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Assessment of Cartilage Oligomeric Matrix Protein Biomarker for Laboratory Diagnosis of Hepatocellular Carcinoma

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Abstract

Background/Aim: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for approximately 782,000 new cancer cases and 745,000 deaths worldwide. Because HCC is usually detected at an advanced stage, the prognosis is worse. As a result, early diagnosis and biomarker discovery may greatly enhance results. **Material/Methods:** In a recent study, the study population include 173 patients (130 males and 43 females, aged 28-71 yrs.), with different degrees of liver fibrosis and 52 patients with HCC. According to the METAVIR scoring system, non-significant fibrosis (F0-F1) was identified in 33 patients (F0 in 22 and F1 in 11 patients), and significant fibrosis (F2-F4) was identified in 88 patients (F2 in 21, F3 in 23, F4 in 44 patients). Serum samples of 173 patients were tested for Cartilage oligomeric matrix protein (COMP) levels using ELISA. Statistical analysis was performed using SPSS. **Results**: The result revealed that there was very highly significant increase ($p< 0.0001$) in ALT, AST, and ALP in group F4 compared to group F0-F1 and F2-F3 but the conc. of AFP (mean = 217.12 ng/ml) very highly significant in HCC compared to F0-F1, F2-F3 and F4. It was discovered that HCC patients' serum levels of COMP (mean = 19.69 pg/mL) were significantly higher ($p < 0.0001$) than in the significant liver fibrosis patients (F2, F3) (mean = 8.83 pg/mL), non-significant liver fibrosis (F0-F1) control group (mean=3.35 pg/mL) and cirrhotic liver patients (F4) (mean = 13.82 pg/mL). **Conclusion**: The HCC-COMP biomarker test offers a very precise diagnostic approach for the identification of HCC.

Keywords: HCC, COMP biomarker, ALT, AST, AFP.

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Introduction

Eighty percent of all liver cancers are hepatocellular carcinomas (HCCs), the most prevalent primary liver cancer and a leading cause of cancer-related deaths globally. Chronic hepatitis B or C virus infections, obesity, alcohol abuse, and nonalcoholic fatty liver disease are the major known risk factors for the development of hepatocellular carcinoma. The global incidence and mortality rates of HCC continue to rise despite progress in our understanding of these disease-related risk factors. Regretfully, a significant percentage of HCC patients are discovered when the disease has progressed. Worldwide variations exist in the rates of both incidence and death, which are impacted by risk factors related to the environment and communication, the availability of healthcare resources, and the efficacy of early detection and treatment. HCC progresses quickly, which increases the risk of premature death due to the disease's poor response to chemotherapy and radiation. Therefore, it is essential to identify precancerous HCC nodules early to improve the 5-year survival rate and maybe lower incidence and mortality (1-2).

On the other hand, liver transplantation and resection are the principal therapies for HCC (3). Nevertheless, poor prognoses stemming from low susceptibility to chemotherapy and radiation, along with high rates of metastases and relapse, restrict the available therapeutic options (4). The primary goal of the screening program is to find nodules before cancerous growths or symptoms appear, allowing for early-stage therapy. Accurate diagnosis and successful treatment of HCC require comprehensive evaluation, which includes imaging, laboratory testing, and histological inspection. Imaging is crucial to diagnosis, and the quality of the imaging instrument, the examiner's skill, and the methodologies used all have a big impact on the results.

Alpha-fetoproteins (AFP) have low sensitivity, often as low as 35% in cases of cirrhosis, making them unsuitable for early-stage HCC screening tests. As a result, it is critical to enhance early detection and find efficient therapies (5). Biomarkers are measurable indicators of physiological or pathological processes, as well as responses to various diagnostic or therapeutic procedures. To date, a number of potential biomarkers for HCC early diagnosis and prognosis prediction have been found. Nevertheless, most of them are currently unsuitable for use in a clinical environment due to issues with measurement validity and reproducibility. More novel biomarkers for the early detection and surveillance of hepatocellular carcinoma will become accessible with the development of genomes and proteomics.

On the other hand, HCC is a complicated illness brought on by several risk factors. It is so challenging to use a single biomarker for patients with hepatocellular cancer. For the early diagnosis and prognosis prediction of HCC, the creation and optimization of biomarker combinations will have greater significance. With better detection technologies and biomarker combination optimization, the promise of early detection and treatment of hepatocellular carcinoma should soon be achieved (6).

A cartilage oligomeric matrix protein (COMP) is a member of the thrombospondin family. Its primary role is to bind type I and type II COMP fibers and catalyze fibrillary COMP assembly in general. It is primarily expressed in cartilage. In recent times, it has also been demonstrated in experimental animal models that COMP controls the deposition of COMP, which in turn causes liver fibrosis. In light of this, we examined in the current study the potential use of the COMP biomarker as a diagnostic tool in patients with HCC and contrasted it with established, widely accepted techniques for determining the stage of liver disease, specifically HCC. Our findings suggest that the COMP biomarker could help improve liver cancer laboratory diagnosis and may be a potential target for therapy monitoring (7-13).

Materials and Methods

Study Patients and sample collection

A total of 173 HCV-infected Egyptian patients (aged 28-71 years) were collected during the period February 2021 to November 2022 from Minia University Hospitals, Minia Oncology Center, ElMinia, EGYPT and Gastrointestinal Surgery Center, Mansoura University, Mansoura, Egypt. They were 33 NS fibrosis (controls), 44 significant fibrosis (F2, F3), 44 cirrhosis (F4), and 52 HCC. For the current investigation, informed consent was obtained from all participants. Each patient underwent a thorough history and clinical examination.

The patients had not undergone any form of preoperative chemotherapy or radiation therapy from the hospitals and centers mentioned above. Moreover, patients who suffered from chronic obstructive pulmonary disease, diabetes mellitus, other site malignancy, and human immunodeficiency virus. This study eliminated kidney illnesses, metabolic liver disease, insufficient liver tissue for fibrosis staging, missing data on liver function tests, and any other illness not related to HCV.

As part of the standard clinical care for these individuals, a liver biopsy was conducted. Hematoxylin-eosin stain was regularly applied to liver biopsy specimens, which were obtained from each patient using a minimum of four liver biopsy needles and an 18-gauge or larger needle. Liver biopsies, except for cirrhosis, for which there was no restriction, had to be at least 15 mm in size and/or have five portal tracts to be deemed suitable for scoring.

Liver fibrosis was categorized into four stages using the METAVIR score (F0-F4). "F0 indicates no liver fibrosis; F1, portal fibrosis alone; F2, portal fibrosis with rare septet; F3, portal fibrosis with frequent septet; and F4, cirrhosis, were the five points used to assess liver fibrosis. Stage F2-F4 fibrosis was referred to as major fibrosis, whereas stage F0-F1 fibrosis was referred to as mild or no significant fibrosis.

Blood samples were collected from consenting patients using standard phlebotomy procedures. A total of 10 mL of blood was collected and placed in additive-free (serum) blood tubes. A portion of the blood sample is tested fresh for routine blood pictures, which include platelet counting. At room temperature, samples were allowed to clot for 10 min. The serum was extracted from the clot with a pipette, poured into a clean tube, and centrifuged at 1000 g for 3 minutes at 4°C. Serum was labeled in tubes and kept at -80°C until the time of analysis.

Laboratory investigations

It included liver function tests (AST, ALT, ALP, and Albumin), kidney function (Creatinine), INR, and prothrombin concentration were measured using standard procedure and a commercially available colorimetric assay kit (Sigma-Aldrich).

Assessment of COMP and AFP using sandwich ELISA

The biomarker COMP and tumor marker alpha-fetoprotein (AFP) were detected using ELIZA kits (Elabscience, Houston, Texas, USA), based on the manufacturer's guidelines.

Ethical approval

The present study protocol was approved by the Ethical Committee of the Faculty of Pharmacy, Minia University, Egypt (P. No. MPEC 240501).

Statistical Analysis

A two-sided $P < 0.05$ was used to determine statistical significance for all statistical analyses, which were performed using the statistical software program SPSS 22.0 for Microsoft Windows. The mean ± SD was used to express numerical data. The t-test was utilized to examine the marker levels and compare them between independent groups.

We computed the Area Under Curve (AUC) for the ROC curve. The AUC varies from 0.5 (indicating a non-informative marker) to 1 (indicating a perfect marker). It represents the likelihood that a randomly

chosen case will have a higher marker value than a randomly chosen control. To investigate if a marker panel can result in better performance, the ROC analysis was first performed on each marker and subsequently on the combination of markers.

Results

Demographic data of study patients

The present study included 52 HCC patients (aged 35 to 81 years), with different stages, grades, tumor sizes, and sub-sites from Minia Oncology Center. Also, 121 patients in different stages of liver fibrosis (aged 18-71 years) are included in the present study. The present study included 130 males (72.2%) and 43 females (27.8%). A liver biopsy was performed for all 173 patients to identify the degree of liver fibrosis.

According to the METAVIR scoring system, the fibrosis stage was F0 in 22 patients (12.2%), F1 in 11 (6.1%), F2 in 21 (11.7%), F3 in 23 (12.8%), F4 in 44 patients (24.4%) and HCC in 52 patients (28.9%). Accordingly, non-significant fibrosis (F0-F1) was identified in 33 samples (20 males & 13 females) and significant fibrosis (F2-F4) was in 88 samples (66 males &22 females). No statistically significant difference ($p > 0.05$) was found between sex distribution in non-significant and significant liver fibrosis. The incidence rates increase sharply with age from 35 to 71 and are rare before the age of 20 years, the incidence rates of liver fibrosis decrease by ages 75 to 80.

Value of indirect laboratory biomarkers in the detection of HCC and liver fibrosis

Indirect biochemical markers

The distribution of evaluated biochemical markers ALT. AST, ALP, and BIL differed significantly. There are statistically highly significant ($p < 0.0001$) differences between ALT, AST, ALP, and BIL in patients with cirrhotic liver (F4) compared with liver fibrosis patients (F2, F3) and non-significant liver fibrosis (F0-F1). However, there was a very highly significant ($p < 0.0001$) of AFP (mean = 217.12± 29.84 ng/ml) in HCC than the groups F0-F1, F2-F3 and F4. They found a slight increase in Albumin in F0-F1 and F2-F3 than the F4 and HCC.

There is no significant ($P > 0.05$) difference between creatinine and AST/ALT ratio with liver fibrosis patients (F2, F3), non-significant liver fibrosis (F0-F1), cirrhotic liver patients (F4) and HCC patients (Table **1**, Fig 1 A-H).

Indirect Hematological markers

The value of hematological markers of Plt was significantly ($p<0.05$) higher in HCC compared to F0-F1, F2-F3, and F4. However, the value of INR slightly increased in HCC compared to the control group (Table 2 and Fig 2 A-B).

Determination of COMP in serum samples

When comparing the COMP levels between controls (n = 33) and patients with HCC (n = 52) by using the mean value of them, it was found that the serum level of COMP in HCC patients (mean = 19.69 pg/mL) was significantly higher ($p < 0.0001$) than in the significant liver fibrosis patients (F2, F3) (mean = 8.83 pg/mL), non-significant liver fibrosis (F0-F1) control group (mean=3.35 pg/mL) and cirrhotic liver patients (F4) (mean = 13.82 pg/mL) (Table 3, Fig 3).

Comparison of COMP with laboratory biomarkers

The laboratory biomarkers (AST, ALT, Bil, INR, ALP, ALB, AFP, PLT, and AST/ALT) are widely used as noninvasive biomarkers for HCC in the same serum samples. The AUC (area under ROC curve) of all laboratory biomarkers for discriminating HCC patients from non-fibrotic patients are shown in (Fig 4).

Table 1. Levels of Indirect Biochemical Markers in liver fibrosis and HCC patients.

Biochemical marker	Mean ± SD				P Value
	F0, F1	F2, F3	F4	HCC	
ALT (IU/L)	49.64 ± 20.12	68.43 ± 37.34	105.48 ± 52.57	50.50 ± 26.02	0.0001
AST (IU/L)	40.09 ± 17.24	63.93 ± 40.93	111.66 ± 63.35	53.87 ± 22.10	0.0001
ALP (IU/L)	66.36 ± 16.43	88.41 ± 54.62	131.46 ± 120.39	98 ± 67.78	0.004
AFP (ng/ml)	3.05 ± 2.18	25.19 ± 36.96	93.76 ± 112.08	217.12 ± 29.84	0.0001
ALB (g/dl)	4.33 ± 0.24	4.17 ± 0.49	3.75 ± 0.57	3.87± 0.63	0.0001
BIL (mg/dl)	1.35 ± 1.52	1.35 ± 0.92	4.58 ± 7.62	1.08 ± 0.57	0.0001
Creatinine (mg/dl)	0.72 ± 0.23	0.71 ± 0.26	0.76 ± 0.49	0.84 ± 0.45	0.387
AST/ALT	0.92 ± 0.56	0.99 ± 0.42	1.10 ± 0.42	1.16 ± 0.34	0.050

Table 2**. Levels of Indirect Hematological Markers in liver fibrosis and HCC patients**

Hematological Marker	Mean ± SD				P Value
	F0, F1	F2, F3	F4	HCC	
P1t	176.73 ± 49.66	175.39 ± 47.44	132.86 ± 29.49	189.71 ± 84.307	0.0001
1NR	1.12 ± 0.10	1.23 ± 0.12	1.42 ± 0.16	1.27 ± 0.18	0.050

Table 3. Determination of COMP in serum samples

Investigated Marker	Mean ± SD				P Value
	F0, F1	F2, F3	F4	HCC	
COMP pg/mL	3.35 ± 1.19	8.83 ± 4.05	13.82 ± 7.65	19.69 ± 9.24	0.0001

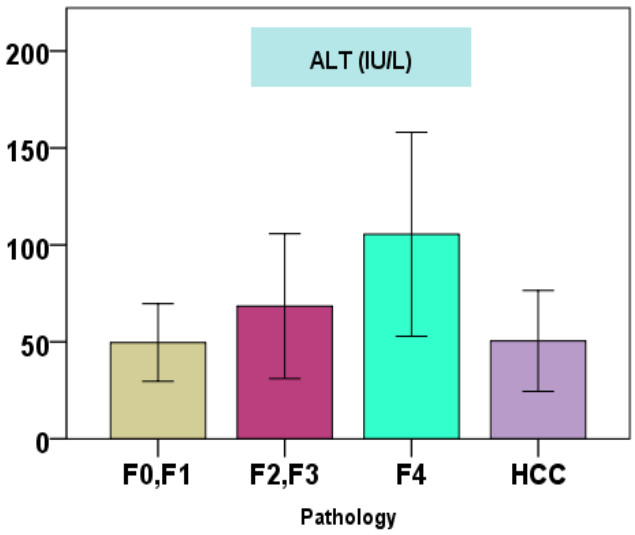


Fig 1 A

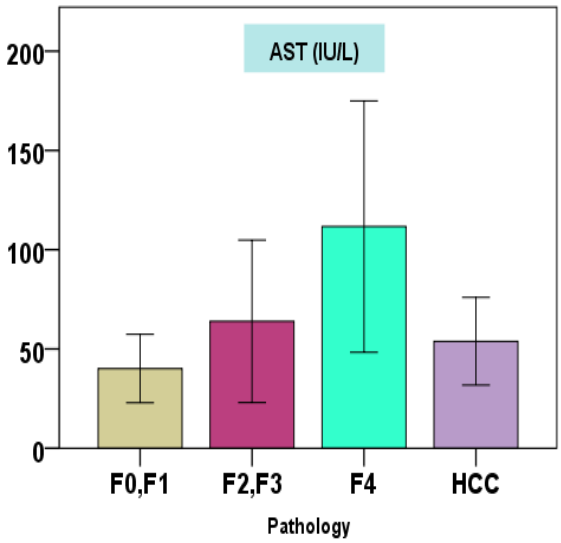


Fig 1 B

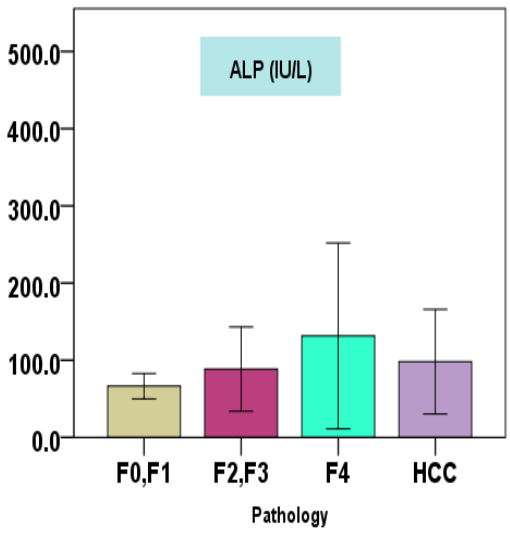


Fig 1 C

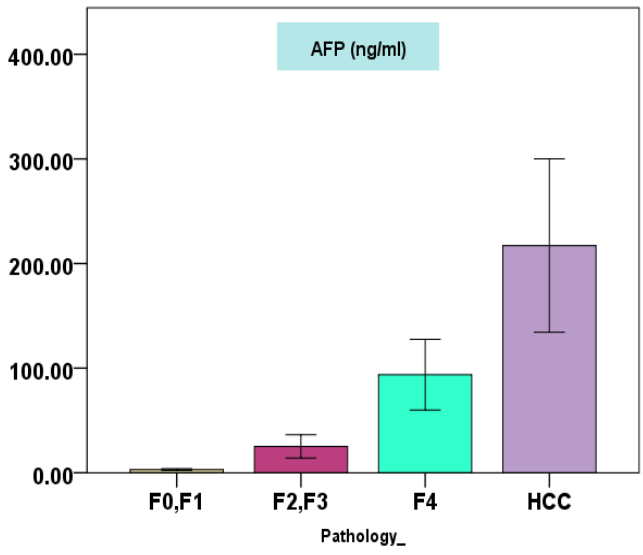
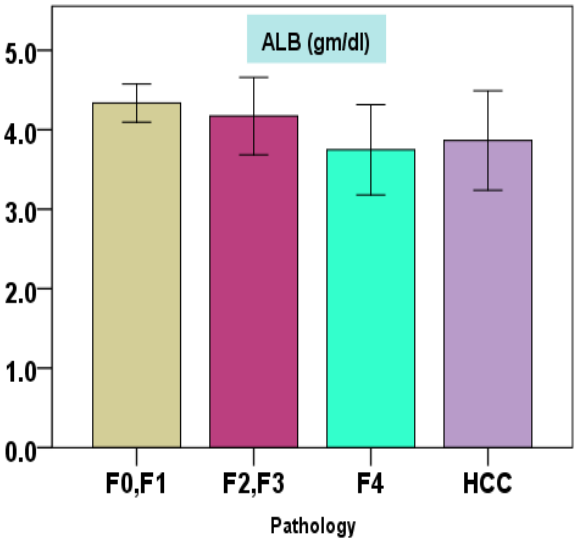


Fig 1 D



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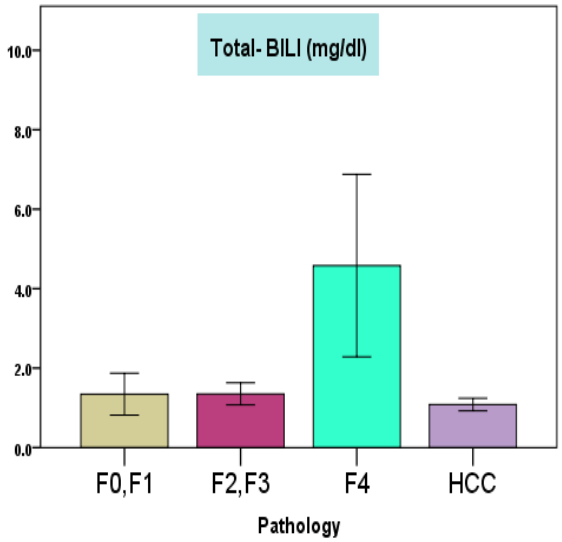

Fig 1 F

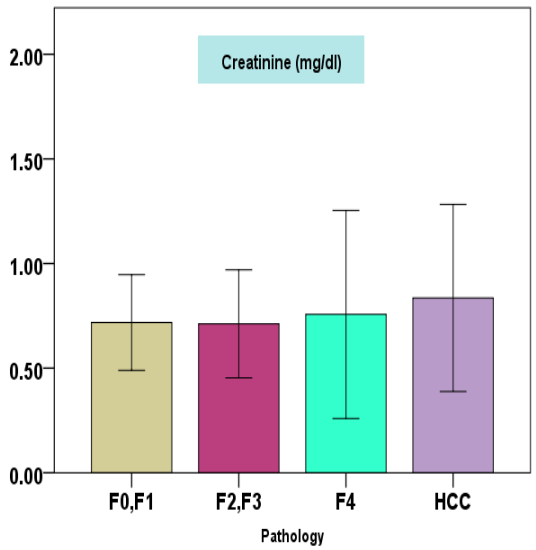

Fig 1 G

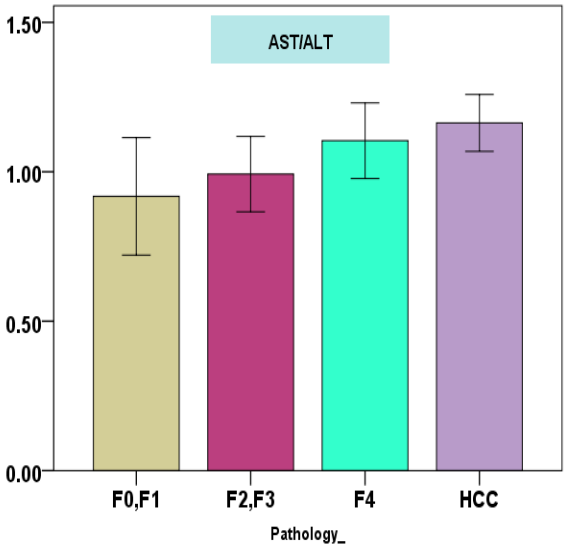

Fig 1 H

Figure 1: Levels of Indirect Biochemical Markers in liver fibrosis and HCC patients.

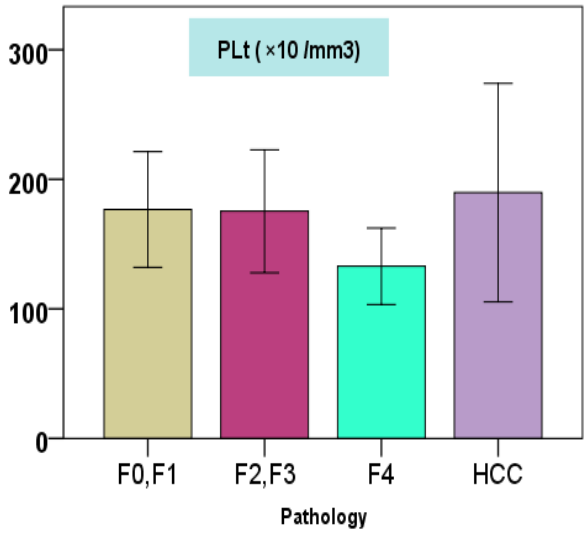

Fig 2 A

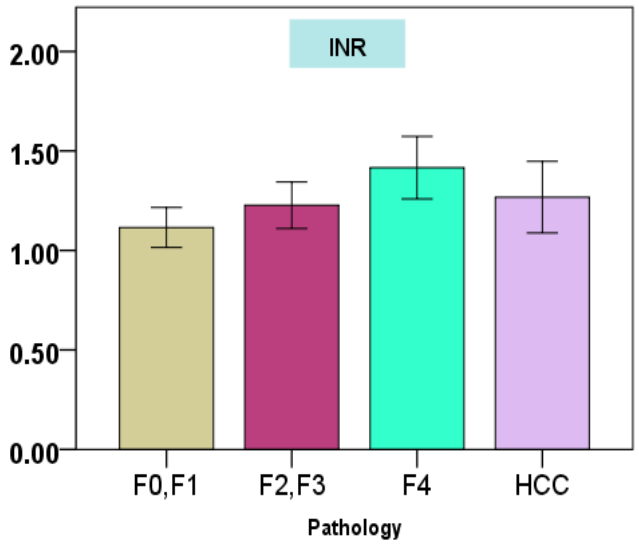

Fig 2 B

Figure 2: Indirect Hematological markers

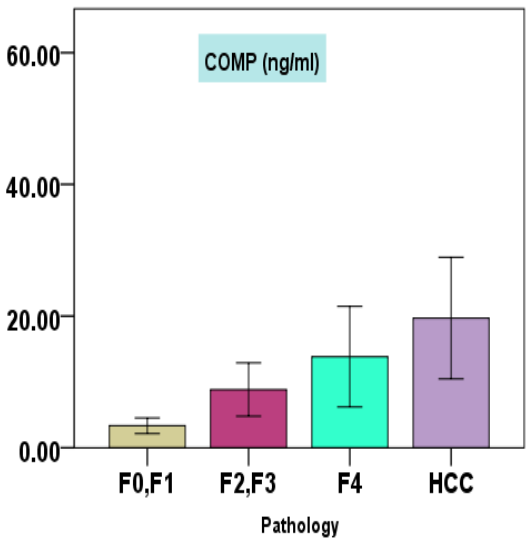

Fig 3

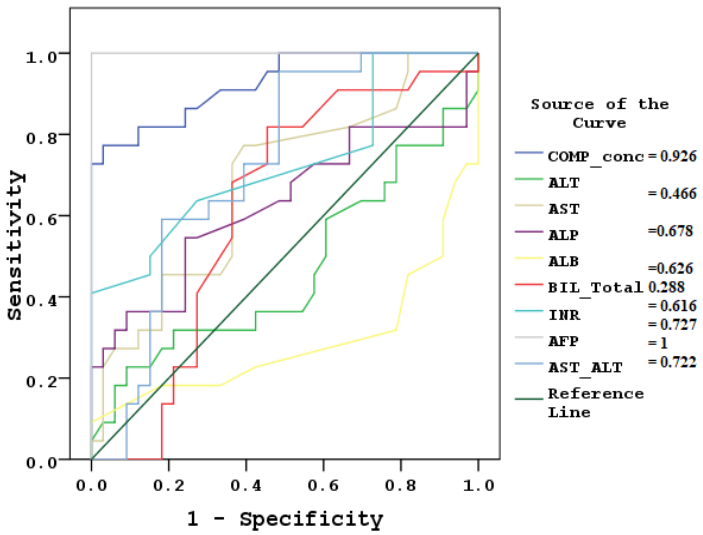

Fig 4 The AUC (area under ROC curve) of investigated markers

Determination of COMP in serum samples

Discussion

Hepatocellular carcinoma ranks as the sixth most frequent cancer globally. Usually the result of either chronic viral hepatitis or other non-viral chronic liver illnesses, HCC develops as a consequence of end-stage liver disease. Advanced liver fibrosis and cirrhosis are the main risk factors for the development of HCC in patients with chronic liver disorders (14-18).

Regardless of the cause, the result of most chronic liver illnesses is liver fibrosis, which severely alters the structure and function of the liver parenchyma. Furthermore, because it forecasts the onset of cirrhosis and HCC, the fibrosis stage is a major predictor of the result. Nonetheless, the majority of HCC cases are sneaky in the early stages, which leads to the identification of HCC in a sizable fraction of patients at an advanced stage, with a generally dismal prognosis and few options for treatment (19-22).

The gold standard for precise staging is still liver biopsy, which also yields useful information about the presence of steatosis, the degree of necro-inflammation, and other concurrent variables that may worsen liver injury (23). Interobserver differences, sampling inaccuracy, and the possibility of problems are some of its possible drawbacks (24). The development of non-invasive serological or imaging techniques, such as the AST-to-platelet ratio index (APRI) (26), Fibrosis-4 score (FIB-4) (27), and transient elastography (TE) (28–29), has decreased the usefulness of routine liver biopsy (25). It is important to note that serum levels of proteins directly linked to the hepatic fibrogenic process may be used as stand-in indicators of liver fibrosis (30-31).

In the present study, we found that liver fibrosis increases sharply with age from 35 to 71, rare before the age of 20 years, and decreases by age 75 to 80 years. Age has been linked to poor outcomes in cases of alcoholic hepatitis and the advancement of fibrosis in hepatitis C (32–34). As a result, it has been proposed that liver fibrosis susceptibility rises with age. The biochemical processes that underlie this tendency are not fully known, though. Reduced tolerance to oxidative damage and increased oxidative stress are typically associated with aging (35).

In our study, there were statistically significant differences between levels of indirect biochemical markers (AST, ALT, ALP, BIL, ALB, and platelet count) in patients with liver fibrosis (F2, F3), non-significant liver fibrosis (F0-F1), cirrhotic liver patients (F4) and HCC patients ($P < 0.001$). However, there were no significant differences between AST/ALT with liver fibrosis patients (F2, F3), non-significant liver (F0-F1), cirrhotic liver patients (F4), and HCC patients ($P < 0.05$). The claims that indirect markers represent changes in hepatic function could account for these findings. These indicators are helpful in the diagnosis, assessment of severity, monitoring of treatment, and prognostication of liver illnesses (36). These comprise quantifying the activity of enzymes such as aminotransferases, alkaline phosphatase (ALP), and γ-glutamyl transferase (γGT), as well as estimating blood levels of albumin and bilirubin (37). These are liver chemistries or liver tests, and they should be referred to as indicators for liver injury rather than hepatic function (38).

The most common cause of elevated transaminase activity in serum is liver disease. When disease processes impair the integrity of liver cells, serum levels of the AST and ALT are increased (36). ALT is a more specific enzyme for liver damage than the other one. Changes in ALT activity last longer than those in AST activity. In liver disorders, both enzyme activities can increase to 100 times the upper reference level. Peak activities are unrelated to prognosis and may decline when a patient's condition deteriorates (39).

Infectious hepatitis and other liver illnesses primarily affecting parenchymal cells usually exhibit just a mild elevation or normal serum ALP activity. An increase could also be seen as a result of the medication's therapeutic effect (40). The liver's

ability to synthesize albumin allows it to hold on to albumin concentrations until parenchymal damage reaches a 50% threshold (36). Measurements of plasma albumin can help determine how severe and chronic a disease is. Its usefulness for this purpose is, however, restricted since acute renal illness also results in a drop in plasma albumin concentration (40).

In our study, HCC patients had considerably greater amounts of serum AFP than control individuals (non-significant fibrosis F0, F1). Moreover, there was a notable distinction between concentrations of AFP in significant fibrosis and cirrhotic liver patients.

Guidelines across Asia establish the evaluation of serum AFP for HCC surveillance in high-risk patients; however, its application is still debatable in other regions. The inclusion of AFP in guidelines has varied. For instance, JSH recommendations urge surveillance but neither EASL nor AASLD guidelines do. While AFP is useful in detecting both early-stage and all-stage HCC in a number of studies, its applicability is restricted because of elevated levels in patients with chronic hepatitis (15–58%) and liver cirrhosis (11%–47%), as well as the fact that up to 50% of patients with HCC do not have elevated levels (41-44).

It is important to note that serum levels of proteins directly linked to the hepatic fibrogenic process may be used as stand-in indicators of liver fibrosis (30-31). They show that the liver has more extracellular matrix deposited there, either because activated stellate cells are producing more of it or because Kupffer and endothelial sinusoidal cells are clearing it more slowly (45). Among the research conducted are hepatic metabolic activity, extracellular matrix remodeling proteins, collagen synthesis, and matrix breakdown (31). Among these proteins is COMP. COMP is a glycoprotein that is mostly present in the cartilage, synovium, ligaments, and tendons' extracellular matrix (46).

The current investigation's findings demonstrated that individuals with HCC had higher mean COMP levels than those with cirrhosis, severe liver fibrosis, and non-significant liver fibrosis (F4) ($P < 0.0001$), these results concur with other authors (38, 47-48). They discovered that HCC patients had higher serum levels of COMP than the healthy control group and that the change from premalignant lesions to HCC was correlated with higher serum levels of COMP (49). However, when COMP was identified by northern blot and western blot studies, it was revealed to be highly overexpressed in HCC tissue samples, but it was absent or seldom expressed in liver cirrhosis and normal liver tissues (41).

COMP was found to be significantly expressed in HCC tumor cells when COMP mRNA and protein expression were localized within the cytoplasm of the tumor cells using in situ hybridization and immunohistochemical analysis. Further, its overexpression is associated with the aggressiveness of several types of solid cancers. This finding raises the possibility that COMP is involved in the pathophysiology of HCC. Additionally, they discovered that COMP was only marginally expressed in cirrhotic liver tissues, suggesting that this gene may have a role in the early stages of liver carcinogenesis. It is generally agreed upon that no single biomarker will provide all necessary information for optimal diagnosis of liver fibrosis (50).

For this reason, we construct the areas under ROC curves for all available biomarkers (AST, ALT, BIL, INR, Plt, and AST/ALT). COMP showed the highest area under curve between all biomarkers while ALB and INR showed the lowest area under curve.

Conclusion

Our findings suggest that the COMP biomarker could help improve liver cancer laboratory diagnosis and may be a potential target for therapy monitoring. Further studies prospective, multicenter studies are needed to validate the results and confirm whether developed index can be used for accurate liver cancer diagnosis.

Conflicts of Interest:

The authors declare no conflict of interest.

Fund:

No funds, grants, or other support were received.

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