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# Diagnostic Outcomes of Soluble Major Histocompatibility Complex Class I Related Chain Molecule A and Des- γ Carboxy Prothrombin versus Alpha-FetoProtein for Hepatitis C Virus-Induced Hepatocellular Carcinoma in Egyptian Patients

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## Abstract

**Objectives**: Hepatitis C virus (HCV) infection is a major threat for developing hepatocellular carcinoma (HCC) in Egypt which represents an increased cause of mortality. HCC usually presents at a very late stage thus many patients miss the best opportunity for treatment because of lack of early symptoms and early reliable diagnostic marker for malignant transformation. This study aimed to perform a head-to-head comparison of the diagnostic performance of soluble major histocompatibility complex class I related chain molecule A (sMICA), Des-γ Carboxy Prothrombin (DCP) and Alpha-Feto Protein (AFP) in HCC patients.

**Subjects and methods**: The study included 250 subjects. They were including 50 chronic hepatitis patients, 50 cirrhotic patients, 100 patients with HCC on top of cirrhosis and 50 apparently healthy control subjects. HCC group was subdivided into two subgroups, 61 patients with tumor size from 2 to 5 cm and 39 patients with tumor size >5cm. Serum levels of sMICA, DCP as well as AFP were measured in the sera of all subjects by Enzyme Immune Assay (EIA).

**Results**: AFP, DCP and sMICA showed statistical significant increased levels in HCC group when compared to other groups ($p \leq 0.05$). However, there was a highly significant increase in AFP levels in other patients groups when compared to control group ($p \leq 0.001$). There was no significant difference in DCP level between chronic hepatitis and liver cirrhosis groups and as well when both were compared to the control group. sMICA levels were mostly increased in HCC patients in comparison to healthy or disease controls ($p \leq 0.001$). The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the diagnostic efficacies of sMICA, DCP and AFP. When employing the ROC curve, the superiority of sMICA [AUC: 0.928] to both AFP [AUC: 0.886] and DCP [AUC: 0.656] was evident in the diagnosis of HCC, in discriminating HCC from LC and CH patients [AUC: 0.908] as well as in discriminating HCC with small focal lesions (tumor size from 2-5cm) from both cirrhotic and CH patients [AUC: 0.917 & sensitivity: 88.5%]. The sensitivity of sMICA was the highest (88.5%) versus (62%) for AFP and (54%) for DCP.

**Conclusion**: sMICA levels showed a stepwise increase from CH to LC and up to the most in HCC. However, AFP levels were increased in HCC and other chronic liver diseases while DCP levels were increased only in HCC. As well, sMICA has superior diagnostic performance for HCV-induced HCC on both AFP and DCP with even better performance for distinguishing HCC with small focal lesions. Consequently, measurement of sMICA as a single marker or beside AFP and/or DCP may be valuable in the screening for early malignant transformation of chronic liver diseases to HCC.

**Keywords:** Hepatocellular carcinoma; Liver cirrhosis; Des- γ carboxy prothrombin; soluble MICA; alpha-feto protein

## Introduction

Hepatitis C virus (HCV) is a major threat to global public health. Approximately 130-150 million people all over the world are chronically infected and at risk for liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1]. About, 500 thousand people die each year from HCV-related liver diseases [2]. HCC is currently the second leading cause of cancer-related death globally, a Figure that is on the rise [3]. Furthermore, HCC is the most common neoplasm among all primary liver cancers which accounts for approximately 90% of cases. In contrast to other human malignancies, the risk factors for HCC are well established. Indeed, HCC is common in patients with advanced hepatic fibrosis or cirrhosis due to chronic liver disease, and in particular with liver damage caused by HBV or HCV infection along with unhealthy alcohol use. The worldwide incidence of HCC parallels that of chronic viral hepatitis [4].

When HCC causes symptoms, the disease is most often at an advanced stage and therefore not amenable to potentially curative treatment. Death usually ensues within a few months. However, HCC has a prolonged subclinical course that provides the opportunity for early detection [5]. These considerations have led to the development

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of protocols for the surveillance of HCC in patients at risk for this cancer. Yet, this surveillance remains controversial because the only RCT that demonstrated decreased mortality was probably statistically incorrectly analyzed [**6**]. The techniques for surveillance are also controversial. There is no question that ultrasound should be part of the algorithm but the use of biomarkers remains controversial. There is some suggestion that biomarkers improve early detection however there is currently no evidence that this leads to improved cure rates compared with ultrasound alone. Alpha-Feto Protein (AFP) is still one of the most widely used markers to diagnose HCC. However, AFP levels may increase in patients with acute hepatitis, chronic active hepatitis or liver cirrhosis. As well, AFP has a limited accuracy with a sensitivity of about 60% at a cut-off value of 20 ng/ml and low specificity [**7-9**]. Most importantly, the majorities of serum biomarkers that are used are more frequently associated with advanced-stage disease than early-stage disease and would therefore be theoretically unsuitable for the detection of early HCC [**8,10**]. Accordingly, so far there is no reliable marker for the early diagnosis of HCC [11,12].

To complement the limitations of AFP, the combined measurement of AFP and DCP or other biomarkers have been suggested [**13-15**]. DCP, also called a protein induced by vitamin K absence (PIVKA-II), is an abnormal prothrombin molecule generated as a result of an acquired defect in the posttranslational carboxylation of the prothrombin precursor in malignant cells. This decarboxylated prothrombin is also produced in the presence of vitamin K deficiency. DCP was discovered in serum of patients during their anticoagulant therapy with a vitamin K antagonist [**13**]. High DCP levels are found in patients with HCC and in cases of HCC recurrence after surgical resection, suggesting the usefulness of DCP as an HCC biomarker. Thus far, we are still in need for a reliable diagnostic and prognostic marker for HCC [**10**].

The major histocompatibility complex class I related chain molecule A (MICA) is a natural ligand for the activating receptor natural killer group 2, member D (NKG2D) expressed on the surface of natural killer (NK) cells [**16**]. In non-pathological situations, expression of MICA is restricted to epithelial cells of the gastrointestinal tract and is only present at very low levels in most normal cells and tissues. However, many malignant carcinoma cells express high levels of MICA on their surface, making them susceptible to targeting and killing by NK cells [**17**]. The engagement of MICA and NKG2D strongly activates NK cells and co-stimulates CD8 T cells, enhancing their cytolytic ability and cytokine production. Additionally, MICA is cleaved proteolytically from tumor cells and appears as soluble forms in sera of patients with malignancy [**18**]. Studies suggest that release of MICA from cancer cells (soluble MICA) constitutes an immune escape mechanism that systemically impairs antitumor immunity and also indicate the possibility that soluble MICA (sMICA) is of diagnostic value in patients with malignancies [**19**].

Previous studies have demonstrated that sMICA levels are significantly correlated with patient prognosis in some cancer types such as ovarian cancers [**20**], lung, breast, gastric, colorectal and squamous cell carcinoma. Indeed, a high level of sMICA is usually related with poor prognosis among cancer patients [**21**]. Recent studies focused on the possible involvement of sMICA in liver carcinogenesis related to hepatitis B virus infection and hepatitis C virus infection [**22**]. These data may indicate that sMICA might serve as a clinical marker in the diagnosis and also prediction of HCC.

The objectives of this study were to assess the serum levels of sMICA, DCP and AFP among patients with chronic hepatitis, liver cirrhosis as well as HCC. Moreover, we aimed to study the diagnostic role of DCP and the novel sMICA as serum tumor markers of HCC by detecting their levels in comparison with the conventional AFP marker.

## Subjects and Methods

### Subjects

This case control study was carried out at the Clinical Pathology, Internal Medicine and Tropical Medicine Departments, Faculty of Medicine, Minia University. A total of 250 subjects were enrolled in this study. They were including 200 patients and 50 apparently healthy volunteers who served as a control group (Group **IV**). Thorough history questionnaires were filled for all subjects plus full clinical examination. Also, written consents were signed by all subjects before their enrolment in the study. Within the control group, the number of males was 28 subjects and the number of females was 22 subjects. Their ages ranged from 20 to 41 years old. All of whom were negative for the markers of hepatitis viruses A, B and C as well as HIV antibodies. Additionally, they had no liver, gallbladder or kidney diseases.

All patients were proved to be negative for hepatitis B virus surface antigen (HBsAg) by enzyme immunoassay (EIA). As well, these patients were proved to be HCV positive by viral markers using EIA and by real time (RT)-PCR. The patients were further divided into three groups. The patients group I included 50 chronic hepatitis C patients. They were 38 males and 12 females and their ages were ranged from 27 to 45 years old. The patients group II was consisted of 50 cirrhotic patients. They were 35 males and 15 females and their ages were ranged from 50 to 77 years old. They were diagnosed by ultrasound, CT scan, clinical and laboratory findings of hepatocellular failure or portal hypertension, decreased synthetic function. Finally, patients group III involved 100 HCC patients without extra hepatic malignancy. They were 80 males and 20 females and their ages were ranged from 54 to 78 years old. The diagnosis of HCC was made by non-invasive radiological techniques using contrast imaging or invasive (biopsy) approaches. A biopsy is required for patients who do not have any particular risks for HCC, for the most part patients without cirrhosis. The recommended algorithm for investigation of lesions in at-risk patients is as follows: for nodules <1cm in size, ultrasound follow-up at 3 months is recommended; for lesions >1cm, the radiological hallmarks of HCC define diagnosis; if the radiology is not typical in at least one of two imaging techniques (CT and MRI), a liver biopsy is recommended [23]. According to tumor size, patients in Group III were subdivided into two more subgroups. Group IIIa which comprised 61 patients with tumor sized from 2 to 5cm and Group IIIb which implicated 39 patients with tumor sized more than 5cm. Patients with other malignancies, other infectious diseases or cholestatic autoimmune diseases were excluded from the study. As well, we exclude patients taking vitamin K or warfarin.

### Blood sampling

Peripheral blood samples were withdrawn from all subjects under complete aseptic conditions. A total of about 8 ml of blood were collected. 2 ml of blood was withdrawn into an EDTA tube for complete blood picture (CBC) which was analyzed with automated cell counter Sysmex KX-21N (TAO Medical incorporation, Japan). 1.8 ml of blood was collected in a citrated tube (3.2% trisodium citrate) for prothrombin time and concentration (PT and PC) which was evaluated by STAGO COMPACT CT Coagulation Analyzer (Diamond Diagnostics, USA). The last 4 ml of blood was evacuated in a plain tube, left to clot and centrifuged at 3000 revolutions per minute (rpm) for 5 minutes. The serum was then separated and liver function tests (AST, ALT, Alkaline phosphatase, bilirubin, total protein and albumin) were detected

immediately using fully automated clinical chemistry auto-analyzer system Konelab 20i (Thermo Electron Incorporation, Finland). The remaining serum was aliquoted and stored at -70°C till used for further assessment of serum AFP, DCP and sMICA.

### Laboratory methods

Serum AFP was determined by EIA kit according to the manufacturer's instructions using anti-AFP antibodies for quantitative detection of human AFP (EIAab-China). Quantitative detection of DCP in serum was performed with EIA kit (EIAab-China) as indicated by the manufacturer's instructions. Likewise, sMICA in serum samples was measured by a commercially available EIA kit (EIAab-China) as stated by the manufacturer's instructions using biotin-conjugated antibodies specific for sMICA.

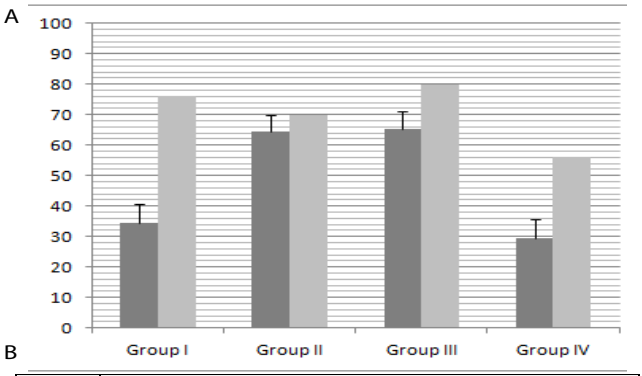
### Statistical analysis

All collected data were analyzed statistically using statistical package for social sciences (SPSS) program version 20.0 (SPSS Inc., Chicago, IL, USA). The quantitative data were presented as mean ± standard deviation (SD) while the qualitative variables were described as number and percentage. Results were expressed as tables and Figures. Graphics were done by Excel Microsoft Office 2010. Student t-test was used to compare results between groups as regards quantitative data. Chi-square test was used to compare qualitative variables between groups. P-values equal to or less than 0.05 are statistically significant. One Way Anova test was used for comparison of parametric quantitative data between more than two groups. Correlation was performed by using Pearson correlation coefficient of variation (r). Moreover, ROC curve was used to evaluate the diagnostic performance of AFP, DCP as well as sMICA in HCC patients group versus different studied groups.

## Results

### Demographic characters and laboratory results of enrolled subjects

A total of 250 subjects were included in this study. They were divided into 200 patients (Group I-III) and 50 apparently healthy control subjects (control Group IV). The patient group I was involving 50 chronic hepatitis patients while the patient group II was consisted of 50 patients with LC. The patients group III comprised 100 subjects who were suffering from HCC. This group was further subdivided into two subgroups (IIIa and IIIb) according to the size of hepatic focal lesion




| | p-values | | | | | |
|---|---|---|---|---|---|---|
| | I vs II | I vs III | I vs IV | II vs III | II vs IV | III vs IV |
| Age (years) | 0.001** | 0.001** | 0.07 | 0.839 | 0.001** | 0.001** |
| Sex (%) | 0.723 | 0.705 | 0.053 | 0.465 | 0.110 | 0.002* |

**Figure 1:** Comparison between different studied groups regarding demographic data. **vs**, versus; * p-value ≤ 0.05; ** p-value ≤ 0.001.

The demographic features of the subjects involved in this study are summarized in Figure 1. There was no significant difference between group I and IV regarding the age (p=0.07). On the other hand, there was highly statistical significant difference between both group II and III when compared to group IV (p<0.001). Moreover, there was highly statistical significant difference between group I, II and III (p<0.001) but no significant difference between group II and III (p=0.839). There was no significant difference in the sex of patients between group III when compared to group I and II (p=0.705 and 0.465 respectively). Also, there was no significant difference when group II was compared to group I and IV (p=0.723 and 0.110 respectively). There was no significant sex difference in group I when compared to group IV (p=0.053) but there was significant difference when group III compared to group IV (p=0.002).

Regarding liver function tests, there was highly statistical significant increase in total bilirubin, ALT and AST levels in group I, II and III patients when compared to group IV (p<0.001). In contrary, there was no significant difference regarding total bilirubin, ALT as well as AST levels between group II and III (p=0.07, 0.07 and 0.08 respectively). Also, there was highly statistical significant increase in total and direct bilirubin in group II and III when compared to group I (p<0.001) and in direct bilirubin when group II and III were compared to group IV (p<0.001). As well, there was significant difference in direct bilirubin when group I compared to group IV (p=0.003) but no significant difference was detected between group II and III (p=0.06). Additionally, there was highly statistical significant increase in ALT and AST levels in group I when compared to group II (p ≤ 0.001) however there was no significant increase in ALT as well as AST levels in group I when compared to group III (p=0.082 and 0.516 respectively) (Tables 1 and 2). Furthermore, there was no significant difference between group I and IV (p=0.430) and between group II and III (p=0.807) concerning serum albumin levels but there was highly statistical significant decrease in albumin in group II and III when compared to group IV (p ≤ 0.001). Also, there was highly statistical significant decrease in albumin in group II and III when compared to group I (p<0.001) (Tables 1 and 2). Moreover, there was a highly statistical significant decrease in PC in group II and III when compared to group IV (p<0.001) and there was significant difference between group I and IV (p=0.04). In contrary, there was no significant difference between group II and III (p=0.062). In addition, there was a highly statistic significant decrease in PC in group II and III when compared to group I (p<0.001) (Tables 1 and 2).

When we come to CBC parameters, there was no significant difference between group I and IV concerning hemoglobin concentration (p=0.807) as well there was no significant difference between group II and III (p=0.456). There was highly statistical significant decrease in HB concentration in both groups II and III when compared with group IV (p ≤ 0.001). There was also highly statistical significant decrease in HB concentration in groups II and III when compared to group I (p ≤ 0.001) (Tables 1 and 2). Additionally, there was no significant difference considering TLC between both group II and III when compared to group I (p=0.057 and 0.826 respectively). However, there was significant difference in TLC between group II and III (p=0.043) and significant decrease in group I and III when compared to group IV (p=0.029 and 0.049 respectively). As well, there was highly significantly decrease in group II when compared to group IV (p ≤ 0.001) (Tables 1 and 2).

| Variables \ Groups | Group I CH (N=50) | Group II LC (N=50) | Group III HCC (N=100) | Group IV HC (N=50) |
|---|---|---|---|---|
| Total Bil. (mg/dl) M ± SD | 0.87±0.29 | 3.3 ± 2.2 | 3.7±2.0 | 0.36±0.1 |
| Direct Bil. (mg/dl) M ± SD | 0.19±0.09 | 1.09±0.83 | 1.4±0.9 | 0.1±0.003 |
| ALT (U/L) M ± SD | 95.5±43.5 | 59.6±25.65 | 84.15±73.03 | 12.25±1.48 |
| AST (U/L) M ± SD | 102.7±53.2 | 80.25±37.03 | 103.9±78.99 | 14.35±2.54 |
| Albumin (g/dl) M ± SD | 4.54 ± 0.29 | 2.51 ± 0.45 | 2.47±0.43 | 4.61±0.29 |
| PC (%) M ± SD | 83.5±8.21 | 48.3±13.82 | 40.23±12.9 | 97.5±4.44 |
| Hb (g/dl) M ± SD | 12.86±2.26 | 9.43±2.31 | 9.03±3.03 | 13.18±0.95 |
| TLC ($1\times10^3$/µl) M ± SD | 5.55±1.93 | 4.5±1.33 | 5.67±1.69 | 6.76±1.69 |
| Platelets ($1\times10^3$/µl) M ± SD | 164.15±42.7 | 103.7±71.89 | 86.75±46.35 | 236.2±48.44 |

**Table 1:** Comparison between different studied groups regarding laboratory data. N- number; M-mean; SD-standard deviation; CH- chronic hepatitis; LC-liver cirrhosis; HCC- Hepatocellular Carcinoma; HC-healthy control; ALT-Alanine Transaminase; AST-Aspartate Transaminase; PC-Prothrombin Concentration; TLC-Total Leucocytes Count; Hb- Haemoglobin.

| Variables \ Groups | p-values | | | | | |
|---|---|---|---|---|---|---|
| | I vs II | I vs III | I vs IV | II vs III | II vs IV | III vs IV |
| Age (years) M ± SD | 0.001** | 0.001** | 0.07 | 0.839 | 0.001** | 0.001** |
| Gender Male/female (%) | 0.723 | 0.705 | 0.053 | 0.465 | 0.110 | 0.002* |
| Total Bil. (mg/dl) M ± SD | <0.001** | <0.001** | <0.001** | 0.07 | <0.001** | <0.001** |
| Direct Bil. (mg/dl) M ± SD | <0.001** | <0.001** | 0.003* | 0.06 | <0.001** | <0.001** |
| ALT (U/L) M ± SD | <0.001** | 0.082 | <0.001** | 0.07 | <0.001** | <0.001** |
| AST (U/L) M ± SD | 0.001* | 0.516 | <0.001** | 0.08 | <0.001** | <0.001** |
| Albumin (g/dl) M ± SD | <0.001** | <0.001** | 0.430 | 0.807 | <0.001** | <0.001** |
| PC (%) M ± SD | <0.001** | <0.001** | 0.04* | 0.062 | <0.001** | <0.001* |
| Hb (g/dl) M ± SD | <0.001** | <0.001** | 0.807 | 0.456 | <0.001** | <0.001** |
| TLC ($1\times10^3$/µl) M ± SD | 0.057 | 0.826 | 0.029* | 0.034* | <0.001** | 0.049* |
| Platelets ($1\times10^3$/µl) M ± SD | 0.002* | <0.001** | <0.001** | 0.755 | <0.001** | <0.001** |

**Table 2:** Statistical difference between studied groups regarding laboratory data.* p-value ≤ 0.05; ** p-value ≤ 0.001; N-number; Mmean-SD-Standard Deviation; CH-Chronic Hepatitis; LC-Liver Cirrhosis; HCC-Hepatocellular Carcinoma; HC-Healthy Control; ALT-Alanine Transaminase; AST-Aspartate Transaminase; PC-Prothrombin Concentration; TLC-Total Leucocytes Count; Hb-Haemoglobin.

Moreover, there was highly significant decrease in platelets count in group I, II and III when compared to group IV (p<0.001) but when group II was compared to group III there was no significant difference (p=0.775). There was significant decrease in platelet count in group II when compared to group I (p=0.002). In contrary, the decrease in platelet count was highly significant when group III compared to group I (p<0.001) (Tables 1 and 2).

## Levels of AFP, DCP and sMICA in different study groups

There was a highly statistical significant increase in AFP levels in group I, II, IIIa and IIIb in comparison to group IV (p<0.001). Also, there was a significant increase in AFP levels within group IIIa and IIIb when compared to group II (p<0.05) and I (p<0.001). In addition, there was highly statistical significant increase in AFP levels in group II when compared to group I (p ≤ 0.001). As well, there was a highly significant difference between group IIIa and IIIb (p<0.001) (Table 3). There was a highly statistical significant increase in DCP levels in group IIIa and IIIb in comparison to group I, II and IV (p<0.001) but there was no significant difference between group II when compared to group I and IV (p=0.051 and 0.06 respectively) and between group I when compared to group IV (p= 0.08). In parallel, there was no significant difference between group IIIa and IIIb (p=0.77) (Table 3). There was a highly statistical significant increase in sMICA levels in group IIIa and IIIb in comparison to group I, II and IV (p<0.001). Also, there was a highly statistical significant increase in sMICA levels in group II in comparison to group I and IV and in group I when compared to group IV (p<0.001). On the other hand, there was no significant difference between group IIIa and IIIb (p=0.26) (Table 3).

| Groups / Variables | Group I CH (n=50) | Group II LC (n=50) | Group III HCC | | Group IV HC (n=50) | p-values | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Goup IIIa (n=61) | Group IIIb (n=39) | | | | | | |
| AFP (ng/ml) M ± SD | 14.6±9.8 | 147.4±30.8 | 247.3±241.4 | 1116± 442 | 1.3±0.53 | I vs II | I vs IIIa | I vs IIIb | I vs IV | II vs IIIa |
| | | | | | | <0.001** | <0.001** | <0.001** | <0.001** | 0.004* |
| | | | | | | II vs IIIb | II vs IV | IIIa vs IIIb | IIIa vs IV | IIIb vs IV |
| | | | | | | <0.001** | <0.001** | <0.001** | <0.001** | <0.001** |
| DCP (ng/ml) M ± SD | 1.6±0.5 | 2.4±1.2 | 380±230 | 385±253 | 1.1±0.4 | I vs II | I vs IIIa | I vs IIIb | I vs IV | II vs IIIa |
| | | | | | | 0.051 | <0.001** | <0.001** | 0.08 | <0.001** |
| | | | | | | II vs IIIb | II vs IV | IIIa vs IIIb | IIIa vs IV | IIIb vs IV |
| | | | | | | <0.001** | 0.06 | 0.77 | <0.001** | <0.001** |
| sMICA (pg/ml) M ± SD | 305±50 | 438±108 | 672±188 | 628±189 | 219±51 | I vs II | I vs IIIa | I vs IIIb | I vs IV | II vs IIIa |
| | | | | | | <0.001** | <0.001** | <0.001** | <0.001** | 0.001* |
| | | | | | | II vs IIIb | II vs IV | IIIa vs IIIb | IIIa vs IV | IIIb vs IV |
| | | | | | | <0.001** | <0.001** | 0.26 | <0.001** | <0.001** |

**Table 3:** Comparison between patients and control groups as regards AFP, DCP and sMICA. N-number; * p-value ≤ 0.05; ** p-value ≤ 0.001; M-Mean; SD-Standard Deviation; CH-Chronic Hepatitis; LC-Liver Cirrhosis; HCC-Hepatocellular Carcinoma; HC-Healthy Control; AFP- Alpha-Feto Protein; DCP-Des-γ CarboxyProthrombin; sMICA-Soluble Major Histocompatibility complex class I related chain molecule A.

## Correlation of DCP with other laboratory data in HCC group

To assess the role of DCP in HCC, we correlated between its serum levels and AFP, sMICA as well as other laboratory parameters in HCC group. Within group IIIa, serum DCP correlation with AFP was fair negative and statistically non-significant (r=-0.304, p=0.337) while this correlation within group IIIb was moderate negative and statistically nonsignificant (r=-0.689, p=0.059) (Table 4). Also, the correlations between serum DCP and sMICA in group IIIa was weak positive and non-significant (r=0.233, p=0.465) and in group IIIb was fair negative and non-significant (r=0.277, p=0.507) (Table 4). As well, DCP correlations with other laboratory findings (ALT, AST, T. bilirubin and PC) in HCC group were shown in Table 4.

| DCP / Variables | r | p-values |
|---|---|---|
| Group IIIa | | |
| AFP | -0.304 | 0.337 |
| sMICA | 0.233 | 0.465 |
| ALT | 0.307 | 0.332 |
| AST | 0.551 | 0.064 |
| T. bilirubin | 0.180 | 0.576 |
| PC | 0.535 | 0.073 |
| Group IIIb | | |
| AFP | -0.689 | 0.059 |
| sMICA | -0.277 | 0.507 |
| ALT | -0.438 | 0.277 |
| AST | -0.361 | 0.380 |
| T. bilirubin | -0.104 | 0.806 |
| PC | 0.126 | 0.766 |

**Table 4:** Correlation between DCP and other laboratory findings within HCC group. r=0.75-1(strong correlation) r=0.5-0.74(moderate correlation), r=0.25-0.49(fair correlation), r=0.1-0.24(weak correlation); HCC, hepatocellular carcinoma; DCP, des-γ carboxyprothrombin; AFP, alpha-fetoprotein; sMICA, soluble major histocompatibility complex class I related chain molecule A; ALT, alanine transaminase; AST, aspartate transaminase; T. bilirubin; total bilirubin; PC, Prothrombin concentration.

## Correlation of sMICA with other laboratory data in HCC group

Additionally, the current study shows the correlation between sMICA and AFP along with other laboratory findings within HCC group. In group IIIa and IIIb, AFP was correlated in a fair positive and non-statistically significant manner (r=0.337, p=0.284 and r=0.392, p=0.336 respectively) (Table 5). Moreover, the correlations between sMICA and ALT, AST, T. bilirubin in addition to PC in HCC group were shown in (Table 5).

| sMICA / Variables | r | p-values |
|---|---|---|
| Group IIIa | | |
| AFP | 0.337 | 0.284 |
| ALT | -0.220 | 0.492 |
| AST | -0.319 | 0.311 |
| T. bilirubin | -0.364 | 0.45 |
| PC | -0.272 | 0.393 |
| Group IIIb | | |
| AFP | 0.392 | 0.336 |
| ALT | -0.145 | 0.733 |
| AST | -0.144 | 0.734 |
| T. bilirubin | -0.180 | 0.669 |
| PC | 0.419 | 0.301 |

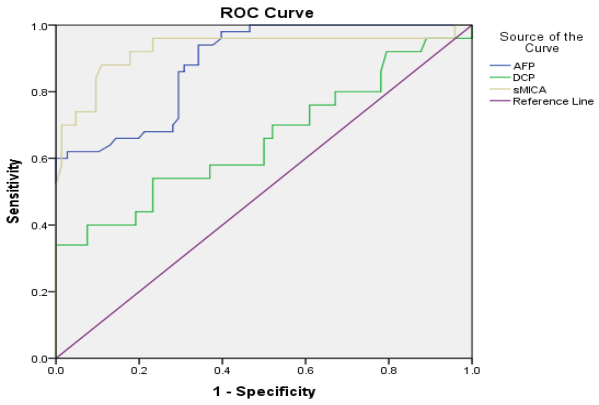
**Table 5:** Correlation between sMICA and other laboratory findings within HCC group; r=0.75-1(strong correlation), r=0.5-0.74(moderate correlation), r=0.25-0.49(fair correlation), r=0.1-0.24(weak correlation)HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein; sMICA, soluble major histocompatibility complex class I related chain molecule A; ALT, alanine transaminase; AST, aspartate transaminase; total billirubin; PC, Prothrombin concentration.

## Diagnostic performance of sMICA and DCP versus AFP in identifying HCC patients

To determine cut-off levels that balanced the false-positive and the false-negative rates with the best positive predictive value, ROC analysis was performed for sMICA, DCP and AFP. ROC curves of sMICA, DCP and AFP for discriminating patients with HCC from all non HCC subjects were shown in (Figure 2A). The AUC value of sMICA was 0.928 [95% confidence interval (CI)=0.888–0.967, p=0.000]. On the other hand, DCP showed an AUC value of 0.656 (95% CI=0.583–0.729, p=0.000) and AFP showed an AUC value of 0.886 (95% CI=0.846–0.925, p=0.000) (Figure 2B).

In addition, ROC curves of sMICA, DCP and AFP for discriminating patients with HCC from DC (CH plus LC) were shown in (Figure 3A). The AUC value of sMICA was 0.908 [95% CI=0.863–0.953, p=0.000] and of DCP was 0.725 [95% CI=0.654–0.796, p=0.000]. Moreover, AFP showed an AUC value of 0.827 (95% CI=0.770–0.883, p=0.000) (Figure 3B).

HCC vs. DC+HC
(A)




(B)

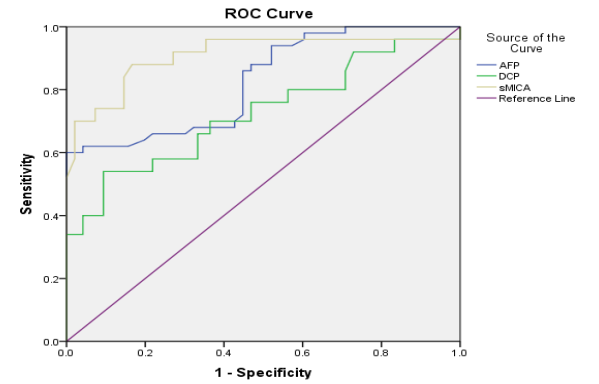
Area Under the Curve

| Test Result Variable(s) | Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| AFP | .886 | .020 | .000 | .846 | .925 |
| DCP | .656 | .037 | .000 | .583 | .729 |
| sMICA | .928 | .020 | .000 | .888 | .967 |

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

**Figure 2:** Diagnostic performances of the sMICA, DCP and AFP in discriminating patients with HCC from both healthy and patient controls. (A) ROC curve obtained by plot at different cut-offs for AFP, DCP and sMICA in HCC versus all controls; (B) The area under the curve is 0.886 for AFP with Std. Error=0.020 and 95% Confidence Interval (CI) from 0.846 to 0.925. The area under the curve is 0.656 for DCP with Std. Error=0.037 and 95% CI from 0.583 to 0.729. The area under the curve is 0.928 for sMICA with Std. Error=0.020 and 95% CI from 0.888 to 0.967.

HCC vs. DC
(A)




(B)

Area Under the Curve

| Test Result Variable(s) | Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| AFP | .827 | .029 | .000 | .770 | .883 |
| DCP | .725 | .036 | .000 | .654 | .796 |
| sMICA | .908 | .023 | .000 | .863 | .953 |

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

**Figure 3:** Diagnostic performances of the sMICA, DCP and AFP for discriminating HCC from patient control. (A) ROC curve obtained by plot at different cut-offs for AFP and sMICA in HCC versus diseased control; (B) The area under the curve is 0.827 for AFP with Std. Error=0.029 and 95% CI from 0.770 to 0.883. The area under the curve is 0.725 for DCP with Std. Error=0.036 and 95% CI from 0.654 to 0.796; The area under the curve is 0.908 for sMICA with Std. Error=0.023 and 95% CI from 0.863 to 0.953.

These ROC curves indicated that a sMICA value of 499.5 pg/ml yielded the best sensitivity and specificity for differentiating patients with HCC from those without HCC as whole, diseased controls (LC plus CH) as well as from LC alone (Table 6). For DCP and AFP, these best cut-off values were 3 ng/ml and 171 ng/ml respectively (Table 6). Likewise, based on these ROC defined cut-off values, the sensitivity of sMICA was 88.5% against all controls, DC or LC with specificity values of 89%, 83.3 and 72% respectively. Additionally, the sensitivity of DCP was 54% while its specificity values were 76.7%, 90.6% and 86.0% respectively when the comparison was versus all non HCC patients, DC or LC. As well, the sensitivity and specificity of AFP was 62% and 93.8% respectively when performed to HC plus DC while when versus DC or LC group the results were the same for sensitivity but for specificities were 90.6% and 82% respectively (Table 6). The PPVs and NPVs of sMICA, DCP beside AFP were shown in Table 6.

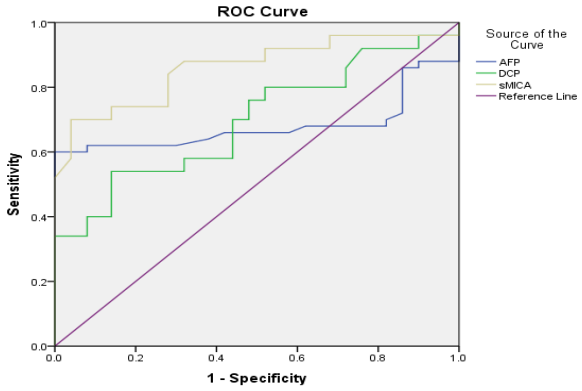
ROC curves of sMICA, DCP and AFP for discriminating patients with HCC from those with LC were shown in (Figure 4A). The AUC value of sMICA was 0.860 [95% CI=0.802–0.918, p=0.000] and of DCP was 0.697 [95% CI=0.614–0.780, p=0.000]. Also, AFP showed an AUC value of 0.680 (95% CI=0.594–0.766, p=0.000) (Figure 4B).

## Diagnostic performance of sMICA and DCP versus AFP for HCC with small sized focal lesions

HCC group was next subdivided into 2 subgroups according to the size of hepatic tumor focal lesion into HCC patients with tumor sized from 2 to 5cm and those with tumor sized more than 5cm. ROC curves of sMICA, DCP and AFP for differentiating patients with small sized focal lesion from all non HCC controls were analyzed (Figure 5A). The AUC value of sMICA was 0.936 [95% CI=0.890-0.982, p=0.000]. As well, DCP showed an AUC value of 0.639 [95% CI=0.549-0.729, p=0.002] and AFP showed an AUC value of 0.813 [95% CI=0.755-0.871, p=0.000] (Figure 5 B).

Furthermore, ROC curves of sMICA, DCP and AFP for differentiating patients with small sized focal lesion HCC from disease

HCC vs. LC
(A)




(B)

Area Under the Curve

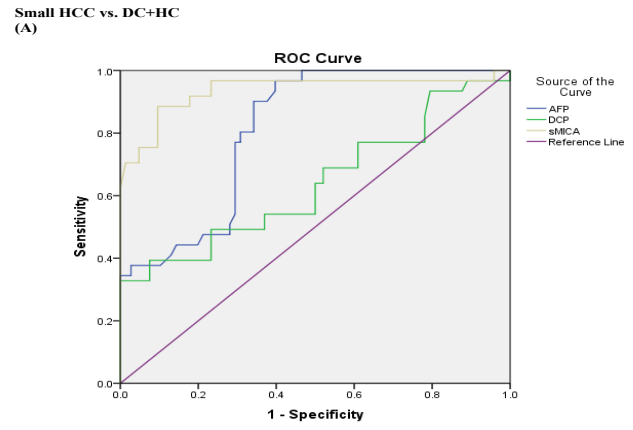
| Test Result Variable(s) | Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| AFP | .680 | .044 | .000 | .594 | .766 |
| DCP | .697 | .042 | .000 | .614 | .780 |
| sMICA | .860 | .030 | .000 | .802 | .918 |

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

**Figure 4:** Diagnostic performances of the sMICA, DCP and AFP for discriminating HCC patients from cirrhotic patients. (A) ROC curve obtained by plot at different cut-offs for AFP and sMICA in HCC versus diseased control; (B) The area under the curve is 0.680 for AFP with Std. Error=0.044 and 95% CI from 0.594 to 0.766. The area under the curve is 0.697 for DCP with Std. Error=0.042 and 95% CI from 0.614 to 0.780. The area under the curve is 0.860 for sMICA with Std. Error=0.030 and 95% CI from 0.802 to 0.918 .HCC, hepatocellular carcinoma; LC- liver cirrhosis; ROC-receiver operating characteristic; AFP- Alpha-fetoprotein; DCP-des-γ carboxyprothrombin; sMICA, soluble major histocompatibility complex class I related chain molecule A.

| Variable | Cut-Off value | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|
| **HCC vs. DC+HC** | | | | | |
| AFP | 171.0 | 62.0% | 93.8% | 86.9% | 77.9% |
| DCP | 3.0 | 54.0% | 76.7% | 56.5% | 70.5% |
| sMICA | 499.5 | 88.5% | 89.0% | 83.0% | 92.3% |
| **HCC vs. DC** | | | | | |
| AFP | 171.0 | 62.0% | 90.6% | 86.9% | 70.5% |
| DCP | 3.0 | 54.0% | 90.6% | 85.2% | 66.4% |
| sMICA | 499.5 | 88.5% | 83.3% | 83.0% | 86.3% |
| **HCC vs. LC** | | | | | |
| AFP | 171.0 | 62.0% | 82.0% | 86.9% | 51.9% |
| DCP | 3.0 | 54.0% | 86.0% | 88.1% | 48.3% |
| sMICA | 499.5 | 88.5% | 72.0% | 83.0% | 71.1% |
| **Small HCC vs. DC+HC** | | | | | |
| AFP | 171.0 | 37.7% | 93.8% | 70.0% | 77.9% |
| DCP | 3.0 | 49.2% | 76.7% | 41.2% | 78.0% |
| sMICA | 499.5 | 88.5% | 89.0% | 74.3% | 95% |
| **Small HCC vs. DC** | | | | | |
| AFP | 171.0 | 37.7% | 90.6% | 70.0% | 70.5% |
| DCP | 3.0 | 49.2% | 90.6% | 75.7% | 74.6% |
| sMICA | 499.5 | 88.5% | 83.3% | 74.3% | 92.1% |
| **Small HCC vs. LC** | | | | | |
| AFP | 171.0 | 37.7% | 82.0% | 70.0% | 51.9% |
| DCP | 3.0 | 49.2% | 86.0% | 80.0% | 58.1% |
| sMICA | 499.5 | 88.5% | 72.0% | 74.3% | 82.1% |

**Table 6:** Results of measurement of AFP, DCP and sMICA in the diagnosis of HCC. HCC-Hepatocellular Carcinoma; DC-Diseased Control; HC-Healthy Control; LC-Liver Cirrhosis; AFP-Alpha-Fetoprotein; DCP-Des-γ Carboxy Prothrombin; sMICA-Soluble Major Histocompatibility Complex Class I related chain molecule A; PPV- Positive Predictive Value; NPV-Negative Predictive Value.



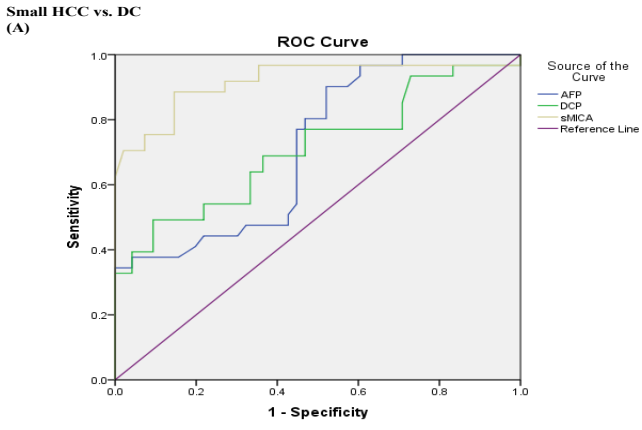

(B)

Area Under the Curve

| Test Result Variable(s) | Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| AFP | .813 | .029 | .000 | .755 | .871 |
| DCP | .639 | .046 | .002 | .549 | .729 |
| sMICA | .936 | .023 | .000 | .890 | .982 |

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

**Figure 5:** Diagnostic performances of the sMICA, DCP and AFP for discriminating patients with small sized HCC lesions from both healthy and patient controls. (A) ROC curve obtained by plot at different cut-offs for AFP, DCP and sMICA in HCC versus all controls; (B) The area under the curve is 0.813 for AFP with Std. Error=0.029 and 95% CI from 0.755 to 0.871. The area under the curve is 0.639 for DCP with Std. Error=0.046 and 95% CI from 0.549 to 0.729. The area under the curve is 0.936 for sMICA with Std. Error=0.023 and 95% CI from 0.890 to 0.982. HCC- hepatocellular carcinoma; DC- disease control; HC-healthy control; ROC-receiver operating characteristic; AFP-alpha-fetoprotein; DCP-des-γ carboxyprothrombin; sMICA-soluble major histocompatibility complex class I related chain molecule A.



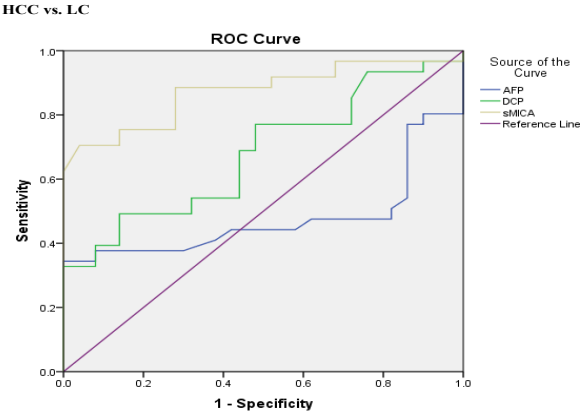

(B)

Area Under the Curve

| Test Result Variable(s) | Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| AFP | .716 | .041 | .000 | .635 | .797 |
| DCP | .711 | .044 | .000 | .624 | .798 |
| sMICA | .917 | .026 | .000 | .866 | .969 |

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

**Figure 6:** Diagnostic performances of the sMICA, DCP and AFP for discriminating patients with small sized HCC lesions from patient control. (A) ROC curve obtained by plot at different cut-offs for AFP and sMICA in HCC versus diseased control; (B) The area under the curve is 0.716 for AFP with Std. Error=0.041 and 95% CI from 0.635 to 0.797. The area under the curve is 0.711 for DCP with Std. Error=0.044 and 95% CI from 0.624 to 0.798. The area under the curve is 0.917 for sMICA with Std. Error=0.026 and 95% CI from 0.866 to 0.969. HCC-Hepatocellular Carcinoma; DC-Disease Control; ROC-Receiver Operating Characteristic; AFP-Alpha-Fetoprotein; DCP-Des-γ Carboxyprothrombin; sMICA-Soluble Major Histocompatibility Complex class I related Chain Molecule A.




**(B)**

**Area Under the Curve**

| Test Result Variable(s) | Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| AFP | .475 | .058 | .657 | .361 | .590 |
| DCP | .679 | .050 | .001 | .580 | .778 |
| Smica | .871 | .034 | .000 | .804 | .939 |

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

**Figure 7:** Diagnostic performances of the sMICA, DCP and AFP for discriminating patients with small sized HCC lesions from cirrhotic patients. (A) ROC curve obtained by plot at different cut-offs for AFP and sMICA in HCC versus diseased control; (B) The area under the curve is 0.475 for AFP with Std. Error=0.058 and 95% CI from 0.361 to 0.590. The area under the curve is 0.679 for DCP with Std. Error=0.050 and 95% CI from 0.580 to 0.778. The area under the curve is 0.871 for sMICA with Std. Error=0.034 and 95% CI from 0.804 to 0.939. HCC-Hepatocellular Carcinoma; LC-Liver Cirrhosis; ROC-Receiver Operating Characteristic; AFP-Alpha-Fetoprotein; DCP-Des-γ CarboxyProthrombin; sMICA-Soluble Major Histocompatibility Complex class I related chain molecule A.

controls (DC) were investigated (Figure 6A). The AUC value of sMICA was 0.917 [95% CI=0.866-0.969, p=0.000] and DCP showed an AUC value of 0.711 [95% CI=0.624-0.798, p=0.000]. Besides, AFP showed an AUC value of 0.716 [95% CI=0.635-0.797, p=0.000] (Figure 6B).

When the analysis were versus LC group (Figure 7A), the AUC value for sMICA was 0.871 [95% CI=0.804-0.939, p=0.000] and for DCP was 0.679 [95% CI=0.580-0.778, p=0.001] while that for AFP was 0.475 [95% CI=0.361-0.590, p=0.657] (Figure 7B).

Sensitivity of sMICA for differentiating HCC from all controls, DC and LC were 88.5% with specificities of 89%, 83.3% and 72% respectively when applying a cut-off level of 499.5 pg/ml, which was the cut-off with the maximal sum of sensitivity and specificity (Table 6). Similarly, sensitivity of DCP for distinguishing these patients from all control, DC and LC patients was 49.2%. The DCP specificity values were 76.7% when the analysis was versus diseased plus healthy controls, 90.6% when versus all DC and 86% when versus LC group only. The cut-off level of 3 ng/ml was the one that showed the maximum value of sensitivity plus specificity (Table 6). As well, sensitivity of AFP for distinguishing these patients from all control, DC and LC patients was 37.7%. The AFP specificity was 93.8%, 90.6% and 82% respectively when the analysis was against all controls, DC and LC group only. The cut-off level of 171 ng/ml was the one that showed the maximum value of sensitivity plus specificity (Table 6). The PPV(s) and NPV(s) for the three markers in identifying HCC patients with small focal lesions between 2-5cms were shown in (Table 6).

## Discussion

HCC is one of the most common cancers worldwide and its incidence is steadily increasing. Most HCC cases develop in patients with a history of CH or LC in which there is continuous inflammation and regeneration of hepatocytes mostly associated with persistent infection with hepatitis B or C viruses [24,25]. Behnke et al., 2012 stated a 20-fold increase in the risk of HCC in patients with HCV infection compared to those without infection [26]. All patients in the current study were HCV positive because Egypt has the highest prevalence (15%-20%) of HCV globally [27]. Accordingly, HCV-related cirrhosis was the underlying cause for 100% of HCC patients in this study.

In the present study, the mean age of HCC patients was 65.5 years old, 64.5 years old in liver cirrhosis patients and 34.5 years old in chronic hepatitis group. These findings were in agreement with Sherman et al., 2005 who showed that the incidence of HCC started to increase above 45 years of age [28]**.** Moreover, Sharma et al., and Leerapum et al., demonstrated a higher mean of age (70 years old) [29,30]. El-Serag, 2002 studied the role of the duration of HCV infection in the development of cirrhosis and HCC. He reported that 3–35% of patients progress to cirrhosis 25 years after infection and 1–3% progress to HCC 30 years after infection [31]. Additionally, male predominance among HCC group (80%) was observed in the current study. These findings are in consistent with Zakhary et al., [32]. Male predominance can be explained by more hepatitis carrier states, exposure to environmental toxins and hepatic effects of androgens [33,34]. These findings confirm the fact that HCC is the most common cancer in men and the eighth most common cancer in women universally 34.

The absence of significant difference between the clinical pictures along with liver function tests in addition to hematological profiles among HCC and LC patients is obvious in our study and in other's as well [35-42]. This is because the disease itself has no unique alarming symptoms. Therefore, despite numerous advances in the treatment of HCC during the last decade, HCC has very bad prognosis and high mortality rates due to late diagnosis. In fact, many HCC patients have already developed locally advanced disease or distant metastasis by the time of presentation which emphasizes the necessity of good screening modalities for detection of HCC [43].

To screen for HCC in high-risk populations, the combination between serum AFP and ultrasound findings continues to be the most commonly used method based on surveillance strategies [23]. To date, AFP is the only HCC biomarker that has been studied through to phase 5 of biomarker development (6). In the present study, AFP levels were highly statistical significant increase in HCC group when compared to other groups ($p \leq 0.05$) as well there was a highly significant increase in AFP levels in other patients groups when compared to control group ($p<0.001$). Regarding tumor size, there was a highly significant difference in AFP levels between HCC patients with tumor sized from 2-5 cm and those with tumors sized more than 5 cm ($p<0.001$). These results were in agreement with Hernández et al., who studied 189 patients. These patients include 22 HCC patients and 167 non HCC patients (HBV, HCV and alcoholic hepatitis) [44]. Also, Ekram et al., and Mukozu et al., and Sterling et al., revealed a statistically significant difference between the mean AFP levels in HCC group when compared to cirrhotic group [45-47]. Likewise, Zakhary et al., reported that AFP levels in HCC group was significantly increased compared to control and HCV groups. They further categorized HCC patients according to tumor size and they found that AFP showed significant changes according to tumors size which concur with our study [32]. Moreover, different studies had revealed a strong correlation between AFP values and tumor dimensions [48,49].

Elevated AFP levels were only observed in about 60%-70% of HCC patients and to a lesser extent (33-65%) in patients with smaller

HCCs which is comparable to what was demonstrated in our study. As well, it was reported that only 10-20% of patients in early stages of HCC has abnormal AFP levels and that its levels vary significantly in the presence of benign liver nodules [50, 51]. Moreover, there is non-specific elevation of AFP in 15%-58% of patients with CH and 11%-47% of patients with LC beside its reported elevation in other cancers [50,52]. Thus, it is obvious that there is still a crucial need for finding more reliable markers for HCC to achieve early diagnosis and hence better prognosis.

DCP is now serving as a complement to other HCC biomarkers such as AFP and AFP-L3% in HCC surveillance and risk assessment [8,10] but its usefulness in different ethnic groups is still an issue of contention. DCP is an abnormal prothrombin protein. It is increased in the sera of patients with HCC. Production of DCP is a result of an acquired defect in the post-translational carboxylation of the prothrombin precursor in malignant cells. The validity of DCP as a tumor marker for HCC patients has been reported by many investigators but very few studies had reported its levels in the Egyptian HCV-induced HCC patients [32]. Additionally, sMICA is suggested by a number of researchers as having a useful diagnostic efficiency for HCC (22). MICA is a natural ligand for activating natural killer group 2, member D (NKG2D) receptor which expressed on the surface of natural killer (NK) cells. The binding of MICA to NKG2D triggers a cascade of signal transduction events that activates NK cells to release cytotoxic molecules and subsequently causes NK cells to identify and lyse target cells [53]. There is a lot of studies suggest that carcinoma cells have a mechanism to shed MICA from the cell surface into the extracellular domain, generating a soluble form of MICA called sMICA [**20**]. This process leads to a decrease in membrane bound MICA and an increase in sMICA [**54**]. Therefore, a possible mechanism by which carcinoma cells escape immune surveillance is the expression and shedding of MICA as sMICA. Previous studies have demonstrated that sMICA levels are significantly correlated with patient prognosis in some cancer types such as ovarian cancer [**20**], squamous cell carcinoma [**21**], colorectal carcinoma, gastric carcinoma and lymphoma [**55**]. Indeed, a high level of sMICA is usually related with poor prognosis among cancer patients. However, sMICA and its diagnostic and prognostic value in HCC are rarely studied.

The present study was designed to elucidate the significance of sMICA in the diagnosis of HCV-induced HCC. As well, we investigated its value in patients with CH and LC on top of HCV infection since being major risk factors for the development of HCC in the Egyptian community. We also evaluated the diagnostic capability of sMICA and DCP among Egyptians in comparison with AFP which is till now the conventional marker that is used as a reference for early detection of HCC.

In this study, there was a highly significant increase in DCP levels in HCC group in comparison to other groups ($p<0.001$). There was no significant difference between LC and CH group when compared to control group ($p=0.06$ and 0.08 respectively). As well, there was no significant difference between LC when compared to CH ($p=0.051$). Concerning tumor size, there was no significant difference between HCC patients with tumor sized from 2-5 cm and those with tumor sized more than 5cms ($p=0.77$). These results were in line with previous studies done by Zakhary et al., 2013; Wesam et al., 2013; Sharma et al., 2010; Durazo et al., 2008 and Tada et al., 2005 who concluded that DCP was not significantly elevated in any patients without HCC [**15,29,32,56,57**]. Sharma et al., and Durazo et al., found that DCP was more specific than AFP for diagnosing HCC. They also categorized HCC patients on the basis of their tumor size. They found that larger tumor size was associated with elevated DCP level which is not in accord with our study [**15,29**]. These differences may be due to population differences and tumor characteristics. DCP also has been reported to predict the progression of HCC patients as those with higher DCP levels had a significantly higher frequency of intrahepatic metastasis, portal or hepatic vein tumor thrombosis and capsular infiltration [**58**]. Furthermore, Zakhary et al., 2013 showed significant gradual elevation of DCP levels within HCC patients correlating with progressive disease grade [32]. El-Assaly et al., 2008 and Durazo et al., reported that DCP levels significantly correlate with histopathological grade of HCC [15,59]. In addition, Inagaki et al., evaluated DCP levels in HCC tissues by immunohistochemical staining using anti DCP antibody together with assessing DCP levels in serum. They found that 31 patients from 74 patients had elevated DCP serum levels and 56 from 74 patients had positive DCP in tissues [60]. Elevated DCP levels in serum were associated with capsule infiltration, vascular invasion, intrahepatic metastases and more advanced TNM (Tumor, Nodes, and Metastasis) staging. Fujiyama et al., 1991 found that normal levels of DCP were observed in 29 out of 30 patients (97%) with small liver tumors measuring 2cm or less in diameter and the authors concluded that the diagnostic application of DCP in small liver tumors is limited. However, in patients with tumors larger than 2cm, the DCP assay may even be more useful than AFP [61]. On contrary, our results disagree with Marrero et al., who found that DCP levels increased according to the stepwise progression of liver disease, i.e. from CH to LC to HCC, and according to tumor stage. They referred that to the possible role of the alterations in vitamin K production secondary to cholestasis, malnutrition, renal failure, or use of medications which alter gut flora in patients with decompensated cirrhosis [62]. DCP is reported to be increased in advanced stages of HCCs as in vascular invasion or metastasis. In addition, false elevation of DCP could be noted in vitamin K deficiency due to liver insufficiency or vitamin K antagonists [8,62]. Though the diagnostic performance of DCP has been studied in some populations, it is essential to note that the FDA-approved DCP for risk-stratification of chronic liver disease patients, rather than in screening for HCC thus we still in need for reliable HCC markers [23].

In the present study, sMICA levels were highly significantly increased in HCC patients when compared to other groups, in LC versus CH and HC as well as in CH versus HC ($p<0.001$). Concerning tumor size, there was no significant difference in sMICA when HCC patients with tumor sized from 2-5 cm were compared to tumor sized more than 5cms. The present study were in agreement with Li et al., 2013 who concluded that levels of sMICA can be used as an independent prognostic factor for advanced HCC and with the study done by Armeanu et al., who detected significantly high MICA levels in liver tumor tissues but failed to detect MICA expression in normal liver tissues [16,63]. In a study done by Jinushi et al., 2005, they assessed serum level of sMICA in 26 patients with HCC. *In vitro* experiments were performed to examine the impact of sMICA on NK cell expression of NKG2D and subsequent dendritic cell (DC) activation. The levels of sMICA were frequently elevated in patients with advanced HCC [64]. Kohgal et al., studied serum levels of sMICA as well as detected MICA by immunohistochemistry. They reported a stepwise increase in the level of sMICA from hepatitis to HCC and they demonstrated that sMICA increases with the progression of chronic liver disease as well as the progression to HCC. They found that sMICA is significantly increased in the sera of patients not only with HCC but also with chronic liver diseases and they stated that MICA is expressed on HCC tissues as well as in LC tissue but normal liver tissues and CH tissue do

not express MICA. They aimed the elevation of sMICA in LC patients to the oxidative and genotoxic stresses accumulation in hepatocytes in chronic liver disease [18]. On the contrary, in the studies done by Li et al., and Kohgal et al., sMICA was significantly related with tumor size which disagrees with our findings but these studies were performed for HCC patients on top of HBV infection and it's established that biomarker performance may vary depending on the etiology of liver disease [16,18,65,66].

Furthermore, it was reported that MICA is rarely expressed by normal cells but is highly expressed in carcinoma cells, such as HCC, prostate cancer, glioma, and others [67-69]. Armeanu et al., detected significantly high MICA levels in liver tumor tissues but failed to detect MICA expression in normal liver tissues [63]. Likewise, Jiang et al., assessed serum levels of sMICA in different malignances and different infectious diseases. They found the elevation of sMICA appears to be especially dramatic in the hepatic cancer patients. They categorized HCC patients according to TNM staging and they found a certain extent of correlation between sMICA levels and the clinical stages of hepatic cancer [70].

In the present study, we constructed for the first time a head-to-head comparison of three diagnostic markers for HCV-induced HCC in Egyptian patients. It is notable that AFP had no significant correlation with either DCP or sMICA and the same was reported between DCP and sMICA. Also, both DCP and sMICA was not correlated significantly with the clinico-pathological features of HCC patients. This is in accordance with Li et al., who reported that sMICA levels were not significantly correlated with AFP levels [16]. Moreover, our results demonstrated that the sensitivity of sMICA was higher than that of both AFP and DCP at the cut-off levels of 499.5 pg/ml (sMICA), 171 ng/ml(AFP) and 3 ng/ml(DCP). The elevated positive and negative predictive values of sMICA are indicating that sMICA is suitable and superior to both AFP and DCP for HCV-related HCC surveillance in Egypt. We further found that compared to AFP and DCP, sMICA had a superior performance in: 1) the identification of HCV-related HCC from HCV-related non HCC patients [AUC 0.908 (95% CI: 0.863-0.953)] vs. both DCP [AUC 0.725 (95% CI: 0.654-0.796)] and AFP [AUC 0.827 (95%CI: 0.770-0.883)], 2) the differential diagnosis between HCC and liver cirrhosis, in particular for HCC patients with focal lesions between 2-5 cm. According to our data, measuring the serum levels of sMICA either alone or in combination with AFP and DCP could have a useful diagnostic tool for surveillance or as a decisional tool in clinical practice to identify HCV-related HCC, differentiate it from HCV-related LC and to predict for early hepatic carcinogenesis. Consequently, augment the proportion of patients with HCC diagnosed in an early tumor stage. In the current study, it is obvious that sMICA always had higher sensitivity over both AFP and DCP however this was not the same regarding specificity. Thus, combining these three markers could be valuable to yield the best sensitivity and specificity ever.

In this study, the control group consisted of the diseased controls including non HCC diseased controls (DC) with CH and LC in addition to apparently healthy controls (HC). The LC control group provides a more stringent and practical comparison for the performance of HCC diagnostic biomarkers as LC is the most predominance premalignant phase in Egypt (27) and all of our patients had cirrhotic liver before developing HCC. Also, most LC patients had elevated levels of AFP but still <200 ng/ml (the established diagnostic point for HCC) which is equal to some HCC patients in their early stages or those with small focal lesions (50) as shown in this study (65% of small HCC, 40% of all HCC). Accordingly, distinguishing HCC patients (in particular those having small sized focal lesions) from LC patients is more challenging. Consequently, we divided the HCC subjects to those having small sized focal lesions (2-5cm) and those having hepatic focal lesions>5 cm. As previously documented, the diagnostic yield of AFP for HCC is significantly lower when using a control group including more advanced liver disease patients [13,71-74], which is comparable to this study.

The current study reported a higher diagnostic performance of AFP (cut-off value 171) over DCP (cut-off value 3) with higher AFP sensitivities (62% for AFP vs. 54% for DCP) except for HCC patients with small focal lesions (3-5cm) where the reverse is found (37.7% for AFP vs. 49.2% for DCP). In lots of case-controlled studies, DCP was found to have sensitivities between 48-62% and specificities between 81-98% for differentiating HCC from LC. In parallel, AFP was found to have sensitivity and specificity values from 40-54% and 88-97% respectively. The performance of DCP versus AFP for HCC diagnosis varies between studies. Some studies showed that DCP is superior to AFP, while others found no significant difference in the relative diagnostic efficacies of the two markers, but the combination between both of them could augment their diagnostic yield and few studies claimed that yet AFP appeared to have greater diagnostic capabilities [8,15,46,47,61,62]. Marrero et al., 2003 reported that DCP at a cut-off of 125 mAU/ml better distinguishes HCC from chronic liver diseases and cirrhosis than AFP at a cut-off of 11 ng/ml whereas Nakamura et al., 2006 reported that AFP outperformed DCP for the diagnosis of HCC<3 cm but DCP had better performance than AFP for the diagnosis of HCC>5cm [62,75]. Additionally, Ji et al., reported an overall better performance of DCP over AFP for HBV-induced HCC which is more ob6ous in the surveillance of early HCC. Moreover, they found that the superiority of DCP to AFP was more profound in AFP-negative HCC and that higher DCP levels were associated with worse clinical behaviour and shorter disease-free survival [76]. As well, the results of Marrero et al., and Li et al., 2014 reported an elevated accuracy of DCP in HCV-related HCC [8,77]. Many reasons might contribute to these differences including population or ethnic differences, tumor characters, etiological difference of the enrolled subjects, different methods of DCP detection between studies [e.g. ELISA **vs**. CLEIA], the use of different marker cut-off values in each study (40, 60, and 100 mAU/mL for DCP and 20-200 ng/ml for AFP) as well as differences in tumor stage [76,78].

To the best of our knowledge, there are no published researches that investigated the clinical impact of sMICA on the diagnoses of HCV-induced HCC via ROC curve either alone or in comparison with other markers. As well, no studies weigh sMICA against either AFP and/or DCP. This adds a point of new to our study and provides it with a clinical significance via helping in the avoidance of the false-negative results of AFP which are reported in 30-40% of HCC patients [79]. Thus, the trial of sMICA in the phases of biomarkers establishment for the screening of HCC on top of HCV-related liver diseases alone or with AFP and/or DCP may enhance their diagnostic performance. Furthermore, more broad research on sMICA expression in HCV-induced HCC would be valuable in studying the possibility of applying sMICA in monitoring HCC therapy.

There are many advantages of our study which make it superior than previous studies and give it a clinical significance: 1- sMICA levels were compared with other common serum HCC biomarkers such as AFP and DCP. 2 -The control patients with normal subjects and non HCC diseased controls including LC and CH patients. 3- The fixed etiological base of all patients which leads to a more or less fair

comparison. However, despite providing these findings, our study was limited by several factors: 1- All patients are from one region in Egypt and thus our results require external validation. 2- sMICA was not assessed as a serum prognostic marker by the follow-up of the cases. 3- sMICA levels were not assessed after treatment which could provide important information on the influence of treatment on changes in sMICA levels. 4- The number of subjects that is still small due to financial issues. Larger sample sizes and follow-up studies are required in future research to further expose more predictive and prognostic values of sMICA. Many factors affecting the diagnostic performance of biomarker should be considered individually in prospective studies including the tumor invasiveness, numbers of tumor focal lesions, tumor differentiation and TNM stage. Finally, the dynamic changes in sMICA, DCP and AFP from CH to LC then to HCC need to be assessed in upcoming studies.

## Conclusion

sMICA has superior diagnostic efficacy with bigger AUC and higher sensitivity than both AFP and DCP. Thus, sMICA could be used as a talented marker for early diagnosis of HCV-induced HCC along with other markers especially for HCC with small sized focal lesions in Egyptian patients. It may have an advantage in improving the therapeutic approaches of HCC through applying immunotherapy for HCC patients or supervising the effectiveness of the already established chemotherapy, especially for those with high level of sMICA. As well, validating the use of sMICA could assist in the prediction of early recurrence of HCC following treatment and after surgical removal of the focal lesions, or liver transplant.

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