Disease Progression in Chronic Liver Disease, *Journal of Carcinogenesis*, *Vol.23, No.1*, 674-683

## 1. INTRODUCTION

Chronic liver disease constitutes a substantial global health burden, with hepatitis B and C viral infections representing the predominant etiological factors contributing to progressive hepatic fibrosis and eventual cirrhotic transformation.[1]

Thrombocytopenia emerges as one of the most prevalent hematological complications encountered in these patients, with reported frequencies reaching up to 78% among cirrhotic individuals.[2] This hematological abnormality significantly complicates clinical management decisions, particularly regarding invasive procedures and antiviral therapeutic interventions.[3]

The pathophysiological mechanisms underlying thrombocytopenia in chronic hepatic disorders involve multiple interconnected processes. Diminished thrombopoietin synthesis by damaged hepatocytes, splenic platelet sequestration secondary to portal hypertension, immune-mediated platelet destruction, and direct bone marrow suppression all contribute to reduced circulating platelet counts.[4,5]

Matrix metalloproteinases and their tissue inhibitors have garnered considerable attention as potential non-invasive markers reflecting hepatic fibrogenic activity. The balance between MMP-2, which degrades extracellular matrix components, and TIMP-1, which inhibits this degradation, fundamentally influences the progression of hepatic fibrosis.[6,7] When this equilibrium shifts toward TIMP-1 predominance, collagen accumulation accelerates, promoting fibrotic progression and its associated complications including portal hypertension and thrombocytopenia.[8]

While substantial research has examined the relationship between hepatitis C virus infection and thrombocytopenia, the specific contribution of hepatitis A virus seropositivity remains inadequately explored.[9] Given the limitations inherent to liver biopsy, including its invasive nature and sampling variability, identification of reliable serum biomarkers capable of predicting thrombocytopenia development assumes considerable clinical importance.[10]

This investigation aimed to evaluate the predictive utility of hepatitis A virus seropositivity, serum TIMP-1, MMP-2, and the TIMP-1/MMP-2 ratio for thrombocytopenia in patients with chronic liver disease, potentially establishing these parameters as accessible non-invasive biomarkers for clinical risk stratification.

## 2. MATERIALS AND METHODS

### Study Design and Setting

This observational cross-sectional investigation was conducted within the hepatology unit of Aswan University Hospital's outpatient department. The institutional review board of Aswan University granted ethical approval for this research protocol. Written informed consent was voluntarily provided by each participant prior to enrollment.

### Participant Selection

The study population comprised 148 individuals with established chronic hepatic disorders. Inclusion criteria encompassed: age exceeding 18 years, confirmed chronic liver disease diagnosis through integrated clinical evaluation, biochemical assessment, and radiological examination, and willingness to participate voluntarily. Individuals declining participation constituted the sole exclusion criterion.

### Sample Size Determination

Sample size calculation employed OpenEpi statistical software, utilizing a 95% confidence level with 5% margin of error. These parameters established the minimum required enrollment at 148 patients.

### Data Collection and Laboratory Evaluation

Comprehensive data acquisition utilized standardized collection instruments capturing demographic characteristics, clinical history, physical examination findings, and laboratory results. Biochemical analyses included complete hematological profiles with platelet enumeration, hepatic function parameters (alanine aminotransferase, aspartate aminotransferase, serum albumin, total bilirubin), coagulation assessment (international normalized ratio), and renal function evaluation.

### Serum TIMP-1 and MMP-2 Measurement

Serum concentrations of tissue inhibitor of metalloproteinase-1 (TIMP-1) and matrix metalloproteinase-2 (MMP-2) were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits following manufacturer protocols. Blood specimens were collected following overnight fasting, centrifuged at 3000 rpm for 15 minutes, and serum aliquots stored at -80°C until batch analysis. The TIMP-1/MMP-2 ratio was subsequently calculated as an index reflecting the balance between matrix deposition and degradation processes.

### Hepatitis A Virus Serological Assessment

Anti-hepatitis A virus immunoglobulin G (anti-HAV IgG) detection employed ELISA methodology, indicating previous viral exposure or vaccination history.

### Operational Definitions and Classifications

Thrombocytopenia was defined as platelet count below $150 \times 10^9$/L, with severity stratification as mild ($100\text{-}149 \times 10^9$/L), moderate ($50\text{-}99 \times 10^9$/L), or severe (below $50 \times 10^9$/L). Hepatic disease severity assessment utilized the Child-Pugh scoring system. Cirrhosis determination integrated clinical presentation, laboratory parameters, and ultrasonographic characteristics.

**Longitudinal Follow-up Protocol**
Prospective assessment extended over a six-month period with evaluations conducted at baseline, three months, and six months. Each assessment encompassed comprehensive hematological analysis including platelet count, mean platelet volume, and platelet-to-lymphocyte ratio determination.

**Statistical Analysis**
Data analysis employed IBM SPSS version 26.0 software. Continuous variables were expressed as mean ± standard deviation for normally distributed data or median with interquartile range for skewed distributions. Categorical variables were presented as frequencies and percentages. Between-group comparisons utilized Mann-Whitney U testing for continuous variables and chi-square or Fisher's exact testing for categorical parameters. One-way analysis of variance with Tukey's post-hoc testing evaluated differences among multiple groups. Pearson correlation coefficients assessed relationships between continuous variables. Receiver operating characteristic curve analysis determined optimal biomarker cut-off values using Youden's index. Multivariate binary logistic regression modeling identified independent thrombocytopenia predictors. Statistical significance was established at $P < 0.05$.

## 3. RESULTS

Table 1 shows that HAV IgG-positive patients were older (median 65 vs. 60 years, p=0.055) with a lower proportion of males (50.0% vs. 67.6%, p=0.076). HAV IgG-positive individuals had significantly higher prevalence of thrombocytopenia (65.0% vs. 42.6%, p=0.025) and lower platelet counts (median 122.5 vs. 185 ×10⁹/L, p=0.010). No significant differences were observed in etiology, comorbidities, or cirrhosis prevalence between groups.

**Table 1: Baseline Characteristics and Thrombocytopenia Stratified by HAV IgG Status**

| Parameter | HAV IgG Positive (n=40) | HAV IgG Negative (n=108) | p-value |
|---|---|---|---|
| Age (years), median (IQR) | 65 (54-73) | 60 (48-69) | 0.055 |
| Male sex, n (%) | 20 (50.0) | 73 (67.6) | 0.076 |
| Etiology of CLD, n (%) | | | |
| HCV | 28 (70.0) | 80 (74.1) | 0.774 |
| HBV | 8 (20.0) | 18 (16.7) | 0.818 |
| Other | 4 (10.0) | 10 (9.2) | 1.000 |
| Comorbidities, n (%) | | | |
| Diabetes mellitus | 25 (62.5) | 57 (52.8) | 0.384 |
| Hypertension | 24 (60.0) | 55 (50.9) | 0.425 |
| Cirrhosis on US, n (%) | 25 (62.5) | 50 (46.3) | 0.117 |
| **Platelet status, n (%)** | | | |
| Normal (≥150 × 10⁹/L) | 14 (35.0) | 62 (57.4) | **0.025*** |
| Thrombocytopenia (<150 × 10⁹/L) | 26 (65.0) | 46 (42.6) | |

**Abbreviations:** IQR = interquartile range; US = ultrasound; CLD = chronic liver disease; HCV = hepatitis C virus; HBV = hepatitis B virus; HAV = hepatitis A virus; IgG = immunoglobulin G.

**Data presented as:** n (%) for categorical variables; median (IQR) for non-normally distributed continuous variables.
**Statistical test:** Chi-square test or Fisher's exact test for categorical variables; Mann-Whitney U test for continuous variables.

**Statistically significant (P < 0.05)*
Table 2 shows that HAV IgG-positive individuals had significantly lower platelet counts and platelet-to-lymphocyte ratios, and higher mean platelet volumes at baseline, 3 months, and 6 months compared to HAV IgG-negative individuals (p<0.05 for all time points).

**Table 2: Platelet Parameters Over 6 Months by HAV IgG Status**

| Time Point | HAV IgG Positive (n=40) | HAV IgG Negative (n=108) | p-value |
|---|---|---|---|
| Platelet Count (×10⁹/L) | | | |
| Baseline | 122.5 ± 78.2 | 185.0 ± 94.3 | **0.010*** |
| 3 months | 138.4 ± 81.6 | 208.2 ± 98.7 | **0.008*** |
| 6 months | 142.8 ± 85.3 | 219.6 ± 101.4 | **0.006*** |

| | | | |
|---|---|---|---|
| Mean Platelet Volume (fL) | | | |
| Baseline | 11.2 ± 1.8 | 9.8 ± 1.4 | **<0.001*** |
| 3 months | 10.8 ± 1.6 | 9.5 ± 1.3 | **0.002*** |
| 6 months | 10.6 ± 1.5 | 9.3 ± 1.2 | **0.004*** |
| Platelet-to-Lymphocyte Ratio | | | |
| Baseline | 68.4 ± 32.1 | 98.6 ± 45.7 | **0.018*** |

**Abbreviations:** HAV = hepatitis A virus; IgG = immunoglobulin G; MPV = mean platelet volume; PLR = platelet-to-lymphocyte ratio.

**Data presented as:** Mean ± SD for normally distributed continuous variables.

**Statistical test:** Independent samples t-test for between-group comparisons.
**Statistically significant (P < 0.05)*
Table 3 shows that patients with thrombocytopenia had significantly lower hemoglobin and albumin levels, and higher INR, ALT, AST, and total bilirubin levels compared to those without thrombocytopenia (p<0.05 for all). WBC was also significantly lower in the thrombocytopenia group (p=0.039).

**Table 3: Laboratory Parameters According to Thrombocytopenia Status**

| Parameter | Thrombocytopenia (n=72) | No Thrombocytopenia (n=76) | p-value |
|---|---|---|---|
| Hemoglobin (g/dL) | 9.2 ± 1.8 | 10.9 ± 2.2 | **<0.001*** |
| WBC (×10⁹/L) | 5.8 ± 3.1 | 6.9 ± 3.3 | **0.039*** |
| ALT (U/L) | 48 (28-268) | 36 (22-228) | **0.028*** |
| AST (U/L) | 56 (38-321) | 38 (25-275) | **<0.001*** |
| Albumin (g/dL) | 2.7 ± 0.5 | 3.2 ± 0.5 | **<0.001*** |
| Total bilirubin (mg/dL) | 2.1 (1.2-11) | 1.2 (0.7-9) | **<0.001*** |
| INR | 1.34 ± 0.32 | 1.11 ± 0.21 | **<0.001*** |
| Creatinine (mg/dL) | 1.31 ± 0.98 | 1.21 ± 0.91 | 0.524 |

**Abbreviations:** ALT = alanine aminotransferase; AST = aspartate aminotransferase; INR = international normalized ratio; WBC = white blood cell count.

**Data presented as:** Mean ± SD for normally distributed variables; median (IQR) for skewed variables (ALT, AST, bilirubin).

**Statistical test:** Independent samples t-test for normally distributed variables; Mann-Whitney U test for skewed variables.
**Statistically significant (P < 0.05)*
Table 4 shows that clinical features and complications were comparable between HAV IgG-positive and -negative thrombocytopenic patients. Although splenomegaly (53.8% vs. 34.8%) and ascites (69.2% vs. 54.3%) were more frequent in HAV IgG-positive individuals, none of the differences reached statistical significance (p>0.05 for all).

**Table 4: Clinical Manifestations and Complications in Thrombocytopenic Patients**

| Clinical Feature | HAV IgG+ with TCP (n=26) | HAV IgG− with TCP (n=46) | p-value |
|---|---|---|---|
| Splenomegaly (clinical), n (%) | 14 (53.8) | 16 (34.8) | 0.184 |
| Splenomegaly (US), n (%) | 13 (50.0) | 18 (39.1) | 0.518 |
| Ascites, n (%) | 18 (69.2) | 25 (54.3) | 0.324 |
| Hepatic encephalopathy, n (%) | 1 (3.8) | 4 (8.7) | 0.768 |
| Variceal bleeding, n (%) | 2 (7.7) | 3 (6.5) | 1.000 |
| Petechiae/Ecchymosis, n (%) | 4 (15.4) | 2 (4.3) | 0.237 |

**Abbreviations:** HAV = hepatitis A virus; IgG = immunoglobulin G; TCP = thrombocytopenia; US = ultrasound.

**Data presented as:** n (%) for categorical variables.

**Statistical test:** Chi-square test or Fisher's exact test.
**Statistically significant (P < 0.05)*
Table 5 shows that TIMP-1 levels were significantly highest in HAV IgG-positive patients with thrombocytopenia (180.46 ± 68.41 ng/mL) compared to all other groups (p<0.001, ANOVA). MMP-2 levels were significantly lower in thrombocytopenic patients regardless of HAV status. The TIMP-1/MMP-2 ratio was significantly elevated in HAV IgG-positive thrombocytopenic patients (2.56 ± 2.30) compared to HAV IgG-negative patients without thrombocytopenia (0.90 ± 2.13, p=0.008). Post-hoc pairwise comparisons using Tukey's HSD test. TIMP-1 levels were significantly higher in HAV+ with TCP compared to HAV+ without TCP (mean difference 52.08, p=0.029), HAV− with TCP (mean difference 42.25, p=0.013), and HAV− without TCP (mean difference 99.56, p<0.001). The TIMP-1/MMP-2 ratio was significantly higher in HAV+ with TCP compared to HAV− without TCP (mean difference 1.66, p=0.008).

**Table 5: Serum TIMP-1 and MMP-2 Levels by HAV IgG and Thrombocytopenia Status**

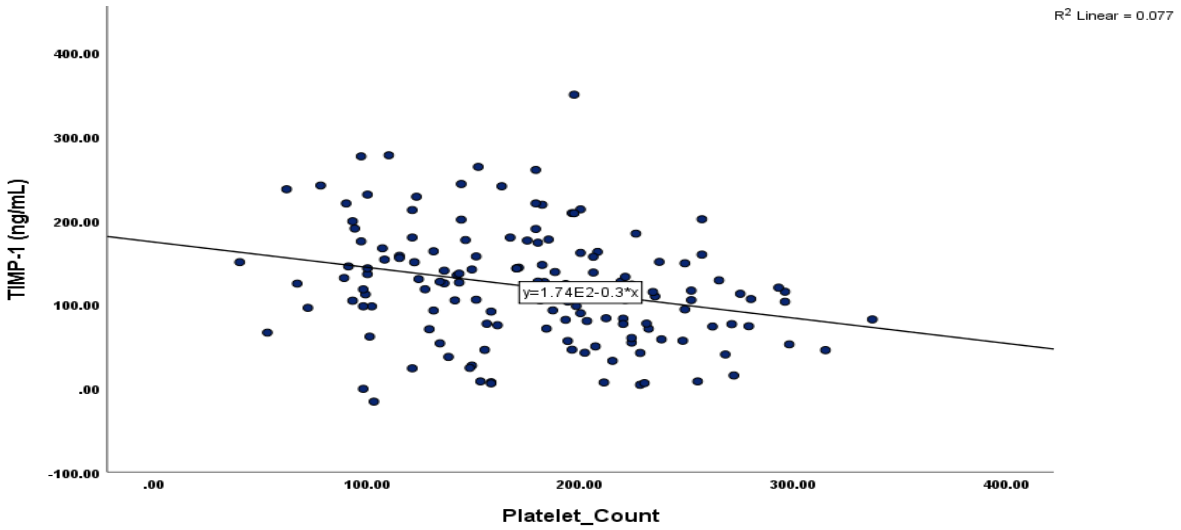
| Parameter | HAV+ with TCP (n=26) | HAV+ without TCP (n=14) | HAV− with TCP (n=46) | HAV− without TCP (n=62) | p-value |
|---|---|---|---|---|---|
| TIMP-1 (ng/mL) | 180.46 ± 68.41 | 128.38 ± 34.27 | 138.20 ± 66.78 | 80.90 ± 44.05 | **<0.001*** |
| MMP-2 (ng/mL) | 97.01 ± 48.56 | 141.20 ± 53.35 | 106.84 ± 59.12 | 153.30 ± 82.12 | **<0.001*** |
| TIMP-1/MMP-2 Ratio | 2.56 ± 2.30 | 1.07 ± 0.57 | 1.91 ± 2.45 | 0.90 ± 2.13 | **0.006*** |

**Abbreviations:** TIMP-1 = tissue inhibitor of metalloproteinase-1; MMP-2 = matrix metalloproteinase-2; HAV = hepatitis A virus; TCP = thrombocytopenia.

**Data presented as:** Mean ± SD.

**Statistical test:** One-way ANOVA with Tukey's post-hoc test for multiple comparisons.
**Statistically significant (P < 0.05)*
Figure (1.A) shows that TIMP-1 levels were significantly negatively correlated with platelet count (r = -0.277, p=0.001), while MMP-2 levels showed a significant positive correlation (r = 0.303, p<0.001) (Figure 1.B). The TIMP-1/MMP-2 ratio demonstrated a significant inverse correlation with platelet count (r = -0.199, p=0.015) (Figure 1.C).

**A**



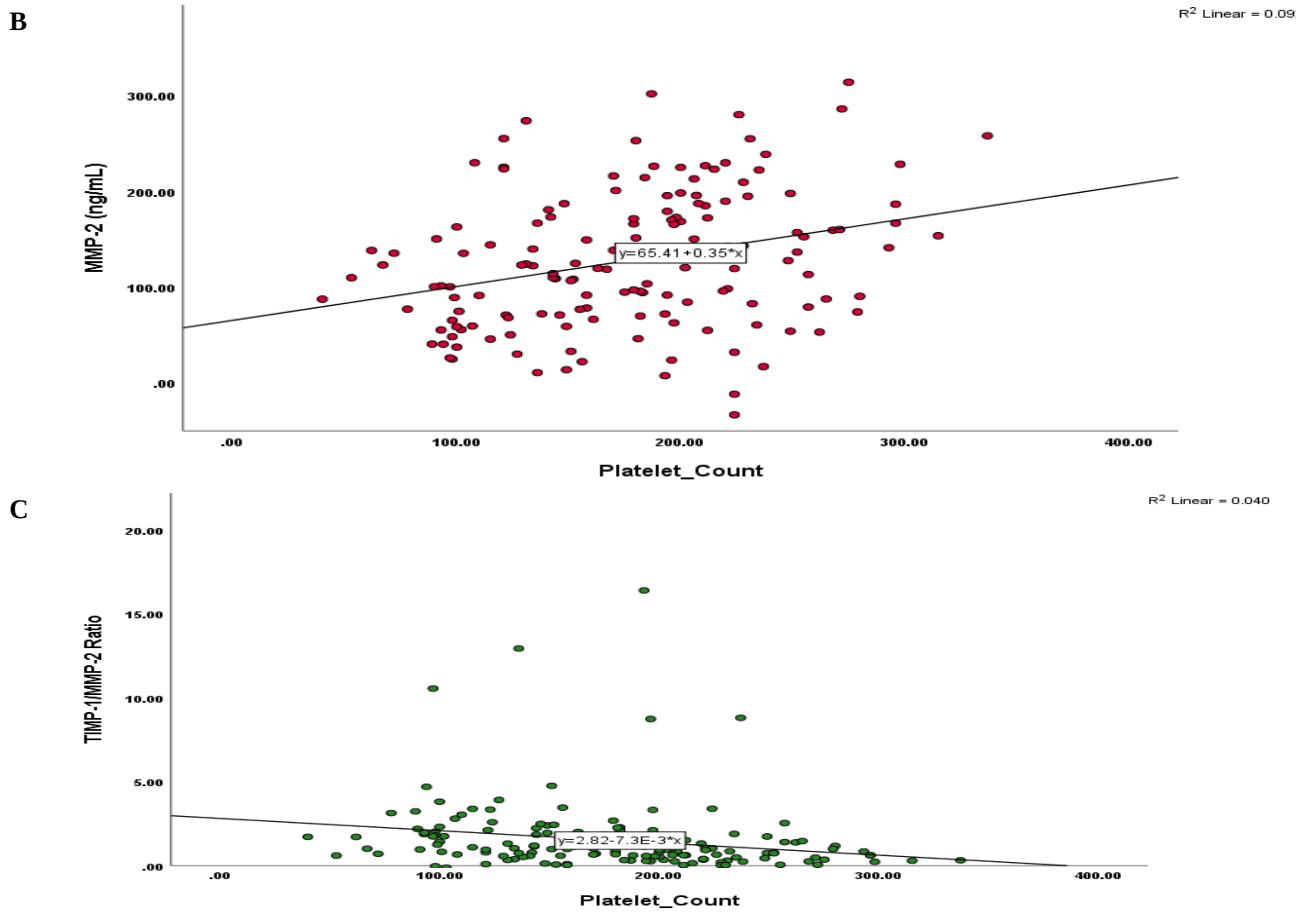



**Figure 1: Correlation Between TIMP-1/MMP-2 Parameters and Platelet Count**

Figure 2 shows that in HAV IgG-positive patients, the TIMP-1/MMP-2 ratio had the highest discriminative ability for thrombocytopenia (AUC = 0.805, p=0.002). At the optimal cut-off value of 1.15, the TIMP-1/MMP-2 ratio achieved 84.6% sensitivity and 71.4% specificity. TIMP-1 alone at cut-off ≥133.6 ng/mL showed 69.2% sensitivity and 57.1% specificity. For MMP-2, lower values predict thrombocytopenia (AUC < 0.5 indicates inverse relationship); the cut-off represents values ≤93.3 ng/mL as predictor of thrombocytopenia.

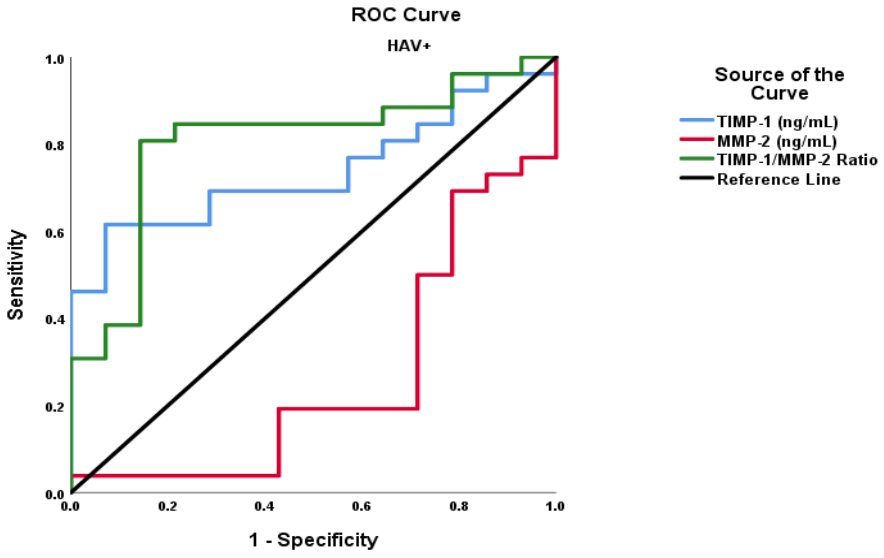



**Figure 2: ROC Analysis for Predicting Thrombocytopenia in HAV IgG-Positive Patients**

Figure 3 shows that in HAV IgG-negative patients, TIMP-1 had the highest discriminative ability for thrombocytopenia (AUC = 0.776, p<0.001). At the optimal cut-off value of ≥105.1 ng/mL, TIMP-1 achieved 76.1% sensitivity and 66.1% specificity. The TIMP-1/MMP-2 ratio at cut-off ≥0.95 demonstrated 71.7% sensitivity and 75.8% specificity. For MMP-2, lower values predict thrombocytopenia (AUC < 0.5 indicates inverse relationship); the cut-off represents values ≤108.6 ng/mL as predictor of thrombocytopenia.

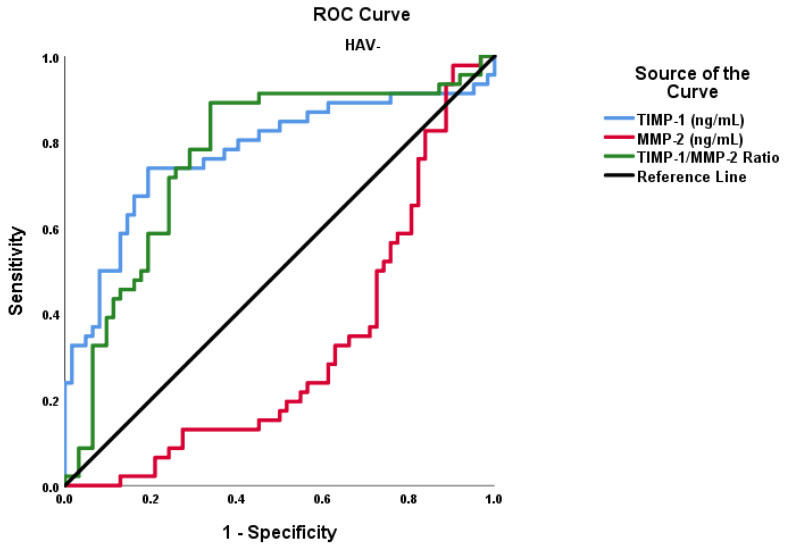



**Figure 3: ROC Analysis for Predicting Thrombocytopenia in HAV IgG-Negative Patients**

Table 6 shows that HAV IgG positivity (OR: 2.28, 95% CI: 1.08–4.82, p=0.031), low serum albumin <3.0 g/dL (OR: 2.94, 95% CI: 1.42–6.08, p=0.004), cirrhosis on ultrasound (OR: 3.67, 95% CI: 1.73–7.79, p=0.001), and splenomegaly (OR: 2.51, 95% CI: 1.21–5.21, p=0.013) were independently associated with increased odds of thrombocytopenia. Additionally, higher serum TIMP-1 levels (OR: 1.021, 95% CI: 1.013–1.030, p<0.001) were positively associated, whereas higher serum MMP-2 levels (OR: 0.988, 95% CI: 0.981–0.995, p<0.001) were inversely associated with thrombocytopenia. In contrast, the TIMP-1/MMP-2 ratio, elevated total bilirubin, and age >60 years were not significantly associated with thrombocytopenia.

**Table 6: Multivariate Logistic Regression Analysis of Factors Associated with Thrombocytopenia**

| Variable | Odds Ratio | 95% CI | p-value |
|---|---|---|---|
| **HAV IgG positive** | 2.28 | 1.08-4.82 | **0.031*** |
| **Serum TIMP-1 (ng/mL)** | 1.021 | 1.013-1.030 | **<0.001*** |
| **Serum MMP-2 (ng/mL)** | 0.988 | 0.981-0.995 | **<0.001*** |
| **TIMP-1/MMP-2 Ratio** | 0.920 | 0.752-1.125 | 0.417 |
| **Albumin <3.0 g/dL** | 2.94 | 1.42-6.08 | **0.004*** |
| **Cirrhosis on US** | 3.67 | 1.73-7.79 | **0.001*** |
| **Splenomegaly** | 2.51 | 1.21-5.21 | **0.013*** |
| **Total bilirubin >2.0 mg/dL** | 1.89 | 0.91-3.92 | 0.087 |
| **Age >60 years** | 1.42 | 0.71-2.84 | 0.318 |

**Abbreviations:** HAV = hepatitis A virus; IgG = immunoglobulin G; TIMP-1 = tissue inhibitor of metalloproteinase-1; MMP-2 = matrix metalloproteinase-2; OR = odds ratio; CI = confidence interval; US = ultrasound.

**Data presented as:** Odds ratio (Exp(B)) with 95% confidence interval.

**Statistical test:** Multivariate binary logistic regression; significance defined as p<0.05 and 95% CI not including 1. *Statistically significant (P < 0.05)

**Interpretation:** Each 1 ng/mL increase in TIMP-1 is associated with 2.1% higher odds of thrombocytopenia (OR=1.021). Each 1 ng/mL increase in MMP-2 is associated with 1.2% lower odds of thrombocytopenia (OR=0.988). The ratio was not independently significant when individual markers were included in the model.

## 4. DISCUSSION

The current investigation sought to determine whether hepatitis A virus seropositivity alongside serum TIMP-1 and MMP-2 concentrations could serve as dependable indicators for anticipating thrombocytopenia among individuals suffering from chronic hepatic disorders. Our data revealed several noteworthy findings that contribute meaningfully to existing knowledge in this field.

Regarding thrombocytopenia frequency, our cohort demonstrated that 65% of HAV IgG-positive patients exhibited reduced platelet counts compared with 42.6% among seronegative individuals. This observation carries substantial clinical relevance. Ayaz and colleagues[11] documented thrombocytopenia prevalence reaching 37.1% in their chronic HCV population, whereas Mehmood et al.[12] reported rates of 36.3% among chronic liver disease patients in Pakistan. The higher frequency observed in our HAV-positive subgroup suggests that prior hepatitis A exposure may confer additional risk beyond what has been traditionally attributed to hepatitis B and C infections alone.

The pathophysiological rationale underlying our observations warrants careful consideration. When examining why HAV seropositivity associates with more pronounced thrombocytopenia, the TIMP-1/MMP-2 pathway offers a compelling explanation. In our results, HAV IgG-positive thrombocytopenic patients displayed markedly elevated TIMP-1 concentrations (180.46 ± 68.41 ng/mL) relative to HAV-negative individuals without thrombocytopenia (80.90 ± 44.05 ng/mL). This pattern aligns with the proposed mechanism wherein chronic immune memory following HAV exposure triggers persistent hepatic stellate cell activation, subsequently upregulating TIMP-1 expression while suppressing MMP-2 activity.[13]

Metwally and coworkers[14] previously established that TIMP-1 levels correlate closely with hepatic disease severity among Egyptian patients. Our findings extend their work by demonstrating that this relationship holds particular significance within the HAV-seropositive population. The mean TIMP-1 value we recorded in HAV-positive thrombocytopenic subjects exceeded that reported by Olusi et al.[7] in their chronic hepatitis cohort, possibly reflecting the additive fibrogenic stimulus contributed by prior HAV infection superimposed on existing chronic liver pathology.

Concerning MMP-2 concentrations, our study documented significantly diminished levels among thrombocytopenic patients irrespective of HAV status. This inverse relationship between MMP-2 and platelet counts stands in agreement with observations by Lichtinghagen et al.[15] who characterized altered MMP/TIMP ratios in chronic hepatitis C. However, in contrast to their suggestion that circulating metalloproteinase levels weakly reflect hepatic fibrosis severity, our multivariate analysis demonstrated that reduced MMP-2 independently predicted thrombocytopenia (OR 0.988 per ng/mL, $p<0.001$). This discrepancy may stem from differences in patient selection criteria or assay methodologies employed.

The TIMP-1/MMP-2 ratio emerged as the most discriminating parameter in our investigation. Among HAV-positive patients, this ratio achieved an area under the curve of 0.805 for thrombocytopenia prediction, with sensitivity reaching 84.6% and specificity 71.4% at the optimal threshold of 1.15. El-Azm and colleagues[16] similarly advocated for combined biomarker approaches in hepatic fibrosis assessment, reporting that marker combinations outperform individual parameters. Our data support this perspective and further suggest that the TIMP-1/MMP-2 ratio possesses particular utility in HAV-seropositive populations.

Walsh et al.[17] demonstrated nearly two decades ago that tissue inhibitors of metalloproteinases surpass MMP-2 and conventional liver enzymes in detecting advanced hepatic disease. The present study corroborates their conclusion while adding the novel observation that this superiority becomes especially pronounced when stratifying patients by HAV serostatus. In our HAV-positive subgroup, the ratio-based approach substantially outperformed either marker considered independently.

From a mechanistic standpoint, elevated TIMP-1 inhibits collagen degradation by blocking MMP activity, thereby promoting extracellular matrix accumulation and accelerating fibrotic progression.[18] This process culminates in portal hypertension development, which subsequently causes splenic platelet sequestration and reduced thrombopoietin production by damaged hepatocytes.[19] The biological cascade we propose—HAV exposure leading to chronic immune activation, hepatic stellate cell stimulation, TIMP-1 upregulation, collagen accumulation, and ultimately thrombocytopenia—represents a testable hypothesis warranting further investigation.

Mitchell and associates[5] comprehensively reviewed thrombocytopenia pathophysiology in chronic liver disease, emphasizing its multifactorial nature involving diminished thrombopoietin synthesis, hypersplenism, immune-mediated destruction, and bone marrow suppression. Our findings complement their framework by identifying the TIMP-1/MMP-2 imbalance as an additional contributing mechanism, particularly relevant among patients with prior HAV exposure.

Dissimilar to most published investigations focusing exclusively on hepatitis B and C populations,[20,21] our study specifically examined HAV seropositivity influence on biomarker profiles and thrombocytopenia risk. This distinction holds clinical importance given widespread HAV exposure throughout endemic regions including Egypt and other developing nations. Physicians managing chronic liver disease patients in these settings should recognize that positive HAV serology may signal heightened thrombocytopenia susceptibility.

The multivariate regression model identified several independent thrombocytopenia predictors beyond the novel biomarkers. Ultrasonographic cirrhosis demonstrated the strongest association (OR 3.67), consistent with findings by Adinolfi et al.[22] who emphasized fibrosis centrality in thrombocytopenia pathogenesis. Hypoalbuminemia below 3.0 g/dL also predicted reduced platelet counts (OR 2.94), reflecting its role as a hepatic synthetic function marker. Notably, TIMP-1 and MMP-2 maintained independent predictive value even after adjustment for these established clinical parameters, suggesting they capture distinct pathophysiological information.

## 5. CONCLUSION

Hepatitis A virus seropositivity, serum TIMP-1, MMP-2, and particularly the TIMP-1/MMP-2 ratio constitute valuable non-invasive biomarkers for predicting thrombocytopenia in chronic liver disease patients. These parameters demonstrate significant diagnostic accuracy and independent predictive value, potentially facilitating early risk stratification and reducing dependence on invasive procedures. Future large-scale longitudinal studies should validate these findings and establish standardized cut-off values for clinical implementation.

## REFERENCES


[1] Liang B, Li Y, Zhao A, Xie F, Guo Z. Clinical utility of serum matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 concentrations in the assessment of liver fibrosis due to chronic hepatitis B. *J Int Med Res.* 2012;40(2):631–639.

[2] Chen Y, Ko P, Lee C, Tseng C, Tseng K. Baseline thrombopoietin level is associated with platelet count improvement in thrombocytopenic chronic hepatitis C patients after successful direct-acting antiviral agent therapy. *BMC Gastroenterol.* 2021;21(1):30.

[3] Khan N, Abro HA, Ahmed S, Shaikh RA, Muneer A. Frequency of thrombocytopenia in chronic hepatitis C. *Indus J Biosci Res.* 2024;3(6):771–775.

[4] Fouad Y. Chronic hepatitis C-associated thrombocytopenia: aetiology and management. *Trop Gastroenterol.* 2013;34(2):58–67.

[5] Mitchell OJ, Feldman DM, Diakow M, Sigal SH. The pathophysiology of thrombocytopenia in chronic liver disease. *Hepatic Med.* 2016;8:39–50.

[6] Scharf RE. Thrombocytopenia and hemostatic changes in acute and chronic liver disease: pathophysiology, clinical and laboratory features, and management. *J Clin Med.* 2021;10(7):1530.

[7] Olusi S, Abdeen S, George S. Associations among serum concentrations of interleukin-18, matrix metalloproteinase-2, tissue inhibitor of metalloproteinase-1 and METAVIR fibrosis score in patients with chronic hepatitis. *Int J Integr Care Med Res.* 2012.

[8] Busk TM, Bendtsen F, Nielsen HJ, Jensen V, Brünner N, Möller S. TIMP-1 in patients with cirrhosis: relation to liver dysfunction, portal hypertension, and hemodynamic changes. *Scand J Gastroenterol.* 2014;49(9):1103–1110.

[9] Aslam R, Jaffer MA, Saleem H, Fatima S, Shakeel M, Hanif M. Frequency of hepatitis C in patients presented with isolated thrombocytopenia. *Prof Med J.* 2024;31(05):764–768.

[10] Abdel-Ghaffar TY, Behairy BE, El-Shaheed AA, Mahdy K, El-Batanony M, Hussein MH, et al. Clinical benefits of biochemical markers of fibrosis in Egyptian children with chronic liver diseases. *Gastroenterol Res.* 2011;3(6):262–271.

[11] Ayaz QUA, Khanzada FA, Sharif A, Farrukh S, Akhtar R, Hamid A. Pattern and burden of thrombocytopenia in chronic hepatitis C virus patients at a tertiary care hospital. *Pak J Pathol.* 2024;35(1).

[12] Mehmood Z, Ather CAA, Shah ZH, Sajad Q, Farooq N, Ch S. Frequency of thrombocytopenia and its severity among patients of chronic liver disease presenting to a tertiary care hospital. *Pak J Med Health Sci.* 2022;16(1):155–156.

[13] Flisiak R, Maxwell PR, Prokopowicz D, Timms P, Panasiuk A. Plasma tissue inhibitor of metalloproteinases-1 and transforming growth factor beta 1—possible non-invasive biomarkers of hepatic fibrosis in patients with chronic B and C hepatitis. *Hepatogastroenterology.* 2002;49(47):1369–1372.

[14] Metwally K, Fouad TR, Shible N, Zaghla H, Sameea EA, Aref MM, et al. Metalloproteinase inhibitor-1 closely correlates with the severity of liver disease in Egyptian patients. *J Gastrointest Dig Syst.* 2017;7:157.

[15] Lichtinghagen R, Huegel O, Seifert T, Haberkorn CI, Michels D, Flemming P, et al. Expression of matrix metalloproteinase-2 and -9 and their inhibitors in peripheral blood cells of patients with chronic hepatitis C. *Clin Chem.* 2000;46(2):183–192.

[16] El-Azm AA, Kobtan AR, Elgendy Z, Mansour N. Can fibrogenesis markers reflect early hepatic histopathology in chronic hepatitis C. *Afro-Egypt J Infect Endemic Dis.* 2014.
[17] Walsh KM, Timms P, Campbell S, MacSween R, Morris AJ. Plasma levels of matrix metalloproteinase-2 and tissue inhibitors of metalloproteinases -1 and -2 as noninvasive markers of liver disease in chronic hepatitis C. *Dig Dis Sci.* 1999;44(3):624–630.
[18] Abdalla NM, Aziz FMA, Deghady A, Abaza MH, Ellakany WI. Tissue inhibitor of matrix metalloprotinase-1 and collagen type IV in HCV-associated cirrhosis and grading of esophageal varices. *Egypt Liver J.* 2024;14:1–11.
[19] Al-Qtaitat A, Al-Dalaen S, Mahgoub S, Alnawaiseh N, Al-Shuneiqat J, Al-Sarayreh S, et al. Plasma thrombopoietin level in thrombocytopenic patients with or without liver cirrhosis chronically infected by the hepatitis C virus. *Br J Med Med Res.* 2015;5(7):864–871.
[20] Karasu Z, Tekin F, Ersoz G, Gunsar F, Batur Y, Ilter T, et al. Liver fibrosis is associated with decreased peripheral platelet count in patients with chronic hepatitis B and C. *Dig Dis Sci.* 2007;52(6):1535–1539.
[21] Awal DMR, Ferdous N, Hakim ML, Uddin DMM, Iqbal MA, Hossain D, et al. Clinical correlates and severity of thrombocytopenia in patients with chronic liver disease due to hepatitis B and C. *SAS J Med.* 2024;11(09):853–859.
[22] Adinolfi LE, Giordano MG, Andreana A, Tripodi MF, Utili R, Cesaro G, et al. Hepatic fibrosis plays a central role in the pathogenesis of thrombocytopenia in patients with chronic viral hepatitis. *Br J Haematol.* 2001;113(3):590–595.