The role of adiponectin, insulin resistance, and vitamin D as predictors of transformation of fatty liver to hepatocellular carcinoma

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Introduction Hepatocellular carcinoma (HCC) is considered the fifth most common cancer in the world and is responsible for 5% of all malignant tumors in humans. Most HCC cases are related to chronic hepatitis C virus (HCV) infection, chronic hepatitis B (HBV) infection, and alcohol abuse. Approximately 15–50% of HCC cases were classified as idiopathic, suggesting that other risk factors are responsible for its rising incidence. Recent studies have suggested that nonalcoholic fatty liver disease (NAFLD) can be associated with these 'idiopathic' cases.

Aim The aim of the study was to evaluate the levels of both serum 25(OH)VD and adiponectin, as well as homeostatic model assessment insulin resistance as predictors of transformation of NAFLD to HCC among patients with NAFLD, HCV, HBV positive patients and HCC patients.

Patients and Methods This is a case–control study which was conducted on 100 patients at the Internal Medicine Department in ElMataryia Teaching Hospital. They were classified into 20 patients with NAFLD, 20 patients with HBV positive, 20 patients with HCV positive, and 20 patients with HCC, in addition to 20 age-matched and sex-matched healthy participants,. Full medical history, clinical examination, and laboratory investigations including complete blood count, alanine transaminase, aspartate transaminase, prothrombin time, international normalized ratio, fasting blood sugar, glycated hemoglobin, homeostatic model assessment insulin resistance, hepatitis B antigen, hepatitis B antibody, serum vitamin D, and serum adiponectin, abdominal ultrasound were done. Statistical analysis using SPSS was done.

Results The level of adiponectin showed a decrease in NAFLD cases (2 μg/ml), but there was an increase in HBV (18 μg/ml), HCV (10.5 μg/ml), and HCC (13 μg/ml) in comparison to the control group (3.75 μg/ml). Further, the cut-off points for adiponectin to detect HCC in NAFLD was found to be greater than 3.2, with a sensitivity of 100% and specificity of 100%.

Conclusion Serum adiponectin is an excellent marker to predict the transformation of NAFLD to HCC.

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Introduction

Fatty liver (steatosis) is the result of an imbalance in the triglyceride metabolism and characterized by accumulation of lipid within the hepatocytes. It affects up to 30% of the population in industrialized countries and is becoming more prevalent in the developing world with the rise in living standards and change in dietary habits. Although most individuals with this condition do not develop any serious liver disease, it is associated with an increased annual incidence of hepatocellular carcinoma (HCC). Several studies have found that patients suffering from cirrhosis preceded by fatty liver are at risk of developing HCC equal to those who develop cirrhosis related to hepatitis C virus (HCV) [1].

The definition of nonalcoholic fatty liver requires evidence of hepatic steatosis by imaging or histology and no causes for secondary hepatic fat accumulation like alcohol [2]. Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of diseases ranging from simple steatosis to nonalcoholic steatohepatitis and cirrhosis [3].

The prevalence of NAFLD has risen rapidly in parallel to the dramatic rise in levels of obesity and diabetes mellitus II. NAFLD is strongly associated with hepatic and adipose tissue insulin resistance (IR) as well as reduced whole-body insulin sensitivity [4].

IR is defined as decreased sensitivity or responsiveness to metabolic actions of insulin such as inhibition of hepatic glucose production. IR is a key factor in the pathogenesis of NAFLD, which is being considered

as the hepatic component of IR or metabolic syndrome [5].

Patients with NAFLD have lower adiponectin levels compared with normal people, which may act as a predictor of transformation to HCC [6].

ADP is an amino acid protein which accounts for 0.01% of total plasma proteins; it is mainly secreted from the adipose tissue, and it modulates a number of metabolic processes, including glucose regulation and fatty acid oxidation. ADP has antiatherogenic, antidiabetic, and anti-inflammatory properties. ADP plays a role in the suppression of the metabolic actions that may result in type II diabetes, obesity, and NAFLD [7].

Vitamin D has a significant immunomodulatory action and is an important mediator of innate and adaptive immune system, The active form of vitamin D1, 25(OH) 2D3, has been shown to exert an antitumor activities, including anti proliferation, anti-inflammation, antiangiogenesis proapoptosis, prodifferentiation, and inhibiting cancer cell invasion. This means that decreased level of vitamin D in NAFLD might have a role in the proliferation of malignant cells and could rise the possibility of developing HCC [8].

HCC is usually asymptomatic in the early stages and tends to be invasive, and most patients are presented with incurable diseases at the time of detection which makes its early diagnosis critical for good prognosis; hence, the aim of the work was to evaluate serum adiponectin, serum vitamin D, and homeostatic model assessment insulin resistance (HOMA-IR) to predict HCC among NAFLD.

Patients and methods

Patients

This study was conducted on 100 participants classified into five groups: group 1 included 20 patients with NAFLD; group 2 included 20 patients with positive hepatitis B virus (HBV); group 3 included 20 patients with positive HCV; group 4 included 20 patients with HCC; group 5 included 20 healthy normal participants (age and sex matched).

All participants were selected from the outpatient clinic of Internal Medicine and Inpatient Medicine Departments of Elmatariya Teaching Hospital; all are Egyptians, their age ranged from 30 to 55 years. The study was conducted in the period from June 2016 to June 2017.

All participants enrolled in the study were subjected to full history taking, full clinical examination including BMI and laboratory tests including (complete blood count, liver function tests: serum glutamate pyruvate transaminase and serum glutamic oxaloacetic transaminase, serum albumin, prothrombin time, and international normalized ratio). viral screening for hepatitis B antigen and hepatitis C antibody, fasting blood sugar, and glycated hemoglobin, serum adiponectin, serum vitamin D, IR (HOMA-IR) and abdominal ultrasound.

An informed consent was taken from the patients and controls which are approved by the ethics committee of Al Azhar University.

Methods

Specimen collection: 7 ml of venous blood was obtained from each patient and from controls with a sterile syringe drawn without anticoagulant, allowed to stand for 2 h at room temperature, and then centrifuged at 3000 rpm for 10 min. The sera samples were collected and each was divided into two aliquots, one for routine laboratory investigations and the other aliquots were stored at −20°C till the time of use, avoiding repeated freezing/thawing.

Serum vitamin D was measured using the enzyme-linked immunosorbent assay technique; serum adiponectin was measured using the enzyme-linked immunosorbent assay technique, fasting serum insulin, and HOMA-index for the calculation of IR, through the following Matthews formula: HOMA-IR=[FPG (mg/dl)×fasting insulin (μIU/l)]/405.

Statistical analysis

The statistical presentation and analysis of the results of our study was conducted using SPSS (SPSS Inc., Chicago, Illinios, USA); χ^2-test was used to compare qualitative variables between groups. Unpaired *t*-test was used to compare quantitative variables in parametric data (SD<50% mean). Mann–Whitney test was used instead of unpaired *t*-test in nonparametric data (SD>50%mean). One-way analysis of variance test was used to compare more than two groups as regards quantitative variables (least significant difference). Spearman's correlation coefficient test was used to rank variables versus each other positively or inversely. Power of significance was evaluated as follows: A *P* value of greater than or equal to 0.05 was considered insignificant. A *P* value of less than 0.05 was considered significant and a *P* value less than 0.01was considered highly significant.

Results

All demographic and anthropometric measures are tabulated, as shown in Table 1; the detailed laboratory data are shown in Table 2.

The study showed that there was a significant decrease in adiponectin level in NAFLD with median and interquartile (IQ) range (2 μg/ml) compared with the control (3.75 μg/ml). The level of adiponectin was increased in HBV (18 μg/ml), HCV (10.5 μg/ml), and HCC (13 μg/ml) compared with the control group.

The study showed a significant decrease in vitamin D level in patients with NAFLD (median IQ range: 10 ng/ml), HBV (8.75 ng/ml), HCV (10.5 ng/ml) compared with the control group. There was a more significant decrease in the level of vitamin D in HCC (5 ng/ml) compared with controls.

We have found that there was a statistically significant difference between all patients groups and the control group regarding HOMA-IR ($P<0.00$). Also there was a statistically significant difference between NAFLD and HCV ($P<0.02$), but no statistically significant difference between NAFLD and HBV ($P>0.76$), and NAFLD and HCC ($P>0.06$) regarding HOMA-IR (Table 3).

Receiver operating characteristic curve between the HCC group and the NAFLD group was done to detect the best marker that predicts HCC among NAFLD; adiponectin was the excellent predictor for HCC among NAFLD with a sensitivity of 100%, specificity of 100% at the best cut-off point (>3.2 mg/ml), followed by vitamin D sensitivity of 85%, specificity of 95% at the best cut-off point (≤6 ng/ml), and lastly HOMA-IR with a sensitivity of 95% and specificity of 35% (Fig. 1, Table 4).

Table 1 shows a statistically significant difference between the control group and patients group regarding weight and BMI, whereas no statistically significant difference was found between the two studied groups regarding sex and height.

Table 2 shows a statistically significant difference between the control group and patients group regarding all laboratory data.

There was a statically significant difference between all patient subgroups and the control group regarding IR (HOMA-IR), adiponectin, and vitamin D levels.

Discussion

NAFLD is rapidly becoming the most common liver condition which implicated in rising HCC incidence, independently or in synergy with cirrhosis. There are multiple postulated mechanisms for the development of HCC in NAFLD.

(1) Hepatic steatosis mainly causes 1-oxidative (via increased reactive oxygen species and carcinogenic metabolites of lipid peroxidation such as trans-4-hydroxy-nonenal).
(2) inflammatory (upregulation of tumor necrosis factor-α, interleukin-6).
(3) Apoptotic and hormonal changes. Also there is increase in hepatic inflammation and apoptosis through downstream modulation of pathways as mitogen-active protein kinase and phosphatidyl-inositol-3 kinase.
(4) Also one of important mechanisms implicated in HCC from NAFLD is decrease in adiponectin levels with subsequent loss of its antiangiogenic and anti-inflammatory effects [9].

Table 1 Comparison between control group and patients group regarding demographic and anthropometric measures

	Control group (*N*=20)	Patient group (*N*=80)	Test values	*P* value	Significance
Sex [*n* (%)]					
Female	11 (55.0)	34 (42.5)	1.010*	0.315	NS
Male	9 (45.0)	46 (57.5)			
Weight (kg)					
Mean±SD	77.35±7.21	82.98±11.90	–2.019•	0.046	S
Range	65–90	65–115			
Height (cm)					
Mean±SD	161.50±5.27	158.70±6.74	1.728•	0.087	NS
Range	152–170	150–175			
BMI (kg/m^2)					
Mean±SD	29.60±2.89	33.26±5.79	–2.731•	0.007	HS
Range	24–37	23–52			

HS, highly significant; S, significant. *Significant. •Non significant.

Table 2 Comparison between control group and patients group regarding laboratory data

	Control group (*N*=20)	Patient group (*N*=80)	Test value	*P* value	Significance
Hb (g/dl)					
Mean±SD	12.41±1.17	10.41±2.29	3.757	0.000	HS
Range	11–14.5	3.8–16			
WBCs ($\times10^3\mu$l)					
Mean±SD	9.09±1.61	6.38±3.38	3.474	0.001	HS
Range	6.3–11.5	2–19.9			
Platelets ($\times10^3\mu$l)					
Mean±SD	212.15±33.34	159.19±103.72	2.247	0.027	S
Range	175–285	45–603			
ALT (mg/dl)					
Median (IQR)	17 (15.5–19.5)	30 (19.5–55)	–4.716	0.000	HS
Range	12–22	14–260			
AST (mg/dl)					
Median (IQR)	20.5 (18–22)	34.5 (20.5–62)	–3.926	0.000	HS
Range	12–25	12–234			
Bilirubin (mg/dl)					
Mean±SD	0.60±0.21	1.44±0.65	–5.718	0.000	HS
Range	0.3–0.9	0.4–3.8			
Albumin (g/dl)					
Mean±SD	4.41±0.44	3.23±0.77	6.513	0.000	HS
Range	3.8–5	1.3–5			
Alpha fetoprotein (ng/ml)					
Median (IQR)	2.85 (2.15–3.4)	11.6 (4.3–17.2)	–5.124	0.000	HS
Range	1.5–5.3	1.7–300			
Fasting sugar (mg/dl)					
Mean±SD	94.25±13.50	108.01±20.94	–2.792	0.006	HS
Range	65–110	70–200			
HA1c					
Mean±SD	4.98±0.35	5.10±0.62	–0.810	0.420	NS
Range	4.1–5.6	3.5–6.5			
PT (s)					
Mean±SD	11.60±0.75	13.61±3.29	–2.699	0.008	HS
Range	11–13	11–33			
INR					
Mean±SD	1.06±0.07	1.32±0.43	–2.700	0.008	HS
Range	1–1.2	1–3.5			
Serum insulin (μIU/ml)					
Median (IQR)	10 (8.9–11.4)	15 (9.85–25)	–3.134	0.002	HS
Range	6.5–15	0.3–80			

ALT, alanine transaminase; AST, aspartate transaminase; HA1c, glycated hemoglobin; Hb, hemoglobin; HS, highly significant; INR, international normalized ratio; IQR, interquartile range; PT, prothrombin time; S, significant; WBC, white blood cell.

Table 3 Comparison between patients subgroup regarding insulin (homeostatic model assessment insulin resistance), adiponectin, vitamin D

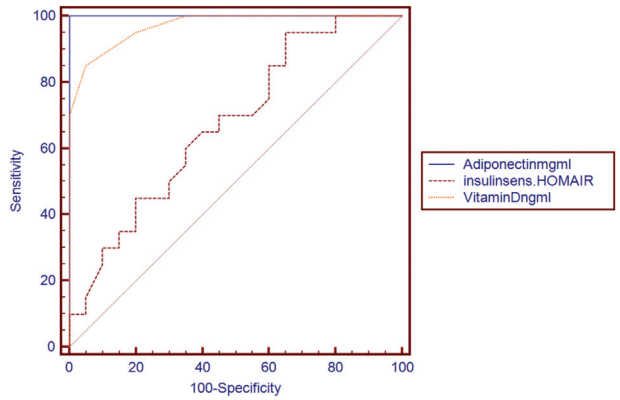
	Group NAFLD (*N*=20)	Group HBV (*N*=20)	Group HCV (*N*=20)	Group HCC (*N*=20)	Test value?	*P* value
Insulin sens (HOMA-IR)						
Median (IQR)	3.25 (1.45–5.5)	3.2 (2.6–4.95)	6.1 (3.1–8.6)	4.85 (2.95–7.4)	8.456	0.037
Range	0.5–9.9	0.06–7.4	1.2–16.5	1.3–14.8		
Adiponectin (mg/ml)						
Median (IQR)	2 (1.95–2.75)	18 (11–22.5)	10.5 (8.5–15.5)	13 (7.75–18)	46.086	0.000
Range	1.5–3.2	3–40	3–25	5.5–25		
Vitamin D (ng/ml)						
Median (IQR)	10 (8–10.5)	8.75 (7–11.5)	10.5 (7.25–12)	5 (4–6)	34.116	0.000
Range	6–14	3–14	5–14	3–8		

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, homeostatic model assessment insulin resistance; IQR, interquartile range; NAFLD, nonalcoholic fatty liver disease.

NAFLD is extremely frequent among diabetic and obese patients. The risk of HCC was found to be increased among diabetic and obese patients even without HBV or HCV infection. Both obesity and diabetes were associated with IR and elevated insulin-like growth factor, which stimulate cell growth and may promote cancer cells proliferation and apoptosis which increase the risk of HCC by threefold [9].

The present study showed that there was a significant decrease in adiponectin level in patients with NAFLD but on the other hand the level increased in HBV positive patients (18 μg/ml), HCV positive patients (10.5 μg/ml), and HCC patients (13 μg/ml) compared to control group. This result showed an agreement with Mohamed *et al.* [10], their study was carried out to evaluate the role of adiponectin, in NAFLD Egyptian patients, they found that plasma adiponectin were significally lower in NAFLD patients than control (3.05±2.65 vs. 10.52±3.35 μg/ml), this mean that high level of adiponectin is a protective against fatty liver and the lower adiponectin level related to higher grade of inflammation suggesting that adiponectin deficiency is an important risk factor for development of liver injury and could accelerate tumor formation in NAFLD. Also our result is in agreement with that reported by Adolph *et al.* [11] who suggested that advanced liver diseases are associated with increased serum adiponectin levels, they found that cirrhotic and noncirrhotic HCC patients demonstrated more increase in serum levels of adiponectin, and high adiponectin serum levels might predict the consecutive development of HCC and higher plasma levels of adiponectin could predict poor HCC survival among patients without liver transplantation, and they explained this by the ability of adiponectin to increase apoptosis of HCC cells through activation of caspase-3, and increased phosphorylation of c-Jun *N*-terminal kinase. Inhibition of c-Jun *N*-terminal kinase-phosphorylation prevented this apoptotic effect of adiponectin.

Figure 1



Receiver operating characteristic curve for hepatocellular carcinoma group vs. nonalcoholic fatty liver disease group.

The present study showed that there was a significant decrease in vitamin D level in patients with NAFLD (median IQ range: 10 ng/ml), HBV (median IQ range: 8.75 ng/ml), HCV (median IQ range:10.5 ng/ml) patients compared to control and there was more significant decrease in the level of vitamin D in HCC patients (median IQ range: 5 ng/ml) compared to control. This result showed an agreements with Cordeiro *et al.* [12]. The results of this study showed that the lowest serum vitamin D concentrations were found in steatohepatitis diagnosed by liver biopsy, and there was more decreases in more histologically severe stages of the disease, suggesting that vitamin D may play a role in the development and progression of liver disease, partly via inhibition of vitamin D anti-inflammatory and immunomodulatory properties.Also we have found that there is no statistically significant difference in IR (HOMA-IR) level in NAFLD patients compared to the control group (P=0.110), and this showed an agreement with results recorded by Signh *et al.* [13] who had reported that IR could not be detected in 45.53% of the 336 NAFLD patients they had studied. Although age, sex, BMI, and transaminase levels were comparable, significantly higher proportion of patients in non-IR group were nonobese, which mean that NAFLD is probably a heterogeneous disease and IR is not the sole factor responsible for NAFLD, and NAFLD may not be associated with IR especially in nonobese lacking criteria of metabolic syndrome.

In view of the expected possible changes in the pattern of chronic hepatic diseases in Egypt after the 'National Plan for HCV Prevention and Eradication', NAFLD with its serious complication HCC, might dominate

Table 4 Receiver operating characteristic curve between hepatocellular carcinoma group and nonalcoholic fatty liver disease group

	Cut-off point	AUC	Sensitivity	Specificity	Positive predictive valve	Negative predictive valve
Adiponectin (mg/ml)	>3.2	1.000	100.00	100.00	100.0	100.0
Insulin sensitivity (HOMA-IR)	>1.7	0.674	95.00	35.00	59.4	87.5
Vitamin D (ng/ml)	≤6	0.970	85.00	95.00	94.4	86.4

AUC, area under the curve; HOMA-IR, homeostatic model assessment insulin resistance.

the picture in the future, especially with the obvious increasing rise of the incidence and prevalence of both obesity and type 2 diabetes among the Egyptians as a result of changing life style and eating habits.

The results in our study might add to the protocol of both prediction and early detection of this serious condition.

Conclusion

We recommend to take NAFLD seriously, as it is emerging as the most common liver disorder in the industrialized countries leading to HCC. Finding a simple noninvasive measures for follow-up patients with NAFLD, using the previous three markers [adiponectin, serum vitamin D, and IR (HOMA-IR)] might give a hope to early detection of HCC in NAFLD patients. Adiponectin level shows an excellent sensitivity and specificity followed by vitamin D then IR (HOMA-IR). Our study adds to the increasing evidence that HCC can occur in patients even with noncirrhotic NAFLD and those who are nonobese.

The main value of our study is that it might add to the protocol of both prediction and early detection HCC in patients with NAFLD, the emerging common chronic liver disease after eradication of HCV in Egypt.

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Conflicts of interest

There are no conflicts of interest.

References

1 Arase Y, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, Akuta N, *et al.* Difference in malignancies of chronic liver disease due to non-alcoholic fatty liver disease or hepatitis C in Japanese elderly patients. *Hepatol Res* 2013; **42**:264–272.

2 Vernon G, Baranova A, Younossi ZM. The epidemiology and natural history or NAFLD. *Aliment Pharmacol Ther* 2012 **34**:274–285.

3 Ounossi ZM, Stepanova M, Afendy M, Fang Y, Younossi Y, Mir H, *et al.* Change in prevalence of the most common causes of chronic liver diseases in the united states from1988 to 2008. *Clin Gastroentrol Hepatol* **2013**; 9:524–530.

4 Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, *et al.* The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**:2005–2023.

5 Tsochatzis FA, Manolakopoulos S, Papatheodoridis GV. Insulin resistance and metabolic syndrome in chronic liver diseases: old entities with new implication. *Scand J Gastroenterol* 2013; **44**:6–14.

6 Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, *et al.* Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2013; **17**:4–12.

7 Fernández-Real JM, López-Bermejo A, Casamitjana R, Ricart A. Novel interactions of adiponectin with the endocrine system and inflammatory parameters. *J Clin Endocrinol Metab* 2013; **88**:2714–2718.

8 Schuster I. Cytochromes P450 are essential players in thevitamin D signaling system. *Biochim Biophys Acta* 2014; **1814**:186–199.

9 Leung C, Yeoh SW, Patrick D, Ket S, Marion K, Gow P, Angus PW. Characteristics of hepatocellular carcinoma in cirrhotic and non-cirrhotic non-alcoholic fatty liver disease. *World J Gastroenterol* 2015; **21**: 1189–1196.

10 Mohamed AA, Shousha W, Shaker O, *et al.* Role of serum adiponectin, *IL-6, and hs-CRP in non-alcoholic fatty liver Egyptian patients. Int J Bioche Res Rev* 2014; **4**: 493–504.

11 Adolph TE, Grander C, Grabherr F, *et al.* Adipokines and non-alcoholic fatty liver disease: multiple interactions. *Int J Mol Sci* 2017; **18**: 1649.

12 Cordeiro A, Pereira S, Saboya CJ, *et al.* Relationship between nonalcoholic fatty liver disease and vitamin D nutritional status in extreme obesity. *Can J Gastroenterol Hepatol* 2017; **2017**: 9456897.

13 Singh SP, Misra B, Kar SK, Panigrahi MK, Misra D, Bhuyan P, *et al.* Nonalcoholic fatty liver disease (NAFLD) without insulin resistance: is it different? *Clin Res Hepatol Gastroenterol.* 2015; **39**:482–488.