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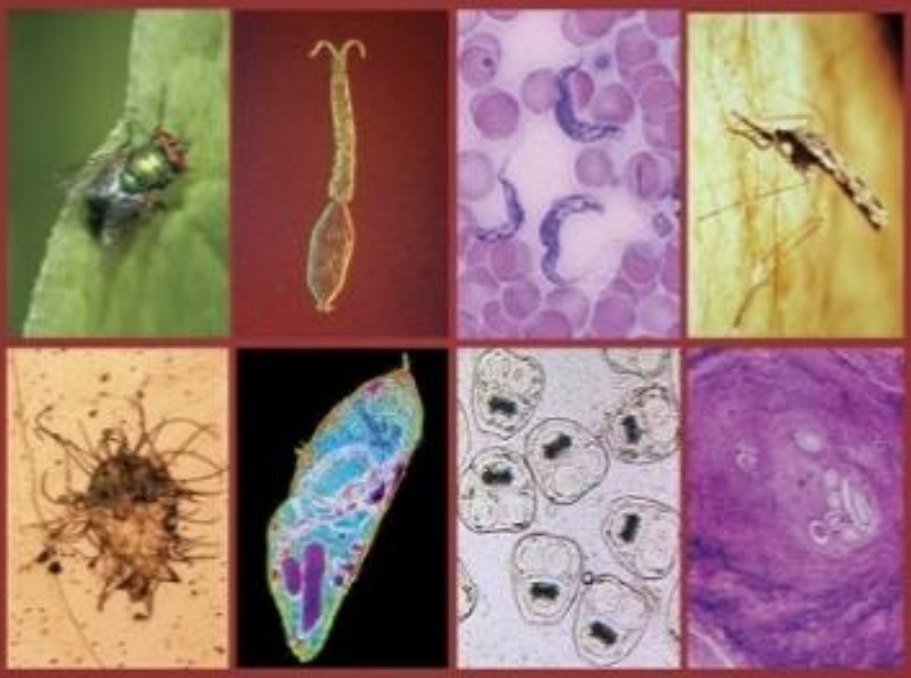

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Insights into TIMP-1, M-CSF, and YKL-40 Serum Biomarkers in Common Tropical Hepatic Diseases Among the Egyptian population

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ABSTRACT

Background: Noninvasive predictive options for circulatory profibrotic biomarkers are increasingly explored in tropical hepatic diseases. This work comparatively assessed tissue inhibitors of metalloproteinases (TIMP), Chitinase-3-Like-Protein-1 (YKL-40), and macrophage colony-stimulating factor (M-CSF) sera levels in non-cirrhotic patients. **Method:** A case-control study of 80 patients with early-stage hepatic diseases, involved schistosomiasis, hydatidosis, HCV and HBV infected groups (each = 20) with 20 healthy controls. Serum TIMP-1, M-CSF, and YKL-40 levels were assessed using the ELISA technique. **Results:** Compared to control, alanine aminotransferase (ALT) showed significant elevatation in all groups ($p < 0.01$), while aspartate aminotranferase (AST) was significantly elevated in HBV and HCV groups ($p < 0.001$). Serum TIMP-1 showed significant elevations in all groups ($p \leq 0.001$). Serum YKL-40 revealed significant elevation in HCV group ($p < 0.001$). M-CSF levels were significantly higher in each group ($p = 0.01$ for hydatidosis, 0.002 for HBV and < 0.001 for both HCV and schistosomiasis). Using pairwise comparisons, TIMP-1 levels were significantly higher in HBV group vs. hydatid and schistosomiasis groups ($p = 0.003$, $p < 0.001$, respectively) and in HCV vs. hydatid and schistosomiasis groups ($p < 0.05$). The HCV-positive group was higher than HBV group in YKL-40 and M-CSF levels (both: p=0.03). **Conclusion:** The increased TIMP-1, YKL-40, and M-CSF sera levels and their significant correlation with ALT may suggest their predictive roles in hepatic fibrogenesis since the early chronic stage of these diseases. HCV recorded the highest serum levels of fibrotic and liver enzymes biomarkers. Therapeutic trials targeting TIMP-1 and M-CSF are recommended.

INTRODUCTION

The liver plays a fundamental role in several vital functions, for instance, immunity and metabolism. Chronic liver injury leads to inflammation, stimulation of resident hepatic stellate cells (HSCs), and the production of extracellular matrix (ECM) proteins that so far led to fibrosis of hepatic parenchyma (Kisseleva and Brenner, 2021). Zoonotic and non-zoonotic infections are still major etiological factors of liver fibrosis and cirrhosis in several areas of the world. Dramatic increases in both parasitic and viral infections have been witnessed owing to poverty, global climate changes, exposure to environmental contaminants, socio-environmental factors, and human-environmental interactions (El Saftawy *et al.,* 2024).

Schistosomiasis affects more than 250 million patients in African, South American, and Asian rural areas and approximately 70 million people suffer Disability-Adjusted Life Years (DALYs) (Aula *et al.,* 2021). Schistosomiasis *mansoni* is an endemic parasitic disease in Egypt. It is transmitted through infected water canals in rural areas. In Egypt, despite governmental efforts, the disease remains a public health concern (El-Kassas *et al.,* 2024). Eggs embolize in liver, triggering granulomatous reactions in the early chronic disease and fibrosis in the late stages (El Saftawy *et al.,* 2022; Fadl *et al.,* 2021).

Hydatidosis is an endemic cyclo-zoonotic disease in the Mediterranean countries, and 1 million people suffer DALYs were recorded (Ito and Budke, 2017; Mathivathani et al., 2023; Gessese, 2020). Hydatid cysts are bladder-like lesions that mostly affect the liver (60% of the patients) and induce fibrotic alterations, forming a thick adventitial layer (El Saftawy *et al.,* 2021a; El Saftawy *et al.,* 2021b).

Hepatitis C affects 71·1 million people and develops into chronic hepatitis in 50–80% of the patients, inducing injury in the hepatic parenchyma, liver dysfunction, fibrosis, cirrhosis, liver failure or hepatocellular carcinoma, and death in advanced stages (Manns et al., 2017).

Hepatitis B is a worldwide viral infectious disease affecting 257 million patients. Hepatitis B infection has high potential to cause cirrhosis, hepatocellular failure, and carcinoma transformation (Koffas *et al.,* 2021; Iannacone and Guidotti, 2022).

Chronic liver disease may develop into cirrhotic changes and portal hypertension if not distinguished early with proper interventions. Historically, liver biopsy has been the gold standard diagnostic and staging tool in liver fibrosis (Jain *et al.,* 2021). Nonetheless, the inevitability of attaining a biopsy to diagnose liver fibrosis remains challenging due to some restrictions, such as patient agreement, specimen variability, and the possible pain. Also, it has been recorded that in one time liver biopsy, 10 %-30 % of patients with hepatic fibrosis might be misdiagnosed (Chen *et al.,* 2022). Simultaneously, reliable non-invasive approaches are increasingly available and introduced in clinical practice to minimize the requirement for liver biopsy (Li *et al.,* 2018).

We hypothesized the effect of HBV, HCV, hydatidosis, and schistosomiasis on circulating liver enzymes and fibrotic biomarkers during early noncirrhotic stages, suggesting their potential utility as proactive diagnostic tools. Hence, the progression of future liver fibrosis may vary among tropical diseases. This might benefit the direct-acting therapeutic agents in these diseases.

Circulatory biomarkers can be categorized into direct (class- I) and indirect (class- II). Class-I markers accompany ECM synthesis and degradation, while class-II markers imitate liver function (Chen *et al.,* 2022). In the new technological era, liver biochemical enzymes involving alanine transaminase (ALT) and aspartate transferase (AST) are

increasingly ordered to detect hepatocellular pattern of liver disease and improve its natural fibrogenic course (Neuschwander-Tetri *et al.,* 2004; Kalas *et al.,* 2021).

Tissue inhibitors of metalloproteinases (TIMP) are determined for the progression of liver fibrosis through the degradation of the epithelial cells and their transformation into mesenchymal cells (Tsomidis *et al.,* 2020). TIMP also plays a role in ECM metabolism. Liver parenchyma possesses both TIMP-1 and TIMP-2; however, TIMP-1 compared with TIMP-2 is more specific and sensitive in diagnosing liver fibrosis (Lefeuvre *et al.,* 2022). Additionally, the cytokine macrophage colony-stimulating factor (M-CSF), partly produced by infiltrating monocytes, has been found to mediate hepatic inflammatory reactions and liver fibrosis. M-CSF contributes to the differentiation of Kupffer cells into macrophages that develop pro-fibrotic properties during persistent liver injury (Tsomidis *et al.,* 2025). YKL-40, Chitinase-3-Like Protein 1 (CHI3L1) is another factor closely associated with liver fibrosis. YKL-40 is synthesized in neutrophils and packed in the lactoferrin-containing granules. YKL-40 through binding to interleukin-13 receptor subunit alpha-2 (IL-13Rα2) triggers several intracellular biological processes e.g., inflammation and immune defense against microorganisms, apoptosis, and degradation or remodeling of ECM (Blazevic *et al.,* 2024; Yoshio and Kanto, 2021).

This study aimed to assess TIMP, M-CSF, and YKL-40 sera levels in tropical liver diseases, including schistosomiasis, hydatidosis, Hepatitis C, and Hepatitis B, which commonly affect the Egyptian population.

MATERIALS AND METHODS

1. Study Population:

The study involved 80 Egyptian patients aged 20-60 years old with early-stage hepatic disease, conducted between June 2024 and January 2025. The patients attended the Gastroenterology, Hepatology, and Infectious Diseases outpatient clinics at the Faculties of Medicine, Beni-Suef University, Al-Zahraa University, and Cairo University, Egypt. The study participants were randomly selected regardless of their age and sex. Inclusion criteria involved patients with no liver fibrosis or cirrhosis (confirmed by abdominal ultrasonography and or CT). Additionally, laboratory assessments revealing increases in AST or ALT, positive hepatitis markers (anti-HCV-Ab or HBs-Ag), high anti-bilharzial antibody titer, or high anti -hydatid antibody titer (>2560). Exclusion criteria involved patients with signs of portal hypertension, positive HIV, immunological diseases, cancer, pregnancy, or exposure to anti-viral hepatitis or anti-parasitic treatments in the previous 6 months. In addition, to avoid biased results, only sole infections were involved, and patients with multiple hepatic diseases were excluded. The Research Ethical Committee of the Faculty of Medicine, Beni-Suef University (FM-BSU REC) has approved the protocol from an ethical point of view (FMBSUREC/ 01092024/ Eid). The committee was organized according to the Declaration of Helsinki guidelines, the International Conference of Harmonization ICH, and the United States (FWA) for the Protection of Human Subjects. An informed written consent was obtained from all participants before enrollment in the study.

2. Study Design:

The current work is a case-control study. All patients were clinically and laboratory selected and subdivided into four groups: HCV infection (n = 20), HBV infection (n = 20), bilharzial infection (n = 20), and hydatid infection (n = 20). Also, 20 healthy subjects without any organic disease and matched gender and age were involved in the study. All the participants read and signed the informed consent.

3. Sample Collection and Preparation:

About 6 ml of venous blood was drawn from each subject and divided into three aliquots in 3 plain tubes each of 2 ml.

The first aliquot was for hepatitis markers enzyme-linked immunosorbent assay (ELISA) detection. The second aliquot was for bilharzial and hydatid antibody detection using the indirect hemagglutination tests (IHA). To assess TIMP-1, M-CSF, and YKL-40 by ELISA, the third aliquot was centrifuged at 3000 rpm for 20 minutes. Then the serum was separated, divided into further aliquots, and stored at −20˚C. To avoid repeated freeze-thaw cycles all fibrotic markers were assessed in one assay (Aladawy *et al.,* 2024).

4. Serological Tests:

4.1. Quantitative Assessment Of Anti-Bilharzia And Anti-Hydatid Antibody Titers By Hemagglutination Technique:

The indirect hemagglutination tests (IHA) were performed using commercially available Fumouze Diagnostics-France. Steps involved the addition of 0.05 ml of serum and 1.95 ml of buffer solution to attain a 1/40 stock dilution of test serum. 50 µl of phosphate buffer solution (PBS) was placed in 7 successive wells in the ELISA plastic plates. Employing a micro pipettor, 50 µl of the 1/40 stock dilution was placed in the 1st well (titer of 1/80), mixed well with the PBS, transferred to the second well, and so on until the end of the 6th well (titer of 1/2560). Finally, 50 µl was eliminated from the 6th well. Reading of the microplate reactions: the opened ring indicated a positive titer while the closed ring was considered negative (El Saftawy, 2021).

4.2. Qualitative Assessment of Hepatitis Markers Using the ELISA Technique:

Hepatitis biomarkers were evaluated using the commercially available Monolisa HBs Ag Ultra kit (No. 72348), and Monolisa HCV Ag-Ab Ultra kit (No. 72562), Bio-Rad Laboratories (CA, USA). Monolisa HCV Ag-Ab solid phase was coated with purified HCV antigens: two from the non-structural region and a peptide from the structural region of the HCV, and a monoclonal antibody against the HCV capsid. The liquid phase comprises two conjugates. The first conjugate consists of a monoclonal antibody against the hepatitis C capsid. The second conjugate was a mixture of peroxidase-labeled anti-human IgG antibodies and peroxidase-labeled streptavidin. The colour developed by adding the substrate, once the reaction had been stopped, the spectrophotometer reading was taken at 450/620 nm. Monolisa HBs Ag ULTRA assay was a one-step enzyme immunoassay based on the principle of the "sandwich" ELISA. The solid phase was coated with monoclonal antibodies. The conjugates were based upon the use of monoclonal antibodies from mouse and polyclonal antibody from goat against the HBs Ag. These antibodies were bound to the peroxidase. The colour developed after adding the substrate. Then the reaction was stopped and reading of the optical densities was taken at 450/620nm

4.3. Quantitative Measurement of The Liver Enzymes:

In one assay, liver enzymes were measured using a Hitachi Cobas c 311 analyzer (Mannheim, Germany, serial no.23R5-05) (Yu et al., 2017). Interpretation of the readings: According to Neuschwander-Tetri et al. (2004), the validated values of the upper limit normal (ULN) should not be applied for ALT and AST due to technical issues related to specimen stability. Alternatively, laboratories should use “the locally-defined reference populations”. Therefore, the results of healthy subjects of the Egyptian population were used as a reference range in this work. Interpretation of the magnitude of ALT and AST: < 5 × ULN refers to mild increases, > 5-< 15 ×ULN is moderate, and > 15 × ULN is severe elevations (Neuschwander-Tetri et al., 2004).

4.4. Quantitative Measurement of The Profibrotic Biomarkers: (TIMP-1, M-CSF, and YKL-40):

The concentration of TIMP-1, M-CSF, and YKL-40 in serum was measured following the manufacturer's instructions by Sandwich ELISA technique with a complete set of ELISA reader das 1851. The human TIMP-1 ELISA kit (KE00166) and AuthentiKine™ Human M-CSF ELISA Kit (KE00184) were obtained from Proteintech, Manchester, UK. The YKL-40 ELISA kit (E2063Hu) was obtained from Bioassay Technology Laboratory, China. At a wavelength of 450 nm ± 2 nm, the optical density (OD) was measured spectrophotometrically.

5. Statistical Methods:

The data obtained were presented in tables as mean ± standard deviation. The difference between any two groups was calculated using the Mann-Whitney U test. Spearman's test was used to test the correlation of variables. All statistical procedures were performed using Jamovi software (Version 2.3) (The jamovi project, 2022).

RESULTS

1. The Demographic Data and The Radiological Imaging of The Selected Study Groups:

Concerning the demographic data, there were no significant differences in mean age among the studied groups. Gender distribution was also comparable across the groups (Table 1). Figure 1, shows ultrasonography and CT imaging of the patients with no fibrosis or cirrhosis of the liver.

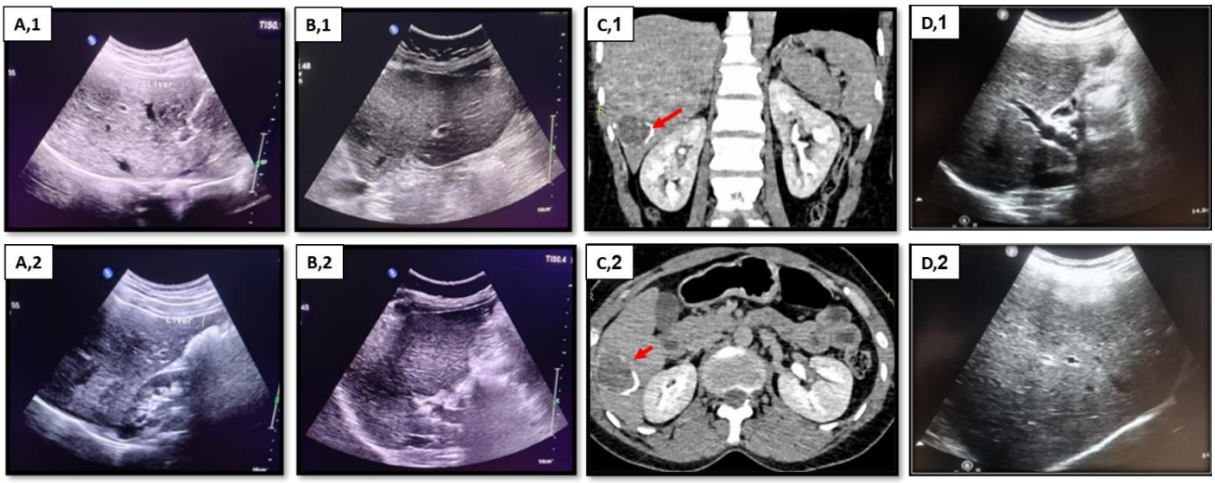

Fig. 1: Radiological imaging of patients with chronic liver diseases. A1&A2: HBV infected patient. Ultrasound shows no fibrotic or cirrhotic changes in the parenchyma. B1&B2: HCV infected patient. Ultrasound shows no fibrotic or cirrhotic parenchymal findings. C1&C2: A post-contrast CT scan of a patient with hydatid disease shows an average-sized liver with normal attenuation features, with a segment VI shows cystic lesions and peripheral calcifications (red arrows), measures about 2.6x2.9cm in diameter. D1&D2: *S. mansoni*-infected patient with normal liver sonographic appearance.

2. Liver Function Parameters:

As shown in Table 1, the HCV-infected group recorded the highest levels in both AST and ALT. Compared to the normal controls, ALT levels significantly increased in each diseased group ($p < 0.001$ for the HCV-infected group, schistosomiasis and hydatidosis ; $p = 0.007$ for HBV). Regarding AST, a significant increase was observed only in the HCV- and HBV-infected groups compared to the normal controls ($p < 0.001$ for both). There was also a significant difference between the HBV-infected group and the hydatidosis group ($p = 0.03$).

3. Serum Fibrosis Markers:

3.1. Overall Study Population:

The results showed a significant increase ($p < 0.001$) in the mean serum levels of YKL-40, TIMP, and M-CSF serum markers in the total patients' groups (**n** = 80)

compared to the controls (**n** = 20): TIMP-1 = 28.8 ± 12.9 vs 12.2 ±4.9 ng/mL, M-CSF = 0.4 ± 0.2 vs 0.2 ± 0.04 ng/mL, and YKL-40 = 41.3 ± 18.7 vs 26.4 ±6.9 ng/mL, respectively.

3.2. Comparison of Fibrosis Markers Between Different Liver Disease Groups And Controls:

3.2.1. Serum TIMP-1. Compared to the control group, the TIMP-1 marker level was significantly elevated in each of the four diseased groups ($p < 0.001$ for HCV, HBV and schistosomiasis; $p = 0.001$ for hydatidosis). The highest mean values were observed in the HCV-positive group (37.6 ± 17 ng/mL), followed by the HBV-positive group (32.6 ± 6.8 ng/mL), as shown in Table 1 and Figure **2.**

3.2.2. Serum YKL-*40*. Although YKL-40 serum levels were elevated in each diseased group compared to controls, no significant differences were found in schistosomiasis, hydatidosis, and HBV-infected patients (***p*** = 0.1, 0.2, 0.4, respectively). However, the HCV-positive group exhibited the highest YKL-40 levels, which were statistically significant ($p < 0.001$) (Table 1 and Fig. **2).**

3.2.3. Serum M-CSF. M-CSF levels were significantly higher in each diseased group compared to the control group ($p = 0.01$ for hydatidosis, 0.002 for HBV, and < 0.001 for both HCV and schistosomiasis) (Table 1 and Fig. 4).

3.3. Comparison of Serum Fibrosis Markers Among Liver Diseases Groups:

Using pairwise comparison, TEMP1 levels were significantly higher in HBV positive group vs. hydatid and schistosomiasis groups ($p = 0.003$, $p < 0.001$, respectively) and in HCV vs. hydatid and schistosomiasis groups ($p = 0.03$, $p = 0.01$, respectively). Additionally, the HCV-positive group exhibited significantly higher serum levels of YKL-40 and M-CSF compared to the HBV-positive group (both: $p = 0.03$) (Table 1 and Figs. 2,3, and 4).

3.4. Intercorrelations of the Fibrosis Serum Markers:

Overall, in the total patients (n = 80), the three serum markers TIMP-1, YKL-40, and M-CSF showed significant strong positive correlations (**p** ≤ 0.001), with **r** values of 0.7 (TIMP-1 and M-CSF), 0.6 (YKL-40 and M-CSF), and 0.5 (TIMP-1 and YKL-40). This data is illustrated in Figure 5

4. Correlation Between Serum Fibrosis Markers and Liver Function Parameters:

The serum markers, TIMP-1, YKL-40, and M-CSF, showed significant positive correlations with ALT (r = 0.3), with *p* values of 0.001, 0.002, and <0.001, respectively. In addition, TIMP-1 positively correlated with AST (r=0.3, p <0.001), Table 2.

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Table 1: Comparison of demographic data, serum fibrosis markers (YKL-40, TIMP1, M-CSF) and liver function tests (ALT, AST) among the study groups**.**

Parameter		**HBV-positive (n=20)**	**HCV-positive (n=20)**	**Hydatid (n=20)**	**Schistosomiasis (n=20)**	**Control (n=20)**
Sex	Male	10	8	14	12	10
	Female	10	12	6	8	10
Age	Mean± SD	54.2± 9.8	54± 10.7	45.3±6.3	50 ± 11.6	48 ± 17.3
ALT (U/L)	Mean± SD	22.4±11.2 P^{*}	26.9 ±12.4 P^{**}	22 ±8.04 P^{**}	25.3 ± 13.9 P^{**}	13.2±3.4
AST (U/L)	Mean± SD	24.4±6.97$^{@}$ P^{**}	26.6 ± 13.2 P^{**}	18.6 ±7.4$^{@}$	20.4 ± 10.99	15.6 ± 4.4
YKL-40 (ng/ml)	Mean± SD	34.4±11.2 $^{@}$	53.1± 23.9$^{@}$ P^{**}	38.2 ±17.2	39.6 ± 15.75	26.4 ±6.9
TIMP1 (ng/ml)	Mean± SD	32.6±6.8$^{\$,\&}$ P^{**}	37.6±17.9$^{@,\#}$ P^{**}	22.9±10.8$^{\#\&}$ P^{**}	21.47±5.8 $^{@,\$}$ P^{**}	12.2 ±4.9
M-CSF (ng/ml)	Mean± SD	0.3 ± 0.1 $^{@}$ P^{*}	0.47 ±0.2 $^{@}$ P^{**}	0.35± 0.2 P^{*}	0.35 ± 0.1 P^{**}	0.2 ± 0.04

@, &, $, #: Denote a significant difference between any 2 disease groups within the same row, sharing the same superscript symbol, as determined by pairwise comparison.

P: Denote a significant difference between the control group and each other group: $P^{*} \leq 0.01$, $P^{**} \leq 0.001$

TEMP1: Tissue inhibitor of metalloproteinases, YKL-40: Chitinase-3-Like Protein, M-CSF**:** Macrophage colony-stimulating factor, AST: Aspartate aminotranferase, ALT: Alanine aminotransferase.

Table 2: Correlation between serum fibrosis markers (YKL-40, TIMP1, M-CSF) and the liver function parameters (ALT, AST*)* in liver disease groups (n= 80).

		TIMP1 (ng/ml)	**YKL-40 (ng/ml)**	**M-CSF (ng/ml)**
ALT (U/L)	R	0.324	0.304	0.339*
	P value	0.001*	0.002*	< 0.001*
AST (U/L)	R	0.345	0.161	0.140
	P value	< 0.001*	0.109	0.166

Statistically significant, *R:* Correlation Coefficient, TEMP1: Tissue inhibitor of metalloproteinases, YKL-40: Chitinase-3-Like Protein, M-CSF**:** Macrophage colony-stimulating factor, AST: Aspartate aminotranferase, ALT: Alanine aminotransferase.

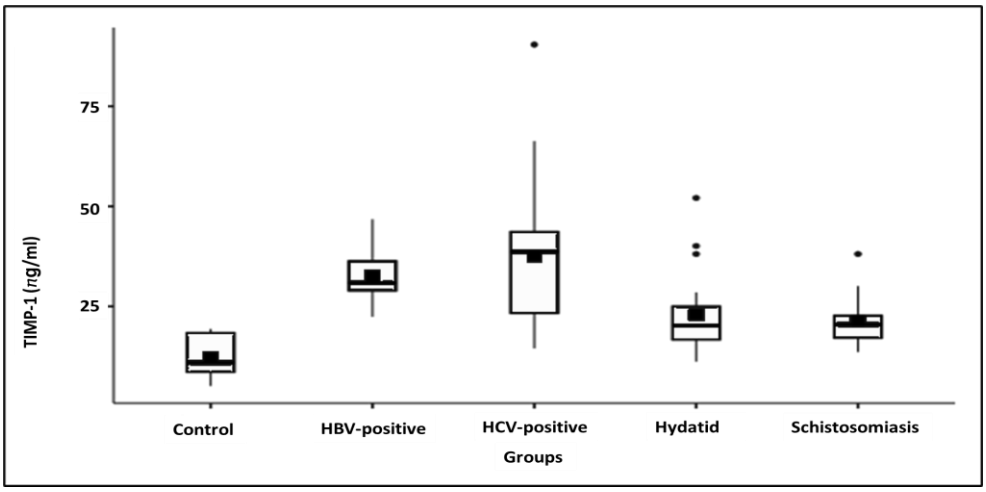

Fig. 2: Box-plot diagram showing serum levels of TIMP-1 marker in the control and diseased groups. The small black square represents the mean value.

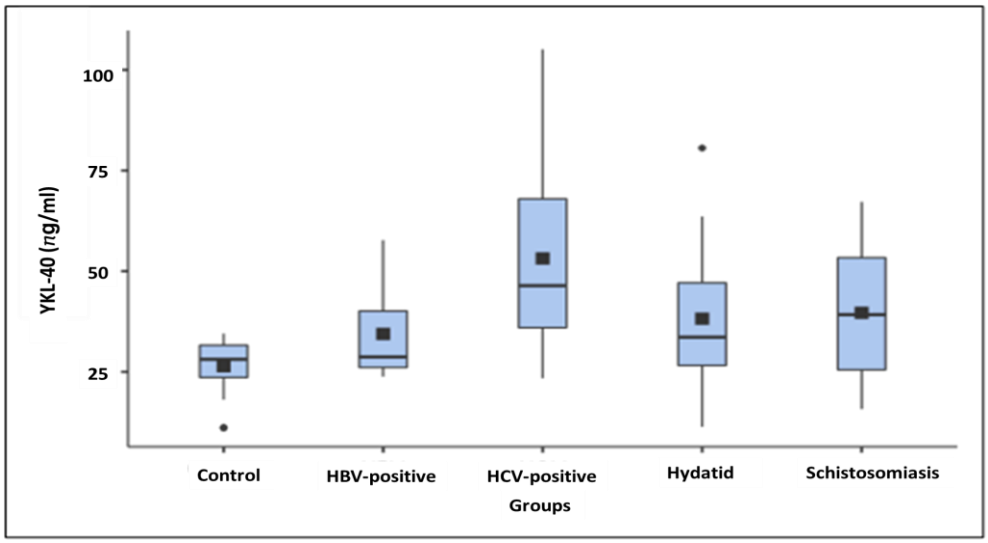

Fig. 3: Box-plot diagram showing serum levels of YKL-40 marker in the control and diseased groups. The small black square represents the mean value.

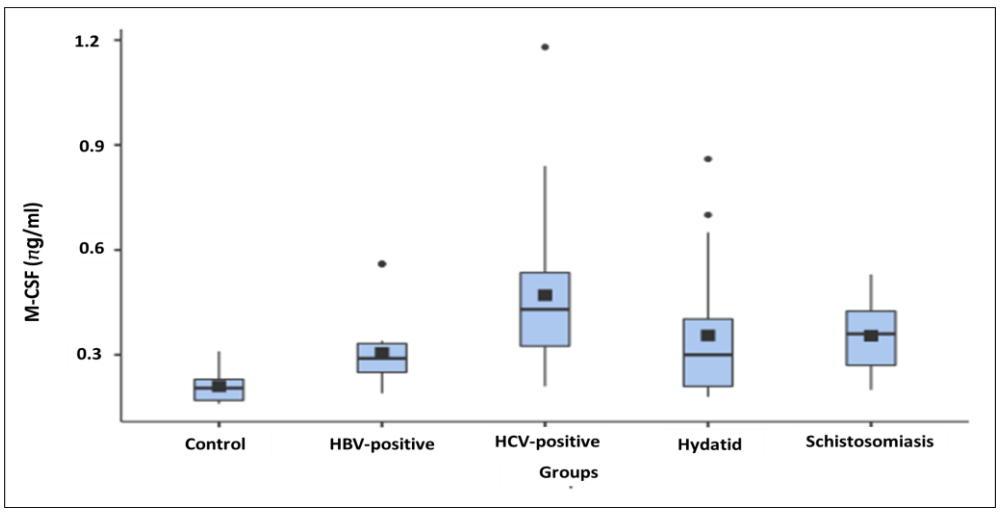

Fig. 4: Box-plot diagram showing serum levels of M-CSF marker in the control and diseased groups. The small black square represents the mean value

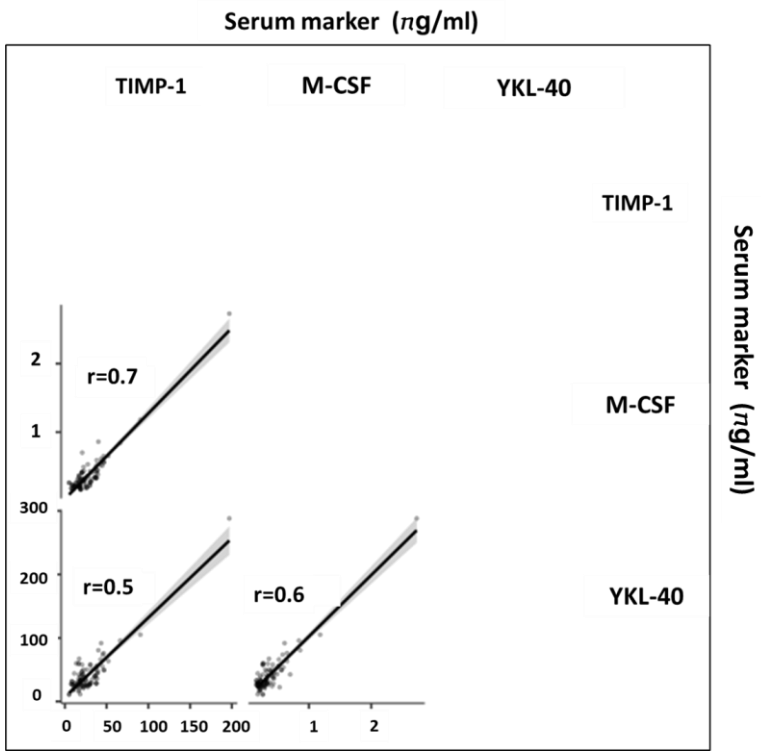

Fig. 5: Scatter plots showing significant positive correlations ($p \leq 0.001$) among the serum markers YKL-40, TIMP-1, and M-CSF in the total liver disease cases (n = 80). **r**: correlation coefficient.

DISCUSSION

Hepatic fibrosis is a complex process that yields chronic hepatocellular damage. The fibrogenic process disrupts the normal balanced deposition and degradation of the ECM involving collagen, glycoprotein, elastin, and proteoglycan [Lin *et al.,* 2018; Chimponda and Mduluz, 2020). Sun *et al.* (2020) determined that the noninvasive methods to trace fibrosis are necessary. Early indication of fibrosis might be useful in reversing the process, thus minimizing parenchyma damage.

In the current work, despite being mildly elevated, ALT levels (in HCV-positive, hydatidosis, and schistosomiasis groups) and AST levels (in HBV and HCV–positive groups) showed significant increases compared to their healthy controls (ULN). Wang *et al.* (2017) considered high-normal ALT in liver injury. Abdulrazzaq *et al.* (2022) assumed that ALT is a more sensitive biomarker of acute hepatic cell injury due to HCV and HBV than AST. In HCV infection, Amjad *et al.* (2021) presumed that ALT is a more specific biomarker of liver damage. In HBV patients, ALT level correlates with HBsAg, HBeAg, and HBV-DNA load (Li *et al.,* 2018). In hydatidosis, Ismail *et al.* (2024) reported patients with cysts > 5 cm have increased levels of the liver enzymes compared with a cyst < 5 cm. Ali and Jihad, (2022) assumed that ALT serum level dramatically elevates as hydatidosis progresses in the liver tissues. Also, in an echinococcosis-infected murine model, the increased AST and ALT serum levels were associated with the oxidative damage of infected liver tissues (Tang et al., 2017). In schistosomiasis, Bisetegn *et al.* (2022) reported higher liver enzymes when compared with healthy subjects. Nevertheless, circulatory ALT showed inverse correlation with the liver egg load in *S. mansoni*-infected murine models (Müller *et al.,* 2024).

In the present study, we investigated and compared the serum levels of TIMP, M-CSF, and YKL-40 biomarkers in Egyptian patients with tropical hepatic

diseases: HBV, HCV, hydatidosis, and *Schistosomiasis mansoni*.

Compared to the control group, there was a significant rise in TIMP-1 marker level in each HCV and HBV- and HBV-infected group. This result may reflect the rapid pathophysiology of blood-borne hepatitis viruses in developing nations (Azzam et al., 2023). Current observations were parallel to those reported by Latronico et al (2016), who demonstrated significantly higher TIMP-1 levels in HCV patients compared to healthy subjects. Dawood et al. (2018) and Chan *et al.* (2022) depicted TIMP as a therapeutic target in HBV and HCV infections.

In schistosomiasis, the elevated TIMP-1 circulatory levels may reflect the activity of HSC around the hepatic granuloma, ultimately contributing to schistosomiasis-induced cirrhosis. Lu et al (2024) suggested that TIMP activity in *S. japonica* is related to the activity of NLRP3 inflammasome. Accordingly, TIMP was speculated as a therapeutic target in *S. japonica* by praziquantel (Niu *et al.,* 2022) and in *S. mansoni* by Xiaochaihu decoction (a Chinese herbal medicine) (Huang *et al.,* 2020) and Schisandrin B (Lam *et al.,* 2021). Shan et al (2023) deduced that restoring matrix metalloproteinases (MMPs)/TIMP1 balance ameliorates liver fibrosis. This might be attributed to the anti-fibrosis role of the MMPs through the degradation of ECM and the correlating effect on the fecal elimination of *S. mansoni* eggs.

Elevations of TIMP-1 in the anti-hydatid anti-sera might elucidate the potential profibrotic role of hydatidosis in the peri-cystic liver parenchyma. Hasanzadeh *et al.* (2022) concluded that in the fibrotic hepatic tissues obtained from 30 hydatid patients, the increased levels of MMPs are counterbalanced by increased TIMP-1 mRNA expression. Interestingly, Hasanzadeh *et al.* (2024) suggested that *E. granulosus* byproducts regulate the host MMPs and thus, are potential biomarkers for the disease prognosis (Hasanzadeh *et al.,* 2024). This may be attributed to an immune background dominated by TGF-β1 and IFN-γ (Mirzavand *et al.,* 2020).

YKL-40 recorded significant elevations in HCV-positive cases, while it relatively increased in schistosomiasis, hydatidosis, and HBV. This might be related to the immunogenicity of the pathogens and the pathophysiology of YKL-40 enrolled by the immune cells and the vascular smooth muscle cells. YKL-40 triggers ECM and tissue remodeling and has been related to hepatic fibrosis staging in HBV and HCV patients (Maroto-García *et al.,* 2024). YKL-40 triggers fibroblast and cell adhesion to ECM (Chimponda and Mduluza, 2020, Hanno *et al.,* 2022). Similar findings were documented in patients with *S. japonica* (Tang *et al.,* 2017) and *S. haematobium* (Sun et al., 2020). Multiple studies demonstrated the feasibility of YKL-40 as a good indicator of liver fibrosis (Zheng *et al.,* 2005; Sun *et al.,* 2020). YKL-40 was also described as a valuable predictor of the progression or regression of liver fibrosis in schistosomiasis, HCV, and HBV liver diseases (Chimponda, and Mduluza, 2020). Moreover, Zheng *et al.* (2005) highlighted the clinical value of YKL-40 as a biomarker for assessing ECM deposition, as its levels correlate with the severity of hepatic fibrosis (Zheng *et al.,* 2005). Additionally, previous immuno-histochemical studies demonstrated active hepatic expression of YKL-40 in fibrosis-affected areas, particularly in Kupffer cells and HSCs (Kumagai et al.,2016). Nevertheless, further studies on the prognostic role of YKL-40 in hydatidosis and HBV infection remain recommended.

The M-CSF levels were significantly higher in each hepatic disease compared with the control group. M-CSF triggers hepatocarcinogenesis by inducing an angiogenic factor produced by liver MΦ (Kono *et al.,* 2016). Another study reported that M-CSF receptor antagonists in murine models suppressed carcinogenesis (Akazawa *et al.,* 2019). Moreover, the expression of the M-CSF factor in liver tissues predicts cancer recurrence (Kono *et*

al., 2016). Early literature related the high levels of M-CSF in the tumor liver tissue to poor survival (Zhu *et al.,* 2008). Seriously, the contribution of HBV and HCV in hepatocellular carcinoma has been documented (Perz *et al.,* 2006). Thus, M-CSF might be a useful therapeutic target against liver cancers in viral hepatitis, and clinical trials to inhibit cancer initiation and progression in HCV and HBV are recommended.

Increased levels of M-CSF in parasitic hepatic diseases (schistosomiasis and hydatidosis) may denote the induced fibrosis caused by M2 macrophage polarization. Indeed, M-CSF triggers monocyte differentiation to macrophages, which are plastic cells that can polarize to either pro-inflammatory M1 or the anti-inflammatory M2 phenotypes (Murray *et al.,* 2017; Sun and Matsukawa, 2024). Furthermore, M-CSF can induce anti-inflammatory M2 phenotype polarization along with other cytokines (Sun and Matsukawa, 2024). M1 macrophages induce a Th1 immune response targeting early parasite clearance. Yet, M2 cells, in the context of the TH2 immune response, induce parasite persistence in tissues and the progression of liver fibrosis in *S. japonicum* infections (Ren *et al.,* 2022; Wang *et al.,* 2023; Sellau *et al.,* 2021). Thus, targeting M-CSF to alleviate parasitic disease-associated fibrosis might be useful in further studies.

The pairwise comparison of the hepatic diseases showed significant elevations of TIMP-1 by viral hepatitis (HBV and HCV) compared with parasitic infections (schistosomiasis and hydatidosis). This might be attributed to the local granulomatous reactions triggered by parasites that seemed to hamper the immunity within the liver micro-environment (Giorgio *et al.,* 2020). For instance, Díaz *et al.* (2018) suggested that eosinophil infiltrations across various *Echinococcus* species are extremely active at killing metacestodes in solid tissues, early and throughout chronic granulomatous reactions (Díaz *et al.,* 2018). Also, in schistosomiasis, the eosinophil-rich granuloma appears to possess a paradoxical role. Peri-oval granuloma, despite being the major etiology of pathology, minimizes collateral damage in the liver and enteric tissues. Furthermore, the granulomatous reaction facilitates the successful egg excretion from the host (Gobbi *et al.,* 2020; Hams *et al.,* 2013). On the contrary, the vigorous and devastating genomic amplification and replication of hepatic viral diseases are the key processes in pathogenesis. Moreover, hepatic granulomas were depicted as rare pathological processes in chronic HCV and HBV (Snyder *et al.,* 2008; Snyder et al., 2008), yet their existence in HCV was related to successful treatment (Snyder *et al.,* 2008).

The pairwise comparison revealed the significant differences in YKL-40 and M-CSF serum levels between HCV and HBV infections. HCV induces YKL-40 expression through ROS-dependent and -independent pathways. In addition, the TNF-α secretion and NF-κB activation are involved. So far, a positive feedback loop has been speculated wherein the YKL-40 protein stimulates HCV replication and thus triggers more release of the hepatic profibrogenic cytokines (Cheng *et al.,* 2021). In contrast, HBcAg appeared to have a strong immunogenic effect, stimulating CD8 T cells that facilitate its elimination. Besides, HBV can upregulate NF-κB and trigger pro-inflammatory cytokines in macrophages (Schuch *et al.,* 2019; Yi *et al.,* 2020). In contrast to our study, Qi *et al.* (2019) reported that HBV-related cirrhotic portal hypertension induces higher YKL-40 levels than those with HCV infection. This may be attributed to variations in the number of study participants or the use of different kits.

Correlation analysis revealed a positive relationship between the three fibrotic markers (TIMP-1, YKL-40, and M-CSF). A better understanding of hepatic fibrogenesis may reveal new diagnostic and

predictive markers and therapeutic targets. For instance, Yao *et al.* (2022) suggested that the elevations in M-CSF and TIMP-1 levels in HBV correspond to the degree of liver fibrosis. Therefore, the combined assessment of these markers might have better diagnostic implications. Lei et al. (2022) deduced that serum TIMP-1 and M-CSF can be vital reference indexes and predictive factors in HBV liver cirrhosis. Collagen and circulatory monocytes expressing the M2 marker can be useful fibrotic biomarkers in chronic HCV (Saha *et al.,* 2016). Hence, TIMP-1, YKL-40, and M-CSF molecules may constitute a useful panel of profibrotic biomarkers for tropical hepatic diseases common in Egypt. However, further longitudinal studies are recommended.

ALT serum levels significantly correlated with TIMP-1, YKL-40, and M-CSF. Also, AST positively correlated only with TIMP-1. Marcellin *et al.* (2002) proposed that high ALT levels are always associated with fibrosis progression. Hui *et al.* (2003) stated that HCV patients with increased ALT are more likely to progress into fibrosis compared with patients with persistently normal levels. Also, Sarin *et al.* (2015) demonstrated guidelines that endorse only ALT, HBV DNA levels, and HBsAg to outline different stages of HBV infection without the need for liver biopsy. In hydatidosis, Tian *et al.* (2020) demonstrated significant elevations in the circulatory transaminases in association with hyperplasia and extension of fibrosis in the portal area. In schistosomiasis, elevation of ALT occurs by the time relying on augmented lymphatic hyperplasia (Müller *et al.,* 2014).

The limitation of the study was the small sample size, as it was restricted by specific inclusion criteria and the low flow of HCV-infected patients, likely due to the mass treatment campaigns conducted in recent years.

5. Conclusion

This study found significantly elevated serum levels of the fibrotic biomarkers YKL-40, TIMP-1, and M-CSF in patients with liver diseases compared to healthy controls. Among the liver disease groups, HCV patients showed the highest serum levels of the three markers. Comparisons revealed higher TIMP-1 levels in viral hepatitis (HBV and HCV) than in parasitic infections (schistosomiasis and hydatidosis). Additionally, HCV patients had notably higher YKL-40 and M-CSF levels than HBV patients. The study also demonstrated significant positive correlations between TIMP-1, YKL-40, and M-CSF and ALT indicating their potential as predictive markers for liver fibrosis. Future research, including larger cohort studies and therapeutic trials targeting TIMP-1 and M-CSF, is recommended to investigate their clinical utility in liver disease management and prognosis.

Declarations:

Ethics Approval and Consent to Participate: The Research Ethical Committee of the Faculty of Medicine, Beni-Suef University (FM-BSU REC) has approved the protocol from an ethical point of view (FMBSUREC/01092024/Eid). The committee was organized according to the Declaration of Helsinki guidelines, the International Conference of Harmonization ICH, and the United States (FWA) for the Protection of Human Subjects. An informed written consent was obtained from all participants before enrollment in the study

Competing interests: The author declares no competing interests.

Author's Contributions: E. E., H. O. and M. A. contributed to the study conception and design. E. E., S. E., M. A. and R. E. contributed to the Methodology. Data collection and analysis were performed by H. O., E. E., M. A., S. E. and F. M. Writing the paper was performed by E. E., H. O., M. A. All authors reviewed and edited the manuscript.

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