British Journal of Medicine & Medical Research
3(4): 1858-1870, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Tissue Biomarkers in the Early Detection of Hepatocellular Carcinoma among Egyptian Patients with Chronic Hepatitis C: A Possible Genetic Profile

Hassan El-Garem[1], Hanan Abdel- Hafez[1*], Ahmed Foaud[1], Wafaa Al Akel[1], Mohey EldienAtia[2], Mona Salah[3], Hany Khatteb[4], Heba Osman[1], Khaled Ragab[2] and Naglaa Zayed[1]

[1]Endemic Medicine and Hepatology Department Faculty of Medicine, Cairo University, Egypt.
[2]Tropical Department, Theodor Bilharz Research Institute, Egypt.
[3]Clinical Pathology Department,Faculty of Medicine, Cairo University, Egypt.
[4]Pathology Department, Faculty of Medicine, Cairo University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author HEG designed the study managed the literature searches. Author HAH did the clinical evaluation of cases as well as their US and biopsies. Author AF shared the patients biopsies. Author WAA performed the statistical analysis, author MEA wrote the protocol. Author MS performed the laboratory work. Author HK did the histopathological examination of cases. Authors HO and KR collected the data. Authors NZ and HAH wrote the first draft of the manuscript as well as the final one. All authors read and approved the final manuscript.

Research Article

Received 1st April 2013
Accepted 31st May 2013
Published 16th June 2013

ABSTRACT

Background and Study Aims: Gene expression of biomarkers involved in hepatocarcinogensis could be used for the early diagnosis of hepatocellular carcinoma (HCC).
Aim: To evaluate the hepatocyte expression of Glypican-3 (GPC-3), paternally expressed gene 10 (PEG-10), Midkine (MDK), Serpin peptidase inhibitor (SERPINI1), and Ubiquinol-cytochrome (QP-C) which can represent a possible genetic profile among hepatitis C virus (HCV)-related HCC patients.

**Corresponding author: Email: hananahh@hotmail.com;*

Patients and Methods: This prospective study was conducted on 70 Egyptian patients with HCV-related chronic liver disease and HCC patients. Patients were categorized into chronic HCV group (n=25), post-HCV cirrhosis group (n=24), HCC group (n=21) in addition to 7 healthy individuals who were candidates for living-donor related transplantation. Liver tissue obtained from all patients was subjected to total RNA extraction, reverse transcription of extracted RNA into cDNA and finally tissue expression of GPC-3, MDK, PEG-10, SERPINI1 and QP-C by qRT-PCR was assessed in each group.
Results: A significant increase in hepatocyte expression of GPC-3, MDK, SERPINI1, and QP-C was detected in cancerous compared to non-cancerous liver tissue; in contrast, PEG-10 was significantly expressed in chronic HCV patients. The ROC curves was able to identify best cutoff values, sensitivity and specificity for GPC-3 (7.26, 81%, 58%), SERPINI1 (0.16, 80%, 70%), MDK (3.8, 60%, 70%) and QP-C (0.45, 65%, 79%) respectively. There was no significant correlation between the tissue expression of these biomarkers and the size of hepatic focal lesion or AFP levels.
Conclusion: Hepatocyte expression of GPC-3, MDK, SERPINI1, and QP-C could represent a potential genetic profile for the early detection of HCC.

Keywords: GPC-3; MDK; SERPINI1; PEG; QP-C; HCC; HCV; Egypt.

1. INTRODUCTION

HCC is one of the most common and aggressive cancers worldwide. It has been the third cancer killer worldwide**.** The high mortality associated with this disease is mainly attributed to the inability to diagnose HCC patients at an early stage [1]. In Egypt, incidence of HCC is currently increasing, which may be the result of a shift in the relative importance of HBV and HCV as primary risk factors [2]. There is a doubling in the incidence rate in the past 10 years [3].

It is estimated that the problem of HCC will increase until it reaches its peak in the year 2018 [4]. The only approach to screen for the presence of HCC in high-risk populations is the combination of serum alpha-fetoprotein (AFP) and ultrasonography [5]. However, elevated serum AFP is only observed in about 60%-70% of HCC patients and, to a lesser extent (33-65%), in patients with smaller HCCs [6]. Moreover, the non-specific elevation of serum AFP in 15%- 58% of patients with chronic hepatitis and 11%-47% of patients with liver cirrhosis have led to the exclusion of AFP as a screening follow up test for HCC patients form the recently published EASL guidelines [7-8].

The possible identification of new biomarkers that have a sufficient sensitivity and specificity for the diagnosis of HCC, especially in AFP-normal and or small lesions for the possible early detection of HCC among high risk patients would be of great impact in the early diagnosis of HCC. A significant increase in the tissue expression of glypican3 (GPC3), paternally expressed gene 10 (PEG 10)*, and midkine (MDK),* Serpine peptidase inhibitor (SERPINI1), and Ubiquinol –cytochrome(QP-C*)* was detected in most of the HCC samples, including those with normal serum AFP and small tumors [9]. The aim of our study was to investigate these possible genetic profile for the early diagnosis of HCC through the assessment of the tissue expression of the candidate genes; glypican3 (GPC3), paternally expressed gene 10 (PEG 10)*, and midkine (MDK),* Serpine peptidase inhibitor (SERPINI1), and Ubiquinol –cytochrome(QP-C*)* in cancerous and non- cancerous liver tissue.

2. PATIENTS AND METHODS

2.1 Study Population

The current study was conducted on 70 patients with HCV-related chronic liver disease form June 2008 to January 2011, as well as 7 healthy controls. The patients recruited from outpatients' clinic of Endemic Medicine Department, Faculty of Medicine, Cairo University, National Cancer Institute and Fatemic Hospital, Ministry of Health and Population (MOHP), Cairo, Egypt. Approval from the institution ethical committee was obtained. A written informed consent was obtained from each patient included in study regarding the study plane or the publication.

2.1.1 Inclusion criteria

-Adult (18 -70 years) patients of both sexes with evidence of chronic HCV infection as diagnosed by positive HCV antibodies in addition to HCV-RNA positivity by PCR and histopathological evidence whenever needed.
-Patients with HCV-related primary HCC.
-Normal healthy individuals who were scheduled for living donor liver transplantation (LDLT).

2.1.2 Exclusion criteria

- Chronic HCV patients who had received Interferon-based therapy.
- HCC patients who had undergone ablative therapy and patients with metastatic liver disease.

2.2 Participants were Classified to Four Groups

Group I: Patients with HCV-related HCC (n=21 patients) who had focal hepatic lesion detected on ultrasonographic examination among patients with HCV-cirrhosis and were diagnosed as primary HCC according to their spiral CT findings and/or their histopathological examination.

Group II: Patients with chronic non-cirrhotic HCV (n=25 patients) who were diagnosed by HCV –RNA PCR and had no clinical, laboratory or histopathological evidence of liver cirrhosis.

Group III: Patients with post-hepatitis C cirrhosis (n=24) who were diagnosed by clinical, laboratory, ultrasonographic and histopathological evidence of liver cirrhosis.

Group IV: Normal healthy individuals (n=7) who were potential donors for living-related liver transplantation.

2.3 All Patients were Subjected to:

1- Informed written consent
2- Full history taking and clinical assessment.
3- Routine laboratory investigations; complete blood picture, renal function tests and blood sugar analysis.

4- Biochemical liver profile; serum bilirubin, serum aminotransaminases (ALT, AST), alkaline phosphatase, serum albumin, prothrombin time & concentration.
5- Assessment of disease severity by Child-Pugh grade [(10)].
6- Hepatitis Markers for HBV and HCV; HBsAg, HBc total Ab and HCV Ab by ELISA & HCV RNA by PCR
7- Serum alpha fetoprotein (ELISA). The clinically acceptable normal serum AFP was defined as >20 ng/mL.
8- Real time abdominal ultrasonography with special emphasis on the ultra-sonographic criteria of cirrhosis and description of any focal hepatic lesion concerning number, site, size, and echogenicity, portal vein diameter and patency. Moreover, the presences of splenomegaly, ascities and abdominal lymph nodes enlargement or masses were also reported.
9- Triphasic CT abdomen for all patients with focal hepatic lesion on ultrasonographic examination. The presence of arterial uptake followed by washout is highly specific for HCC [11]
10- Barcelona Clinic Liver Cancer (BCLC) staging for HCC [12].
11- US-guided liver biopsy for:
    a. Patients with chronic HCV patients with & without cirrhosis whenever possible.
    b. Normal healthy subject as a pre-requisite for the protocol for living-donor liver transplantation
    c. Patients with HCC if CT scan findings were non-conclusive for the diagnosis of HCC.
12. Histopathological examination of the liver biopsies.
    I. Grading of HCV according to the METAVIR score [13].
    II. Grading of HCC according to the Edmondson and Steiner [14] grading system.

2.4 Specified Candidate Markers

The expression of GPC3, PEG10, MDK, SERPINI1 and QP-C in the studied patients and control specimens was evaluated by Quantitative real time RT-PCR (qRT-PCR).

Fresh specimens were stabilized in RNA later RNA Stabilization Reagent. (Qiagen, Cat. No. 76106). Total RNA extraction was done using RNeasy mini kit (Qiagen, Cat. No.74104) and automated QIAcube extraction system (from the tumour tissue for HCC patients). For qRT-PCR, 2µg isolated total RNA was converted to cDNA using the High Capacity cDNA Archive Kit (Applied Biosystems, PN 4374966). Real time PCR reactions were performed with the ABI 7500 Real Time PCR System (Applied Biosystems). Amplification was carried out in duplicate for each sample and each gene. An RNA pool from seven disease-free normal liver donors was used as a calibrator. 20X TaqMan Gene Expression Assay Mix for target genes were purchased from Applied Biosystems (Assays on Demand; GPC3: Hs00170471_m1; MDK: Hs00171064_m1; QP-C: Hs00429571_g1; PEG10: Hs00248288_s1; SERPINI1: Hs00192380_m1). Human 18S rRNA labeled with VIC reporter dye was used as an endogenous control for each sample (Applied Biosystems P/N 4308329).

To obtain quantitative results, the relative standard curve method was used for relative quantitation of the studied genes and the endogenous control (rRNA) in the unknown samples. The cycle threshold (Ct) was interpolated versus the Log ng of RNA dilutions used in the standard curves. For each test sample, the amount of targets and endogenous control were determined from the corresponding standard curves. The ratio between ng of each target gene and ng of rRNA represent the normalized gene expression for each sample. A calibrator was used as the basis for comparing results. The used calibrator for tissue

expression of the studied genes was a pooled RNA sample extracted from normal liver tissue specimens. The target quantity in the calibrator was determined by interpolating from the corresponding standard curve and it was also normalized to the endogenous control. Because the test sample quantity is divided by the calibrator quantity, the unit from the standard curve is cancelled out. Target quantities in the unknown samples were expressed as an n-fold difference relative to the calibrator.

2.5 Statistical Analysis

Data was statistically analyzed using SPSS (statistical package for social science) program version 13 for windows, for all the analysis p value < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curves were performed to determine the best cutoff sensitivity and specificity values for the candidate genes that could differentiate HCC from non-cancerous liver tissue (chronic hepatitis C and cirrhotic group).

3. RESULTS

This study included 70 patients with different HCV-related chronic liver disease with male predominance in all groups and significant age difference towards the development of HCC compared to non-HCC patients, p value <0.001 as shown in Table 1. Liver transaminases; AST and ALT; showed 1 to 2 fold elevation among all groups with a significant P value (p = 0.02). Moreover, serum bilirubin, albumin and ALP levels showed a statistically significant difference, with p values of 0.001, 0.000, 0.002, respectively". Serum AFP levels were elevated in HCC groups compared to the non-HCC groups with a trend towards significance, p value of 0.08 as shown in Table 1.

Table 1. The demographic and laboratory features of the studied patients (n=70)

Features	Non-HCC		HCC group (n= 21)
	HCV group (n=25)	Cirrhosis group (n= 24)	
Age (years)	36.08±10.731 [a]	45.83±9.416 [b]	56.05±5.352 [c]
Gender (n) M/F	16/9 [a]	16/8 [a]	14/7 [a]
ALT (0-41 U/L)	54.56±32.25 [a]	61.13 ±19.03 [a]	57.00 ±43.51 [a]
AST (0-37 U/L)	44.64 ±23.56 [a]	61.88 ±20.96 [b]	72.38 ±39.16 [b]
ALP (40-104 U/L)	67.47 ±24.44 [a]	248.95±147.74 [b]	256.60 ±245.56 [b]
Albumin (3.4-5.2 g/dl)	4.18 ± 0.24 [a]	4.12 ±0.27 [a]	3.45 ±0.55 [b]
Bilirubin (0.1 – 1 mg/dl)	0.77 ± 0.28 [a]	0.79 ± 0.27 [a]	1.21 ±0.67 [a]
AFP ng/ml	3.35 ± 3.00 [a]	15.27±14.34 [a]	207.53±720.53 [b]

** Different letters are significant at P value ≤ 0.05*

AFP: alpha-fetoprotein, ALT: alanine aminotransferase, AST: Aspartate aminotransferase, ALP: alkaline phosphatase.

In the present series, 21 biopsy-proven HCV-HCC patients were enrolled whose characteristic features are shown in Table 2. Liver disease severity was assessed by performance status (PS) where 81% had a PS 0 while 19% had PS 1-2. Most (85.7%) of HCC patients had Child-Pugh class A while none were class C. The ultrasonographic features of hepatic focal lesions (HFLs) revealed that 66% of patients had right lobe lesion, size was < 3m in 52% and almost 50% had multiple lesions. Staging of HCC according to the BCLC [(13)] scoring system demonstrated stage A in 57%, stage B in 42.9% while none had stage C or D. Histopathological grading of HCC according to the criteria of Edmondson

and Steiber [14], revealed that 90.5% were grade II. There was no evidence of portal vein thrombosis or extra-hepatic spread or metastasis.AFP levels demonstrated that 52.4% of HCC patients had elevated serum AFP as the clinically acceptable normal serum AFP was defined at >20 ng/ml.

Table 2. Characteristics of HFL among the HCC group (n=21)

Characteristics of HFL	Number (%)
AFP level(ng/ml)	
<20	10(47.6%)
20-300	10(47.6%)
>300	1(4.8%)
Child-Pugh score	
A	18(85.7%)
B	3(14.3%)
C	0
Number of HFL	
Single/multiple	11/10
Site of HFL	
Right lobe	14(66.6%)
Left lobe	2(9.6%)
Both lobes	5(23.8%)
Size of HFL	
<3cm	11(52.4%)
3-5cm	4(19%)
>5m	6(28.5%)
BCLC	
Stage 0	0
Stage A	12(57.1%)
Stage B	9(42.9%)
Stage C/ Stage D	0/0
Grade of differentiation I / II / III	1(4.8%)/19(90.5)/1(4.8%)

BCLC: Barcelona Clinic Liver Cancer
HFL: hepatic focal lesion

The highest median level of hepatocyte expression for GPC-3, MDK, SERPINI-1 and QP-C mRNA was found in HCC followed by chronic hepatitis C and cirrhotic liver tissue with a statistically significant difference; ($p = 0.05$, <0.01, <0.01, and <0.01 respectively), whereas hepatocyte expression of PEG-10 mRNA showed its statistical significant highest median level in chronic hepatitis C followed by HCC then cirrhotic liver tissue (P value <0.01). Fig. 1 (A-E).

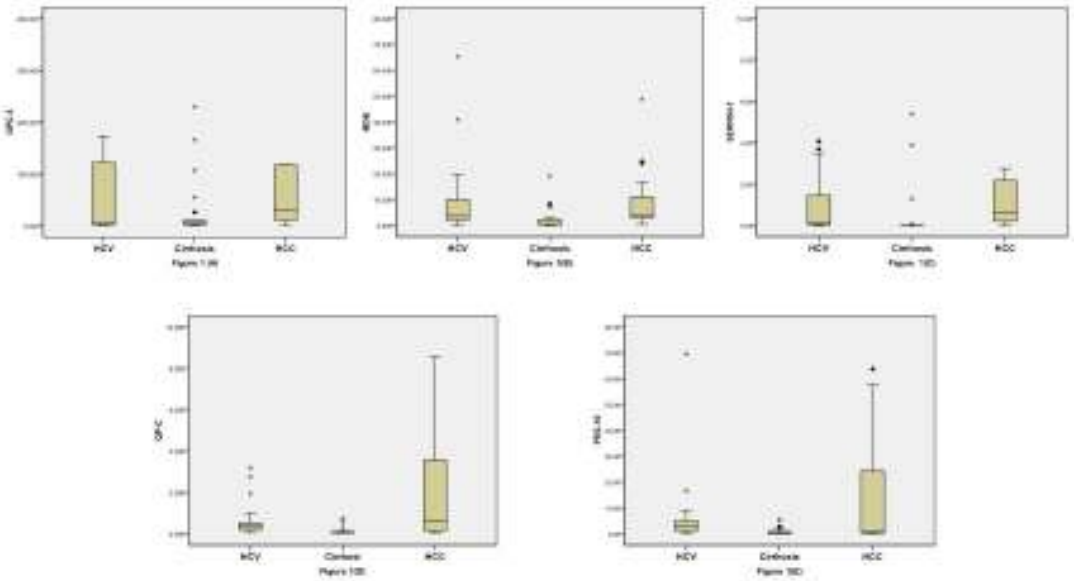

Fig. 1. (A-E): Genetic tissue expression for GPC3, MDK, PEG-10, SERPINI1, and QP-C among the different studied groups

GPC-3: tissue glypican-3; MDK, tissue Midkine; PEG10, tissue paternally expressed 10, *SERPINI1, tissue-serpin peptidase inhibitor, QP-C, tissue Ubiquinol –cytochrome.*

The ROC curve analysis of tissue markers expression in HCC group versus non HCC groups (HCV group and cirrhotic group) was able to identify best cutoff values, sensitivity and specificity for GPC-3 (7.26, 81%, 58%), SERPINI1 (0.16, 80%, 70%), MDK (3.8, 60%, 70%) and QP-C (0.45, 65%, 79%) respectively. (Table 3 and Fig. 2).

Table 3. Diagnostic performance of the studied markers in HCC group versus non HCC

Tissue expression	AUC	Std. Error	P value	Best cutoff	Sensitivity	Specificity	PPV	NPV
GPC3	0.79	0.08	0.002	7.26	81%,	58%	32%	100%
MDK	0.89	0.06	0.000	3.8	60%	70%	37.5%	37.5%
PEG 10	0.77	0.08	0.004	0.16	90%	53%	32%	100%
SERPINI1	0.76	0.06	0.001	0.16	80%	70%	53%	89%
QP-C	0.72	0.07	0.006	0.45	65%	79%	61%	84%
AFP	0.82	0.05	<0.01	11.35	71%	0.75%	56%	86%

AUC: area under the curve, GPC-3: glypican-3, MD: Midkine , negative predictive value(NPV), PEG10: tissue paternally expressed 10, positive predictive value(PPV), QP-C: Ubiquinol–cytochrome, SERPINI1:serpin peptidase inhibitor.AFP:alphafetoprotein.

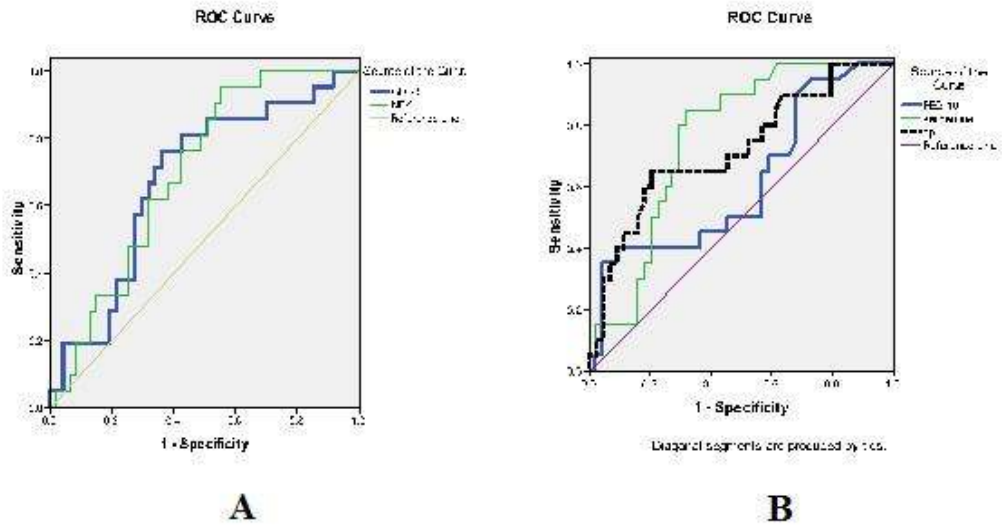

Fig. 2. A+B: The diagnostic performance of the studied markers

GPC-3: glypican-3, MD: Midkine, PEG10: tissue patemally expressed 10, QP-C: Ubiquinol-cytochrome, SERPINII: serpin peptidase inhibitor.

It is observed that specificity is very much impaired and slight significant improving NPV with added AFP to the diagnostic tissue expression markers as presented in Table 4.

Table 4. Diagnostic values of the markers in comparison with addition of AFP at the level of 11.35 to their levels

Tissue expression	Sensitivity	P1	Specificity	P2
GPC3, GPC3 and AFP	81% , 86%	0.72 (NS)	58%, 44%	<0.01 (S)
MDK, MDK and AFP	62%, 86%	0.16 (NS)	69%, 49%	0.06
PEG 10, PEG 10 and AFP	86%,95%	0.61 (NS)	32%, 20%	<0.01 (S)
SERPINI1, SERPINI1 and AFP	80%, 85%	1.0 (NS)	70%, 49%	0.06
QP-C, QP-C and AFP	67%, 86%	0.27 (NS)	80%, 57%	0.02 (S)

Tissue expression for the studied markers in all patients showed a significant correlation between PEG-10 and both GPC-3 (p value 0.01) and MDK (p value 0.009). On the other hand, GPC-3 showed non significant correlation with MDK; p value 0.07. GPC3 tissue expression showed positive significant correlation with the level of albumin and bilirubin (p value 0.01 and 0.007 respectively). On the other hand, other laboratory parameters did not show such significance.

Neither tumour size nor AFP protein level was significantly correlated with any of tissue markers expression. Simultaneous measurement of AFP and our new markers in HCC group versus non HCC group showed that the sensitivity and the specificity did not improve. Addition of SERPINI and QP-C to each other improved their performance; as they represented sensitivity of 100%, specificity 70%, PPV 77% and NPV 100%.

4. DISCUSSION

In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients [15]. The number of hepatocellular carcinoma has been postulated to increase for the next three decades owing to the heavy burden with HCV (14.7%) among the Egyptian population [16].

Traditionally, the most commonly used serum marker for HCC is AFP. However, its use has been questioned due to a reported sensitivity of 39% to 65% and specificity of 65% to 94%. Moreover, multiple limitations exists when applied to chronic HCV patients who might express high levels of AFP which have been related to the hepatic fibrosis and necro-inflammation resulting from the natural process of disease progression in HCV and unrelated to HCC [17]. Recently, the American and the European Associations for the Study of Liver Diseases guidelines for HCC screening have recognized the overall poor performance of AFP and have excluded it from the screening recommendations [8]. Therefore, it is necessary to identify new HCC biomarkers that have a sufficient sensitivity and specificity for the diagnosis of HCC patients. GPC3, MDK, PEG-10, SERPINI1, and QP-C; were validated by qRT-PCR as biomarkers to distinguish tumor samples from non-cancerous hepatic tissues These genetic markers expressed a significant increase in their tissue expression in most of the HCC samples, including those with normal serum AFP and small tumours [9]. Thus we propose the identification of a possible genetic profile for the early diagnosis of HCC through the evaluation of hepatocyte expression of GPC3, MDK, PEG-10, SERPINI1, and QP-C.

In the present series, HCC patients expressed male predominance and significant old age, 47.6% of patients had a level of AFP of < 20ng/ml, and a similar percent to the patients had AFP level of 20-300.Moreover, patients had Child-Pugh A-B with BCLC stage 1and 2 and almost 70% had HFLs of average diameter ≤3-5cm. No significant difference was found between AFP values in relation to HFL size and number.

The incidence of HCC was more prevalent among males and was found to increase progressively with advancing age in almost all areas. Male predominance was more obvious in populations at high risk of the tumor than those in low or intermediate risk [18].

Similarly, previous studies have reported that about two thirds of HCC patients with nodules < 4 cm have serum AFP levels < 200 ng/ml and up to 20% HCC patients do not produce AFP [19] with no significant correlation was observed between the size of the tumour and serum AFP levels [20].This could be explained by the fact that tumor differentiation and its ability to secrete AFP are more important than the tumour size in determining the level of AFP produced by HCC.

Based on the ROC analysis to achieve high sensitivity and specificity, the optimal cutoff value to differentiate HCC from non-malignant chronic liver disease was 7.26 ng/ml (81% sensitivity) for GPC3, 3.8 (60% sensitivity) for MDK, 0.16 (90% sensitivity) for PEG-10, 0.16 (80% sensitivity) for SERPINI1 and 0.45(79% Sensitivity) for QP-C. 100% Combined AFP & (GPC3 or PEG-10) showed sensitivity of 100%, specificity of 22%.

In our series it was found that there is no correlation between the tissue expression of the GPC3, MDK, PEG-10, SERPINI1, or QP-C and AFP levels. The absence of significant correlation between AFP and GPC3 was observed by Nakatsura et al. [21].

Median levels for tissue expression of GPC3 were higher in the HCC group compared to its level in non-cancerous liver tissue (cirrhosis and chronic HCV groups) with *p* value <0.05. The over-expression of GPC3 has been recently demonstrated to be a reliable diagnostic indicator in HCC patient with sensitivity and specificity values that exceeds alpha-fetoprotein due to its important role in cell growth, differentiation, and tumourigenesis [22]. Moreover GPC3 was found to regulate cell proliferation by enhancing the resistance to apoptosis through the dysfunction of the Bax/Bcl-2/cytochrome c/caspase-3 signaling pathway [23].

Our results were in agreement with a similar study which was done to evaluate the clinical utility of tissue GPC3 mRNA as a diagnostic and prognostic marker in Egyptian HCC patients by immuno-histochemical, Youssef [24] and his colleagues concluded that GPC-3 mRNA was significantly up-regulated in HCC (n=40) as compared to normal (n=20) and non-malignant liver samples (n=20), and hence, GPC-3 could serve as a molecular marker for early HCC. Glypicans interact with growth factors can modulate their activities, and hence, play an important role in cell growth, differentiation and migration [25]. Moreover, GPC3 can confer tumourgenesis through the interaction between insulin like growth factor-II (IGF-II) and its receptor and subsequent activation of IGF-pathway [26].

The diagnostic performance of Glypican-3 was previously studied and was able to differentiate between HCC and liver cirrhosis with good sensitivity and specificity. This supports its importance as a novel tumor marker in HCV-induced liver cirrhosis which is considered as a pre malignant condition [27].

We found that the median level of MDK showed higher level in tumourous liver tissue compared to non-cancerous liver tissue (cirrhosis and chronic HCV groups) with p value of <0.01. Similarly, HCC expressed increased MDK at the messenger RNA and protein level as detected by immunohistochemical analysis [28], and by PCR-Southern blot analysis, a sensitive PCR analysis [29].The over-expression of MDK at the mRNA and protein level were 74.2% and 75.8% respectively in carcinoma tissue, which were higher than in para-tumourous liver tissue and normal controls ($p < 0.01$) [30]. Moreover, our results matched the study done by Kato et al. [28] in which 77 primary HCC specimens from patients aged 17-72 years (63 men and 14 women) were examined. MDK was found to exert cancer-related activities in the process of carcinogenesis, including transformation, fibrinolysis, cell migration, cell survival, anti-apoptosis, and angiogenesis

A rather similar prospective study on 285 patients, 144 in complete remission and 141 at risk for developing de novo HCC, evaluated the changes in serum midkine level. MDK levels were in parallel with disease activity in about 81% of patients with HCC and rapidly rising serum MDK levels occurred in patients in the terminal stage. A sharp rise of serum MDK signals was inversely correlated with survival days [31].

MDK has expressed nearly the same diagnostic performance as GPC3; it could help in differentiation between HCC and liver cirrhosis at a cutoff value of 1.58. MDK showed 90% sensitivity and 79% specificity. (*p* value 0.000), and at a cutoff value of 3.80 it could also help in differentiation between HCC and non HCC, with lower sensitivity and specificity60% sensitivity, 70% specificity (*p* value= 0.167) , this is because non HCC group includes liver cirrhosis patients and HCV patients and MDK could not significantly differentiate between the later group and HCC patients.

Concerning the tissue expression of PEG-10, highest levels were evident in chronic HCV patients followed by HCC and liver cirrhosis, (*p* value <0.01). At a cutoff value of 0.16 It

could significantly differentiate HCC from liver cirrhosis with 90% sensitivity and 53% specificity (*p* value =0.004), but it could not significantly differentiate between HCC and either HCV or non HCC. PEG-10 has a functional role in the growth-promoting activities in HCC cells [32]. Moreover, the activation of PEG-10 expression could also be regulated by onco-proteins, c-MYC, that is commonly over-expressed in HCC Feitelson et al. [33]. In contrast to our results, Li et al. [34] studied 87 patients with HCC with serological evidence of HBV carriage. They observed a significant progressive increase in PEG-10 mRNA levels from the putative pre-malignant adjacent livers to early resectable HCC tumours, and to late inoperable HCCs. This difference may be related to the different etiological factor for HCC either HBV or HCV.

Gene expression of SERPINI1 was significantly higher in the HCC group as demonstrated by Spano et al. [35] who revealed a molecular signature of 11 genes up-regulation in HCC, SERPINI1 was one of them. The up-regulation of *SERPINI1* gene is in agreement with its location on chromosomal region gained in HCC.

QP-C at cutoff 0.45 represented sensitivity 65%, and specificity 79%, to our knowledge, data about the QP-C gene expression in cancer is very limited and studies are scarce. An increased expression of QP-C is associated with most HCC samples, including those with normal serum AFP and small tumour size [9].

In the present study we tried to study hepatocyte expression of GPC3, PEG-10, MDK, SERPINI1, and QP-C in order to represent a genetic profile for HCC patients. A diagnostic signature approach using a combined score of biomarkers rather than a single marker may improve the prediction accuracy of HCC patients. We were able to report a significant correlation between PEG-10 and both GPC-3 (*p* value 0.01) and MDK (*p* value 0.009). On the other hand, a trend towards significance correlation was observed between GPC-3 and MDK; *p* value 0.07. These findings may highlight the clinical utility of combined assessment of these genetic markers in the early detection of HCC. However, this study carried some limitations as these findings warrant extensive validation of these biomarkers as clinical diagnostic tools by further investigation in a large case-control study. Furthermore a combined expression score of these five candidate genes may be of high value in the clinical setting of hepatocellular carcinoma with a proper reprehensive sample size population.

5. CONCLUSION

High levels of hepatocyte expression of GPC3, MDK, SERPINI1, and QP-C in cancerous compared to non-cancerous liver tissue could emphasize the potential utility of these biomarkers as a genetic profile for the early detection of HCC among HCV patients.

COMPETING INTERESTS

Authors declare that there is no competing interests exist.

REFERENCES

1. Yuen MF, Lai CL. Serological markers of liver cancer. Best Pract Res Clin Gastroentenrol. 2005;19:91–99.
2. EL Zayadi AR, Badran HM, Barakat EM, et al. Hepatocellular carcinoma in Egypt: a single centre study over a decade. World J Gastroenterol. 2005;11(33):5193-5198.

3. Anwar WA, Khaled HM, Amra HA, et al. Factors changing pattern of hepatocellular carcinoma (HCC) and its risk in Egypt: possibilities for prevention. Mutat Res. *2008;*659(1-2):176-184.
4. Esmat G. Hepatocellular carcinoma: the magnitude of the problem in Egypt, 6th international conference of oncology, surgery and gastroenterology. Towards a Healthy Liver in Egypt; 2002.
5. Befeler AS, Di Bisceglie AM. Hepatocellular carcinoma: diagnosis and treatment. Gastroenterology. 2002;122:1609-1619.
6. Johnson PJ. The role of serum a-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liver Dis. 2001;5:145-159.
7. Kim JW, Ye Q, Forgues M, et al. Cancer-associated molecular signature in the tissue samples of patients with cirrhosis. Hepatology. 2004;39(2):518–527.
8. Bruix J, Sherman M. Management of hepatocellular carcinoma: An Update Hepatol. 2011;53(3):1020-2.
9. Jia HL, Ye QH, Qin LX et al. Gene expression profiling reveals potential biomarkers of human hepatocellular carcinoma. Clin. Cancer Res. 2007;13:1133–1139.
10. Pugh RN, Murray-Lyon IM, Dawson JL, et al. Transection ofthe oesophagus for bleeding oesophageal varices. Br J Surg; 1973;60:646–649.
11. Burrel M, Llovet JM, Ayuso C, et al. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. Hepatology. 2003;38:1034–1042.
12. Liovet JM, Burroughs A, Bruix J, et al. Hepatocellular carcinoma. Lancet. 2003;362:1907–1917.
13. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol Res. 1995;23:178-184.
14. Edmondson HA and Steiner PE. Primary carcinoma of the liver. A study of 100 cases among 48,900 necropsies. Cancer. 1954;7:462–503.
15. El Zayadi AR, Badran HM, Shawky S. Effect of surveillance for hepatocellular carcinoma on tumor staging and treatment decisions in Egyptian patients. Hepatol Int. 2009;9170-9177.
16. El-Zanaty, Fatma, Ann Way. Egypt Demographic and Health Survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International Cairo. p 2009;431. Egypt Demographic Health Survey: Final Report - June, 2009.
17. Daniele B, Bencivenga A, Megna AS, et al. Alfa Fetoprotein and ultrasonography screening for hepatocellular carcinoma. Gastroenterol. 2004;127(5 suppl 1)1:108-112.
18. El-Serag HB, Rudolph KL. Hepatocellular Carcinoma: Epidemiology and Molecular Carcinogenesis. Gastroenterol. 2007;132:2557–2576.
19. Khan A, Asmaa I, Edward L, et al. Diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2009;15(11):1301-1314.
20. Darwish A. Assessment of Clinical Significance of Serum Squamous Cell Carcinoma Antigen in Patients with Liver Cirrhosis and Hepatocellular Carcinoma. Thesis submitted for partial fulfillment of the M.Sc. degree in tropical medicine, Faculty of medicine, Cairo University; 2006.
21. Nakatsura T, Yoshitake O, Senju S, et al. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. Biochemical and Biophysical Research Communications. 2003;306:16–25.
22. Li CM, Margolin AA, Salas M, et al. PEG10 is a c-MYC target gene in cancer cells, Cancer Res. 2006;66:665–672.
23. Liu S, Li Y, Chen W, Zheng P, Liu T, et al. Silencing glypican-3 expression induces apoptosis in human hepatocellular carcinoma cells. Biochem Biophys Res Commun. 2012;419(4):656-661.

24. Youssef ME, El-Sharkawy SL, Naglaa F, et al. Clinical utility of glypican3 in hepatocellular carcinoma. International journal of integrative biology; 2010;10(1):41.
25. Dina KH, Kumarasen CM. Glypican 3 a noval diagnostic marker for hepatocellular carcinoma and more. Advances in Anatomic Pathology. 2009;16(2):125-129.
26. Cheng W, Tseng CJ, et al. Glypican 3 mediated ontogenesis involves the insulin – like growth factor-signaling pathway. Carcinogenesis. 2008;29(7):1319-1326.
27. Ozkan H, Erdal H, Koçak E, Tutkak H, Karaeren Z, et al. Diagnostic and prognostic role of serum glypican 3 in patients with hepatocellular carcinoma. J Clin Lab Anal. 2011;25(5):350-353.
28. Kato M, Shinozawa T, Kato S, Awaya A, Terada T. Increased midkine expression in hepatocellular carcinoma. Arch Pathol Lab Med. 2000;124(6):848-852.
29. Koide N, Hada H, Shinji T, Ujike K, Hirasaki S, Yumoto Y, et al. Expression of the midkine gene in human hepatocellular carcinomas. Hepatogastroenterology. 1999;46(30):3189-3196.
30. Dai LC, Yao X, Lu YL, Ping JL, Zhang BW, et al. Expression of midkine *and its* relationship with HBV infection in hepatocellular carcinomas. Zhonghua Yi Xue Za Zhi. 2003;83(19):1691-1693.
31. Hung YJ, Lin ZH, Cheng TI, Liang CT, Kuo TM, et al. Serum midkine as a prognostic biomarker for patients with hepatocellular carcinoma. Am J Clin Pathol. 2011;136(4):594-603.
32. Okabe H, Satoh S, Furukawa Y, et al. Involvement of PEG10 in human hepatocellular carcinogenesis through interaction with SIAH1, Cancer Res. 2003;63:3043–3048.
33. Feitelson MA. c-myc overexpression in hepatocarcinogenesis. Hum. Pathol. 2004;35: 1299–1302.
34. Li L, Jin R, Zhang X, Lv F, Liu L, et al. Oncogenic activation of GPC3 by c-Myc in human hepatocellular carcinoma. Hepatology. 2012;18(10):5891-5899.
35. Spano D, Russo R, Di Maso D, Rosso N, Terracciano LM, Roncalli M, et al. Galectin-1 and Its Involvement in Hepatocellular Carcinoma Aggressiveness. Mol Med. 2010;16(3-4):102–115.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=205&id=12&aid=1513