Noninvasive prediction of hepatitis C-associated hepatocellular carcinoma using circulating apolipoproteins

Hasan El Garem[a], Gamal Esmat[a], Rabab Salama[a], Hanan Fouad[b], Dina Sabry[b], Yomna Mostafa[c], Dalia Omran[a], Heba Omar[a]

Departments of [a]Endemic Medicine and Hepatogastroenterology, [b]Medical Biochemistry, Faculty of Medicine, Cairo University, [c]Department of Hepatogastroenterology, Egypt Air Hospital, Cairo, Egypt

Correspondence to Heba Omar, MD, GCSRT, Faculty of Medicine, Cairo University, Cairo, 11652, Egypt. Tel: +202- 25326439; e-mail: hebaomar1202@hotmail.com

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Background and aims
Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related mortality worldwide. We investigated the potential usefulness of circulating apolipoproteins (Apo-A1 and Apo-A4) in HCC screening and diagnosis.
Patients and methods
We included 60 adult patients with hepatitis C virus-related chronic liver disease including HCC, in addition to 20 healthy controls. Patients were stratified into three equal groups, with 20 patients each: chronic hepatitis C, posthepatitis C cirrhosis (liver cirrhosis), and HCC. All patients and controls underwent full clinical assessment, laboratory investigations, and evaluation of candidate apolipoproteins by enzyme-linked immunoassay.
Results
Significantly higher Apo-A1 and Apo-A4 levels were detected in patients with HCC than in those with liver cirrhosis ($P<0.001$). Receiver operator characteristic curve showed that for HCC diagnosis, a cutoff of 78.6 mg/dl for Apo-A1 yielded 90% sensitivity and 100% specificity and a cutoff of 16.5 mg/dl for Apo-A4 yielded 85% sensitivity and 80% specificity. Furthermore, within HCC group, Apo-A1 was significantly higher in patients with small HCC (>2 cm) than those with large tumors ($P=0.01$). Lower Apo-A1 level correlated significantly with pylethrombosis ($P=0.007$).
Conclusion
Apo-A1 and Apo-A4 are novel biomarkers for HCC screening and diagnosis, with a special discriminative ability for Apo-A1 for those with small tumors and those with pylethrombosis.

Keywords:
apolipoprotein-A1, apolipoprotein-A4, diagnosis, pylethrombosis, screening, small hepatocellular carcinoma

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Introduction

Chronic hepatitis C (CHC) infection is a global health problem that infects between 130 and 150 million people worldwide. The link between CHC infection and the development of hepatocellular carcinoma (HCC), which is the most common type of primary hepatic tumors (90% of all primary hepatic malignancies), is well known [1], with an estimated 17-fold increase in the risk of HCC development among hepatitis C virus (HCV)-infected patients [1]. Annually, there are more than 620 000 newly diagnosed HCC cases [2], of them 22% (>100 000) were attributed to CHC infection [3].

In Egypt, the incidence rate of HCC has doubled in the past decade. This could be attributed to high prevalence of HCV in Egyptian population, with an estimated prevalence of HCC among patients with liver cirrhosis (LC) of 21% [4].

Early detection of HCC by the screening of at-risk population is considered one of the best strategies to reduce disease-related mortality and mortality [5–7]. Although serum biomarkers are minimally invasive and acceptable, the sensitivity and specificity of biomarkers like alpha-fetoprotein (AFP) for early detection of HCC are marginal [8].

Proteins perform and regulate most biological cell functions. Several recent proteomic analyses had pointed to the potential role of apolipoproteins especially ApoA-1 and Apo-A4 in the diagnosis of HCC [9].

The current study aimed at determination of the diagnostic accuracy of apolipoproteins (Apo-A1 and Apo-A4) as noninvasive biomarkers for HCC screening and diagnosis and to set a cutoff value of

those apolipoproteins at which further investigations for HCC become of high importance.

Patient and methods

Research design and study population

This was a case–control study using prospectively collected data from 60 treatment-naïve HCV patients within the spectrum of HCV-related chronic liver disease, including HCC. All included patients had confirmed positivity of their HCV antibody and HCV RNA by PCR. The presence or absence of LC was judged base on their clinical, laboratory, and ultrasound findings. Patients with HCC were diagnosed by typical enhancement (concurrent wash-in in the arterial phase and wash-out in delayed/venous phase) of hepatic focal lesion more than 1 cm on four-phase multidetector computed tomography or dynamic contrast-enhanced MRI examination of cirrhotic liver according to EASL guidelines [10].

Patients were categorized into three equal groups of 20 patients each (CHC without LC group, LC group, and posthepatitis C HCC group). In addition, 20 healthy volunteers were included as a control group. Participants were consecutively recruited from the outpatient clinic of Endemic Medicine and Hepatology Department, Faculty of Medicine, Cairo University, during the period from May 2015 to March 2016. Patients with concomitant chronic HBV or HIV infection, patients with any other diagnosed malignancy or metastasis, and those who received any treatment for their diagnosed HCC were excluded.

The study was conducted according to guidelines laid down in Declaration of Helsinki 1975 after its approval by the clinical research ethics committee of the Endemic Medicine and Hepatology Department, Faculty of Medicine, Cairo University. All study participants agreed to give written informed consent before participation in the study.

Outcomes

The primary outcome of interest for the current work was to determine the diagnostic accuracy of serum expression levels of apolipoproteins (Apo-A1 and Apo-A4) as noninvasive diagnostic biomarkers for posthepatitis C HCC and to set the best cutoff value for those biomarkers at which HCC diagnosis is highly suspected. Peripheral blood levels of Apo-A1 and Apo-A4 were evaluated using enzyme-linked immunoassay (ELISA) kits Apo-A1 ELISA kit E0604h and Apo-AIV ELISA kit E1967h, respectively, according to the manufacturer's instructions (EIAab Reference Database online at www.eiaab.com).

Covariates

Data regarding the following variables were also obtained from all patients included in our study.

Demographics and clinical data

Age, sex, comorbidities (diabetes), smoking practice, risk factors for acquiring HCV infection (previous parenteral antischistosomal therapy, operations, or blood transfusion) were noted. For patients with LC, their Child–Pugh classification [11] was calculated.

Laboratory data

Laboratory data were collected at the time of enrollment and included complete blood count, hepatic necroinflammatory markers (aspartate and alanine aminotransferases), indicators of liver synthetic functions (total bilirubin, albumin, and international normalized ration), and AFP.

Statistical analyses

Data were entered, validated, and analyzed using STATA 14 (College Station, Texas, USA) software. Patients' demographic and routine laboratory values data were expressed as number (%) for categorical variables and as mean±SD or median (interquartile range) for continuous variables. Continuous variables were tested for normality using histograms and sktest. Those variables with a small P value less than 0.05 on sktest were considered not normally distributed. All quantitative parametric and nonparametric variables were analyzed using either Student t test or Mann–Whitney test for comparison of two groups whenever appropriate. A comparison of three groups was done using Kruskal–Wallis for nonparametric or analysis of variance test for parametric variables whenever appropriate [12]. χ^2 test was used for comparison of categorical data and Fisher's exact test for categorical data with lower number of samples [13]. Associations between HCC diagnosis and candidate apolipoproteins were investigated using univariate logistic regression models. Data were presented as odds ratios (OR) with 95% confidence intervals (95% CI). The diagnostic performance of Apo-A1and Apo-A4 in the prediction of HCC was evaluated using receiver operator characteristic (ROC) curves. The area under the ROC curves and the 95% CI were used as indexes of accuracy ROC curve. All statistical analyses were based on two-sided hypothesis tests with a significance level of P value less than 0.05.

Results

Characteristics of the included participants

Table 1 shows demographics, clinical, and laboratory characteristics of enrolled participants. A total of 60 patients met the study inclusion criteria: 20 had chronic hepatitis, 20 had cirrhosis of the liver, and 20 had HCC. In addition, 20 normal participants were analyzed. There was significant male predominance across all patient groups; males represented 35, 75, and 85% of CHC, LC, and HCC groups, respectively ($P<0.001$). The mean±SD age of all patients was in the seventh decade of life (61.11±11.1), with the youngest mean age in the LC group at 58.90±10.72 years and oldest age in HCC at 64.85±7.16 years, with significant difference in age among the studied groups ($P<0.001$).

Circulating Apo-A1 and Apo-A4 levels in patients with liver diseases and healthy controls

Circulating Apo-A1 and Apo-A4 were measured in the blood of patients with HCV-related chronic liver disease as well as controls using ELISA. Mean serum expression level of Apo-A1 was significantly higher ($P<0.001$) in HCC group (212.12±101.24 mg/dl) compared with LC (30.25±12.71 mg/dl) and control (32.65±2.70 mg/dl) groups. Apo-A4 expression was significantly higher in patients with HCC (19.00±4.13 mg/dl) compared with patients with LC (11.22±6.63 mg/dl) and significantly lower in patients with HCC than controls (23.17 ±1.56 mg/dl) ($P<0.001$). In relation to LC group, CHC group had significant higher level of Apo-A1 (30.25±12.71 vs. 100.50±31.31 mg/dl, respectively, $P\leq0.001$) and significant lower levels of Apo-A4 (11.22±6.63 vs. 38.39±17.05 mg/dl, respectively, $P\leq0.001$), as shown in Table 2.

Features of patients with hepatocellular carcinoma

Clinical characteristics of the 20 patients with HCC (17 male and three female) are shown in Table 3. Overall, 70% (14) of patients with HCC were older than 60 years, a similar percent (14) had single focal lesion, 20% had small focal lesion (<2 cm in largest diameter), 85% had

Table 1 Demographic, clinical, and laboratory data of studied participants (*N*=80)

Variables	Control (*N*=20)	CH (*N*=20)	LC (*N*=20)	HCC (*N*=20)	*P* value
Age (years)	38.05±7.53	59.65±14.04	58.90±10.72	64.85±7.16	<0.001
Male	9 (45)	16 (80)	15 (75)	17 (85)	0.024
Diabetes (yes)	2 (10)	7 (35)	8 (40)	5 (25)	0.002
Smoking (yes)	4 (20)	3 (15)	1 (5)	1 (5)	<0.001
Parenteral antischistosomal therapy	1 (5)	4 (20)	7 (35)	7 (35)	<0.001
Blood transfusion	9 (45)	7 (35)	3 (15)	4 (20)	0.001
Operation	0	3 (15)	7 (35)	5 (25)	0.001
CTP score					
A			1 (5)	6 (30)	<0.001
B	NA	NA	9 (45)	8 (40)	
C			10 (50)	6 (30)	
Hemoglobin (g/dl)	13.25±1.60	12.07±3.08	10.29±1.56	10.59±1.97	<0.001
Median WBC ($\times10^3/mm^3$)	8.25 (7.2–9.9)	7.50 (4.6–9.3)	4.70 (2.7–7.85)	5.50 (3.7–8.05)	0.006
Platelets, ($\times10/mm^3$)	272.50±28.86	169.45±52.83	66.20±26.0	96.80±57.43	<0.001
Median ALT, ($\times10^3/mm^3$) (0–42)	20.00 (16.6–25)	35.00 (22.5–47.5)	27.00 (27–41.5)	51.50 (32–79.5)	<0.001
Median AST, (IU/l) (0–42)	25.00 (20–30)	34.00 (28–70)	48.50 (35.5–75)	68.00 (42.5–103)	<0.001
Median total bilirubin, (mg/dl)	0.30 (0.25–0.3)	0.75 (0.5–1.1)	2.20 (1.05–4.5)	2.05 (1.1–3.2)	<0.001
Albumin (g/dl) (3.5–5.5)	4.13±0.22	3.78±0.58	2.61±0.59	2.96±0.74	<0.001
INR	1.01±0.02	1.15±0.16	1.91±1.01	1.43±0.30	<0.001
Log_{10} AFP (ng/dl)	NA	2.2±1.52	1.82± 0.86	4.26±3.14	0.0009

Data are expressed as mean±SD, median (range), or *n* (%). AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartate transaminase; CH, chronic hepatitis CTP, Child–Turcotte–Pugh; HCC, hepatocellular carcinoma; INR, international normalized ration; liver cirrhosis; WBC, white blood cell.

Table 2 Apolipoprotein-A1 and apolipoprotein-A4 levels in different patient groups

Groups	Patients (*N*)	Apo-A1 (mean±SD)	*P* value	Apo-A4 (mean±SD)	*P* value
All HCC	20	212.12±101.24	<0.001*	19.00±4.13	<0.001*
Cirrhosis	20	30.25±12.71	<0.001**	11.22±6.63	<0.001**
Chronic hepatitis	20	100.50±31.31	<0.001***	38.39±17.05	0.0003***
Controls	20	32.65±2.70	<0.001$	23.17±1.56	0.001$
Small HCC	4	319.23±108.58	<0.001$$	21.18 ±4.41	0.009$$

CH, chronic hepatitis; HCC, hepatocellular carcinoma; LC, liver cirrhosis. **P* (HCC vs. cirrhosis). ***P* (LC vs. CH). ****P* (CH vs. control). $*P* (control vs. HCC). $$*P* (small HCC vs. cirrhosis).

no vascular invasion, 85% had no lymphadenopathy, and 60% had Barcelona clinic liver cancer stage 0-B. Apo-A1 level was significantly higher in patients with small HCC than those with large tumors (319.23±108.58 vs. 185.35 ±82.47 mg/dl, respectively, *P*=0.01). On the contrary, Apo-A4 showed nonsignificant higher values in patients with small HCC compared with those with large HCC (21.18±4.41 vs. 18.46±4.01 mg/dl, respectively, *P*=0.25). Apo-A1 had significantly lower expression level in patients with portal vascular thrombosis (*n*=3) than patients without pylethrombosis (188.18±86.45 vs. 347.83±71.33 mg/dl, respectively, *P*=0.007). On the contrary, Apo-A4 had nonsignificant lower levels in patients with pylethrombosis compared with those without pylethrombosis (18.64± 4.35 vs. 21.07 ±1.68 mg/dl, *P*=0.36).

Diagnostic value of Apo-A1 and Apo-A4

We analyzed the properties of Apo-A1 and Apo-A4 for HCC diagnosis using univariate logistic regression model, with '0' representing cirrhosis group and '1' representing HCC group. Logistic regression analysis revealed that Apo-A1 (OR, 1.10; 95% CI, 1.02–1.20; *P*=0.03) and Apo-A4 (OR, 1.28; 95% CI, 1.09–1.50; *P*<0.001) were significantly associated with increased risk of HCC. However, AFP was not significantly associated with risk of HCC (OR, 1.02; 95% CI, 0.99–1.06; *P*=0.22) (Table 4).

ROC curve showed that for HCC diagnosis, a cutoff of 78.6 mg/dl for Apo-A1 yielded 90% sensitivity and 100% specificity with 100% positive predictive value and 86.96% negative predictive value (C statistics=0.99) and at a cutoff of 16.5 mg/dl for Apo-A4 yielded 85% sensitivity and 80% specificity with 80% positive predictive value and 80% negative predictive value (C statistics=0.84), as depicted in Fig. 1.

Discussion

In the current case–control study, we investigated the diagnostic utility of circulating Apo-A1 and Apo-A4 for prediction of HCV-related HCC. Apo-A1 and Apo-A4 showed superiority to AFP in the diagnosis of HCV-related HCC (C statistics, 0.99 and 0.84, respectively). Furthermore, Apo-A1 could significantly identify patients with small HCC (<2 cm in its largest diameter) as well as those with pylethrombosis.

HCC is a life-threating tumor that accounts for more than 90% of primary liver malignancies. It ranks the fifth most common cancer in males and the seventh in

Table 4 Univariate logistic regression analysis for candidate biomarkers

	OR	95% CI	*P* value
Apo-A1 (mg/dl)	1.10	1.02–1.20	0.03
Apo-A4 (mg/dl)	1.28	1.09–1.50	<0.001
AFP	1.02	0.99–1.06	0.22

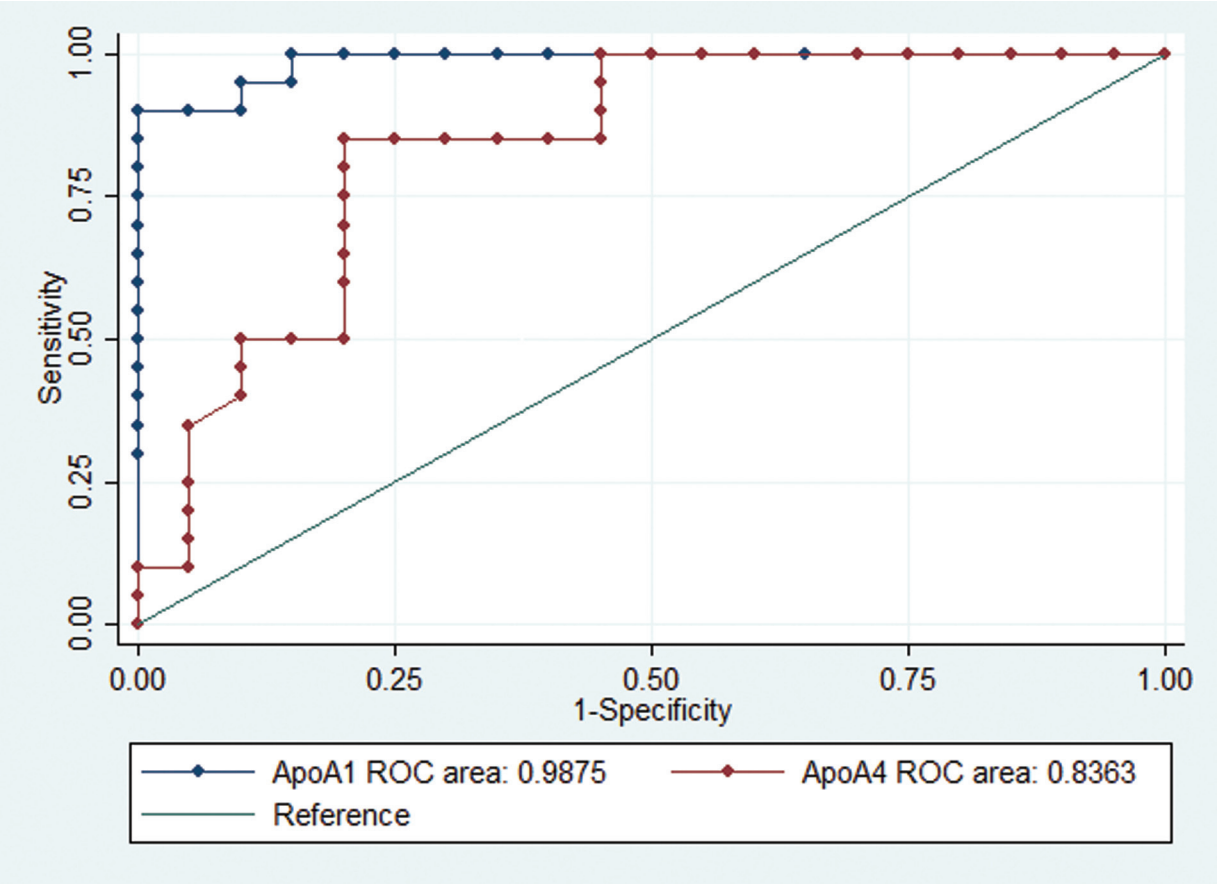
AFP, alpha-fetoprotein.

Table 3 Clinical characteristics of patients with hepatocellular carcinoma (*N*=20)

Parameters	Patients [*n* (%)]	Apo-A1	*P* value	Apo-A4	*P* value
Age (years)					
≤60	6 (30)	186.38±118.34	0.47	19.67±5.3	0.65
>60	14 (70)	223.16± 95.69		18.71±3.67	
Sex					
Male	17 (85)	193.64±97.03	0.05	19.13±4.44	0.75
Female	3 (15)	316.87±51.94		18.27±1.75	
Number of focal lesions					
Single	14 (70)	212.88±104.81	0.96	19.11±4.85	0.86
Multiple	6 (30)	210.37±101.90		18.73±1.88	
Largest diameter (cm)					
<2	4 (20)	319.23±108.58	0.01	21.18±4.41	0.25
>2	16 (80)	185.35±82.47		18.46±4.01	
Portal vein status					
Thrombosis	3 (15)	188.18±86.45	0.007	18.64±4.35	0.36
Patent portal vein	17 (85)	347.83±71.33		21.07±1.68	
Lymphadenopathy					
Yes	3(15)	150.93±87.52	0.27	16.57±2.74	0.28
No	17(85)	222.92±101.92		19.43±4.24	
BCLC classification					
0-B	12 (60)	203.09±87.19	0.64	19.98±4.12	0.20
C-D	8 (40)	225.68±124.60		17.54±3.93	

Data are expressed in mean±SD. BCLC, Barcelona clinic liver cancer.

Figure 1



ROC curve for the diagnostic performance of apolipoproteins (Apo-A1 and Apo-A4) in HCC. HCC, hepatocellular carcinoma; ROC, receiver operator characteristic.

females worldwide. Owing to its very poor prognosis, it has been regarded as the third most common leading cause of death owing to malignancies worldwide [14]. The prognosis of HCC remains unsatisfying with an overall 5-year survival rate of less than 10% owing to a lack of early detecting methods [15]. Progression of HCV-related liver disease from chronic infection to HCC through LC is associated with alterations in circulating proteins as well as proteins present in hepatic tissues [16].

Apo-A1 is a major constituent of high-density lipoproteins encoded on chromosome 11q23-q24 [17,18] and has a well-known anti-atherogenic function that protects against cardiovascular diseases [19–23]. The role of Apo-A1 in HCC is still unclear, but few recent reports have discussed the potential diagnostic role of Apo-A1 for early detection of HCV-related HCC [24,25].

Most apolipoproteins, lipids, and lipoproteins are formed in hepatocytes. Thus, hepatic injury or chronic liver diseases including HCC may result in abnormal circulating patterns of those molecules secondary to alterations of many cytokines and/ or metabolic cellular substances, or tumor factors [26], but the exact mechanisms are not fully understood [27]. In the present work, high levels of circulating Apo-A1 and Apo-A4 were detected in patients with HCC compared with patients with LC (212.12±101.24 vs. 30.25±12.71 mg/dl, respectively, and 19.00±4.13 vs. 11.22±6.63 mg/dl, respectively). Both were associated with increased risk of HCC (OR, 1.10 and 1.28, respectively). Pleguezuelo *et al.* [9] reported an increase in risk for HCC with increased Apo-A1 and decreased Apo-A4 levels. Nonmatching Apo-A4 results between Pleguezuelo study and ours could be explained to the different HCC etiology in both studies. Liver is the main site for the formation, storage, transportation, and breakdown of some Apo [27]. Each Apo might be affected by liver disease in a different way.

Furthermore, within HCC group, we found that Apo-A1 level was significantly higher in patients with small HCC than those with large tumors (319.23±108.58 vs. 185.35±82.47 mg/dl, respectively, P=0.01). The prognosis of HCC could be markedly improved if it is detected at an early potentially curable stage. However,

precise identification of early-stage HCC is still clinically difficult, even with the help of advanced radiology technology. These results may suggest that Apo-A1 might be a useful marker for diagnosis of small HCC [28].Interestingly, we found that within HCC group, lower Apo-A1 level correlated significantly with the occurrence of pylethrombosis (188.18±86.45 vs. 347.83±71.33 mg/dl, respectively, P=0.007). High-density lipoprotein had multiple direct and indirect antithrombotic effects, directly through enhancement of endothelial nitric oxide synthase activity and decrease leukocytes endothelial adhesiveness [29] and indirectly through induction of anticoagulant action of the protein C pathway with subsequent to downregulation of thrombin production [30]. Matching our results, Xu *et al*. [31] reported lower levels of Apo A-I expressed in most HCC tumors with pylethrombosis (n=20) compared with HCC tumors without pylethrombosis (n=20). Eichinger *et al*. [32] found that high levels of Apo A-I meant a decreased risk of recurrent venous thromboembolism. Dissimilar profiles of up- and down-regulated apolipoprotein have been identified; such discrepant results could be attributed to different disease etiology and pathological differentiation of the analyzed HCC. However, it could be also owing to molecular heterogeneity of HCC [9].

The major strength of the current study is being among very few Egyptian reports on the performance of apolipoproteins in the diagnosis of HCV-related HCC patients. A limitation of the present study was the relatively small sample.

Conclusion

Apo-A1 and Apo-A4 are novel biomarkers for HCC screening and diagnosis with special importance for Apo-A1, which could be a valuable biomarker for identification of patients with HCC with small tumors as well as those with pylethrombosis.

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Conflicts of interest

There are no conflicts of interest.

References

1 Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, *et al.* Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002; 155:323–331.

2 Schutte K, Bornschein J, Malfertheiner P. Hepatocellular carcinoma – epidemiological trends and risk factors. Dig Dis Sci 2009; 27:80–92.

3 World Health Organization. Geneva, Switzerland: World Health Report; 1996.

4 Atti EA. HCC burden in Egypt. Gastroenterol Hepatol Open Access 2015; 2:00045.

5 Trevisani F, Cantarini MC, Labate AM, De Notariis S, Rapaccini G, Farinati F, *et al.* Italian Liver Cancer (ITALICA) group. Surveillance for hepatocellular carcinoma in elderly Italian patients with cirrhosis: effects on cancer staging and patient survival. Am J Gastroenterol 2004; 99:1470–1476.

6 Yu EW, Chie WC, Chen TH. Does screening or surveillance for primary hepatocellular carcinoma with ultrasonography improve the prognosis of patients? Cancer J 2004; 10:317–325.

7 Wong LL, Limm WM, Severino R, Wong LM. Improved survival with screening for hepatocellular carcinoma. Liver Transpl 2000; 6:320–325.

8 Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology 2016; 150:835–853.

9 Pleguezuelo M, Lopez-Sanchez LM, Rodriguez-Ariza A, Montero JL, Briceno J, Ciria R, *et al.* Proteomic analysis for developing new biomarkers of hepatocellular carcinoma. World J Hepatol 2010; 2:127–135.

10 EASL-EORTC Clinical Practice Guidelines: Management of Hepatocellular Carcinoma. European Association for the study of the liver1; European Organisation for research and treatment of cancer. J Hepatol 2012; 56:908–943.

11 Child CG, Turcotte JG. Surgery and portal hypertension. In: Child CG ed. The liver and portal hypertension. Philadelphia, PA: Saunders 1964 50–64.

12 Chan YH. Biostatistics 102: quantitative data – parametric & non-parametric tests. Singapore Med J 2003; 44:391–396.

13 Chan YH. Biostatistics 103: qualitative data – tests of independence. Singapore Med J 2003; 44:498–503.

14 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65:87–108.

15 Eggert T, McGlynn KA, Duffy A, Manns MP, Greten TF, Altekruse SF. Epidemiology of fibrolamellar hepatocellular carcinoma in the USA, 2000–10. Gut 2013; 62:1667–1668.

16 Nguyen MH, Keeffe EB. Screening for hepatocellular carcinoma. J Clin Gastroenterol 2002; 35(5 Suppl 2):S86–S91.

17 Liu Z, Xiao Y, Tang L, Jiang L, Wang Y, Zhang R, *et al.* Apolipoprotein A1-75 G/A and +83C/T polymorphisms and renal cancer risk. Lipids Health Dis 2015; 14:143.

18 Das M, Wilson CJ, Mei X, Wales T, Engen JR, Gursky O. Structural stability and local dynamics in disease causing mutants of human apolipoprotein a-i: what makes the protein amyloidogenic?. J Mol Biol 2016; 428 (Part B):449–462.

19 Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011; 473:317–325.

20 Degoma EM, Rader DJ. Novel HDL-directed pharmacotherapeutic strategies. Nat Rev Cardiol 2011; 8:266–277.

21 Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol Med 2011; 17:594–603.

22 Chammem H, Hafaid I, Bohli N, Garcia A, Meilhac O, Abdelghani A, Mora L. A disposable electrochemical sensor based on protein G for high-density lipoprotein (HDL) detection. Talanta 2015; 144:466–473.

23 Kasumov T, Li L, Li M, Gulshan K, Kirwan JP, Liu X, *et al.* Ceramide as a mediator of non-alcoholic fatty liver disease and associated atherosclerosis. PLoS One 2015; 10:e0126910.

24 Steel LF, Shumpert D, Trotter M, Seeholzer SH, Evans AA, London WT, *et al.* A strategy for the comparative analysis of serum proteomes for the discovery of biomarkers for hepatocellular carcinoma. Proteomics 2003; 3:601–609.

25 Mustafa MG, Petersen JR, Ju H, Cicalese L, Snyder N, Haidacher SJ, *et al*. Biomarker discovery for early detection of hepatocellular carcinoma in hepatitis C-infected patients. Mol Cell Proteomics 2013; 12:3640–3652.

26 Jiang JT, Wu CP, Xu N, Zhang XG. Mechanisms and significance of lipoprotein (a) in hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2009; 8:25–28.

27 Lewis GF, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. Circ Res 2005; 96:1221–1232.

28 Qian EN, Han SY, Ding SZ, Lv X. Expression and diagnostic value of CCT3 and IQGAP3 in hepatocellular carcinoma. Cancer Cell Int 2016; 16:55.

29 Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. Circ Res 2006; 98:1352–1364.

30 Griffin JH, Kojima K, Banka CL, Curtiss LK, Fernandez JA. Highdensity lipoprotein enhancement of anticoagulant activities of plasma protein S and activated protein C. J Clin Invest 1999; 103:219–227.

31 Xu X, Wei X, Ling Q, Cheng J, Zhou B, Xie H, Zhou L, Zheng S. Identification of two portal vein tumor thrombosis associated proteins in hepatocellular carcinoma: protein disulfide-isomerase A6 and apolipoprotein A-I. J Gastroenterol Hepatol 2011; 26:1787–1794.

32 Eichinger S, Pecheniuk NM, Hron G, Deguchi H, Schemper M, Kyrle PA, Griffin JH. High-density lipoprotein and the risk of recurrent venous thromboembolism. Circulation 2007; 115:1609–1614.