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ORIGINAL ARTICLE

Evaluation of nitric oxide as a novel diagnostic marker for hepatocellular carcinoma

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KEYWORDS

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Abstract *Introduction:* Liver cancer is the sixth most common cancer worldwide. HCC is the most common primary tumor of the liver. The National Comprehensive Cancer Network (NCCN) clinical practice guidelines for treatment of hepatobiliary cancers propose surveillance for the early detection of HCC by liver ultrasonography every 3–6 months and evaluation of AFP. AFP >200 ng/ml is considered diagnostic for HCC, although fewer than half of patients of HCC may generate levels that are high, so that the specificity of AFP is close to 100% but the sensitivity is 45%. Nitrite/Nitrate is a stable end product of nitric oxide increase in patients with HCC.
Aim: It was to evaluate nitric oxide as a novel diagnostic marker for hepatocellular carcinoma.
Methods: Eighty patients and 15 normal individuals enrolled in the study: Group (1) 15 normal individuals. Group (2) 30 patients with chronic liver disease without HCC. Group (3) 50 patients with HCC. History taking, clinical examination, (detection of liver masses, ascites, spleen size, and grade of encepathalopathy), and Child-pugh scoring. Laboratory investigation: (AlT, AST, bil-

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irubin, albumin, prothrombin, GGT, platelet count, AFP, nitric oxide, HBs-Ag, and HCV-Ab). Abdominal ultrasonography and spiral CT.

Results: The median level of nitric oxide was significantly higher in Group (3) (170 μmol/l) than in Group (2) (56 μmol/l) than in Group (1) (22 μmol/l), with a sensitivity of (68%) and specificity of (90%) at a cutoff level of 110 μmol/l and area under the curve of (0.810).While AFP, at a cutoff level of 200 ng/ml had a sensitivity of (52%), specificity of (100%) and area under the curve (0.855). Indeed nitric oxide was high in 42% of AFP-negative HCC patients.

Conclusion: Nitric oxide is a novel diagnostic marker for hepatocellular carcinoma, the simultaneous determination of serum nitric oxide and AFP gave significant improvement in detection of HCC patients compared to that of AFP alone.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancer with an incidence of 4–5/100,000 in Western countries compared with 120/100,000 in Asia and Africa. HCC is one of the leading causes world wide of cancer mortality due to late diagnosis.[1] Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) has been involved in about 80% of cases world wide of HCC.[2] Alfa-feto protein (AFP) is a glycoprotein formed initially within the yolk sac and later in the liver and gastrointestinal tract of the fetus. Serum values of about 70,000 μg/l are found in neonates, decreasing to the normal value of less than 10 μg/l within 9–12 months. AFP had a limited value for the early detection of HCC because about one-third of HCC patients are presented with normal levels.[2] Higher values can be detected in liver cell regeneration, thus a continuos increase in AFP values arouses suspicion: a value of more than 100 μg/l is highly suspicious for HCC. There is only a moderate correlation between AFP and respective tumor size and doubling time. Serum AFP marker has low sensitivity of about 39–64% and high specificity is between 76% and 91%. AFP values in the normal range exclude HCC in 90–95%.[3–6]

The two major features of the natural history of HCV infection are viral persistence and hepatic damage. Nitric oxide (NO) is one of the most versatile mediators in control of viral infections, being the earliest host antiviral response.[7] NO acts as a pro-apoptotic inducer in some cell types or as antiapoptotic modulator in other cell types including hepatocytes.[8] Also, it was found that in HCV infected patients there is an enhanced inducible nitric oxide synthetase (iNOS) expression, implying excessive NO formation that positively correlates with viral load and hepatic inflammation.[9–11] The most striking feature of hepatitis C is its marked tendency toward chronicity. NO may impair antiviral response by suppressing type 1 helper T-cell response.[12] Also, NO enhances viral escape mutations thus allowing viral persistence.[12]

NO contributes to viral persistence by means of its antiapoptotic effect in hepatocytes[8] and HCV increases liver cell survival by preventing apoptosis through activation of NF-κB signaling pathway.[13] The upregulated INOS gene in chronic HCV infection leads to oxidative stress and reactive NO species (RNOS) such as peroxynitrite and nitrogen oxide leads to cytotoxicity and DNA damage. There is preliminary data that the non structural HCV protein NS5A and the core protein are able to induce INOS gene expression and that HCV and NO interact in a synergistic manner to deliver a potent oncogenic signal to infected hepatocytes.[14]

2. Methods

The study included 80 patients and 15 normal subjects. They were grouped as follows: Group (1) 50 patients with hepatocellular carcinoma. Group (2) 30 patients with chronic hepatitis C. Group (3) 15 normal subjects. Complete history taking, clinical examination stressing on (liver and spleen size, ascites, jaundice, Encepathalopathy, and liver masses). Laboratory testing after overnight fasting (CBP,[15] ALT, AST,[16,17] BIL, Albumin, HBS Ag,[18] HCV AB,[19] and alpha-fetoprotein[20]) and nitric oxide.[21] Child pugh score.[22] Abdominal ultrasound for detecting for hepatic lesions.[23] Triphasic CT for diagnosis of focal hepatic lesions as hepatocellular carcinoma with the characteristic pattern.[24] Statistical analyses were performed using The SPSS soft ware.[25]

The qualitative variables are presented as number and percentages. The quantitative variables are presented as mean ± standard deviation and median (interquartile range)

Table 1 Clinical and radiologic characteristics of Group (1) HCC patients.

Characters	*N*	%
Child class grade		
A	16	32.0
B	25	50.0
C	9	18.0
Vascular invasion		
Yes	16	32.0
No	34	68.0
Spleen enlargement		
Yes	26	52.0
No	24	48.0
Ascites		
Tense	2	4.0
Moderate	4	8.0
Mild	10	20.0
No	34	68.0
edema		
Yes	14	28.0
No	36	72.0
Jaundice		
Yes	14	28.0
No	36	72.0
CT classic features		
Yes	49	98.0
No	1	2.0
Total	50	100.0

Table 2 Laboratory data of different studied groups.

	Group (1) HCC cases	Group (2) chronic liver disease	Group (3) control	Kruskal Wallis test	
				Value	*p*
NO (μmol/L)					
Mean ± SD	495.66 ± 625.72	66.90 ± 43.75	22.2 ± 2.04		
Median (IQR)	170 (759.25)	56 (23.25)	22 (5)	50.905*	< 0.001
AFP (ng/L)				Z#	*p*
Mean ± SD	5339,65 ± 25646.35	5.95 ± 4.36			
Median (IQR)	220.50 (986.25)	4.80 (3.63)		5.289*	< 0.001
AST (U/L)					
Mean ± SD	73.59 ± 44.94	48.10 ± 28.49	19.40 ± 6.43		
Median (IQR)	64 (71)	47 (33.50)	20 (12)	28.812	< 0.001
ALT (U/L)					
Mean ± SD	41.44 ± 33.12	40.23 ± 21.74	17.47 ± 10.10		
Median (IQR)	29.50 (37)	39.50 (27.50)	14 (8)	15.102*	0.001
GGT (U/L)					
Mean ± SD	115.82 ± 120.55	39.07 ± 11.31	23.33 ± 8.04		
Median (IQR)	75.50 (111.50)	38.50 (13.75)	28 (16)	36.457*	< 0.001
Albumin (gm/dl)					
Mean ± SD	2.93 ± 0.59	3.81 ± 0.55	3.99 ± 0.29		
Median (IQR)	2.84 (0.75)	3.85 (0.83)	3.90 (0.20)	43.882*	< 0.001
Total bilirubin (mg/dl)					
Mean ± SD	2.16 ± 2.11	1.16 ± 0.47	0.54 ± 0.17		
Median (IQR)	1.50 (2.12)	1 (0.75)	0.50 (0.20)	31.443*	< 0.001
Platelets/cmm					
Mean ± SD	192,140 ± 104 037	270,500 ± 819 62.46	290,666 ± 382 59.76		
Median (IQR)	204,500 (189 250)	255,000 (142,500)	300,000 (700,00)	15.523*	< 0.001

Z#, Mann–Whitney test.

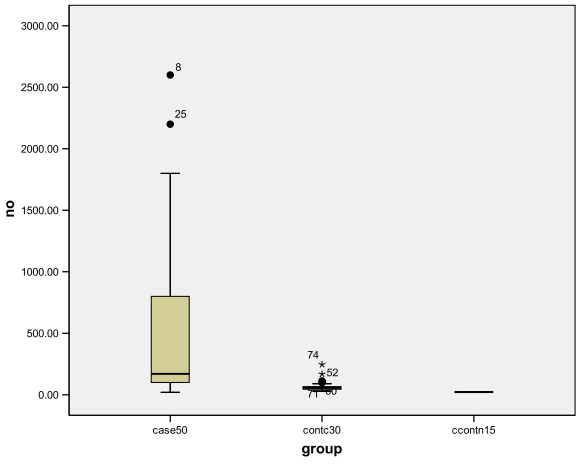
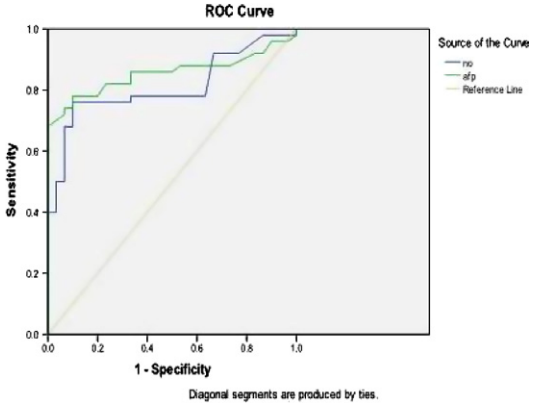


Figure 1 Box plot presentation of nitric oxide (NO) among studied groups.



Tests	Area under the curve	P
NO(μmol/L)	0.81	<0.001
AFP(ng/ml)	0.855	<0.001

Figure 2 ROC curve for the relation between NO and AFP among patients.

as the distribution is abnormal, and presented graphically as box plot. Non parametric testes are used namely the Kruskal Wallis and Mann Whitney tests. Accuracy of the tests (NO and AFP) is determined by plotting the ROC curve for the quantitative values and calculation of sensitivities, specificities, and predictive values for its categories. The 5% level of significance is chosen.

3. Results

Results, Table 1 shows the clinical and radiographic characteristics in Group (1) HCC patients. 50% of cases were Child B, 32% were Child A and 18% were Child C. The classic wash in/

Table 3 Sensitivity, specificity, positive and negative predicitive value for AFP among patients in the two groups.

Sensitivity = 52%	Total	Group (2) Chronic liver disease	Group (1) HCC pateints	AFP (ng/ml)
Specificity = 100%	26	0	26	> 200
+ ve predicitive value (100%)	54	30	24	< 200

Table 4 Sensitivity, specificity, positive and negative predictive value of nitric oxide (NO) in studied patients groups.

Sensitivity = 68%	Total	Group (2) chronic liver disease	Group (1) HCC patients	Nitric oxide (NO) (mol/L)
Specificity = 90%	37	3	34	> 110.65
Positive predictive value = 91.9%	43	27	16	< 110.65
Negative predictive value = 62.8%	80	30	50	Total

washout pattern of HCC was present in 98% of cases. Major vascular invasion was present in 32% of cases. (Table 2) The median of (NO) was statistically significantly higher in Group (1) HCC (170 μmol/l) than in Group (2) (56 μmol/l) than in Group (3) (22 μmol/l), where $p < 0.001$ (Table 2). The median of AFP was significantly higher in Group (1) HCC patients (220.5 ng/ml) than in Group (2) patients (4.8 ng/ml) and $p < 0.001$. The median level of AST was significantly higher in Group (1) (64 U/l) than in Group (2) (47 U/l) than in Group (3) (20 U/l). The median of ALT was significantly higher in Groups (1) and (2) (29.5 and 39.5 U/l) than in Group (3) (14 U/l). The median level of (GGT) was significantly higher in Group (1) (75.5 U/l) than in Group (2) (38.5 U/l) than in Group (3) (28 U/l). The median level of serum albumin was significantly lower in Group (1) (2.84 gm/dl) than in Group (2) (3.85 gm/dl) than in Group (3) (3.90 gm/dl). The median level of serum bilirubin was significantly higher in Group (1) (1.5 mg/dl) than in Group (2) (1 mg/dl) than in Group (3) (0.5 mg/dl) (Fig. 1). The median level of platelet count was significantly lower in Group (1) (204,500/cmm) than in Group (2) (255,000/cmm) than in Group (3) (300,000/cmm). (Fig. 2): The ROC for simultaneous determination of (AFP) and (NO) showed an area under ROC curve of (0.855) and (0.81), respectively. Table 3: serum AFP at a cut off of 200 ng/ml, had a sensitivity of (52%), specificity of (100%), positive predictive value of (100%) and a negative predictive value of (55.6%). Table 4: serum (NO) at a cut off of (110.65 μmol/l) had a sensitivity of (68%), specificity of (90%), positive predictive value of (91.9%) and negative predictive value of (62.8%). Box plot presentation of nitric oxide among studied groups showed that the median value of NO in HCC was significantly higher compared to chronic liver disease and control group by the Kruskal–Wallis test ($p < 0.001$).

4. Disscussion

Identification of early HCC which is potentially amenable to aggressive intervention and improved survival is the rationale behind screening for HCC. An effective screening program, however, requires certain criteria to be successful, including the following: a common disease with substantial mortality, an identifiable target group, acceptable tests with high sensitivity and specificity, and available treatment.[26] Bolondi et al.[27] demonstrated a median survival of 30 months in patients whose HCC was detected by surveillance versus 15 months in those discovered by chance. Other studies have been less convincing.[28] Regardless, it has become common practice among hepatologists to apply one of several surveillance methods to their high-risk patients.[29] Surveillance intervals for HCC are based on a balance between the tumor doubling time and the cost of the screening tests. Doubling time of HCC ranges from 1 to 19 months with a median of 4–6 months.[30] Most study protocols conduct screening every 6 months. Diagnostic tools commonly used include the serum tumor marker alfa-fetoprotein (AFP), radiographic imaging, and liver biopsy. Ultrasound (US) imaging is commonly applied in addition to, or in place of, AFP to help detect small hepatic tumors < 3 cm. Its widespread use as a surveillance tool relates to its noninvasive nature, high availability, and low cost. In combination with AFP the PPV can be as high as 94%.[31]

This study showed that AFP had a sensitivity of 52%, specificity of 100%, positive predictive value of 100% and a negative predictive value of 55%. Other studies have shown similar results with a specificity of AFP close to 100% but at a cost to the sensitivity which falls below 45%.[32] Another study showed that using 20 ng/ml as the cut-off point, the sensitivity rose to 78.9% and a specificity declined to 78.1%.[33] For the difference in results we used the new cut-off diagnostic level of HCC, which is 200 ng/ml. The positive predictive value (PPV) of AFP is low, ranging from 9% to 32%.[34] The median value of Nitric oxide was significantly higher in HCC Group (1) patients (170 μmol/l) than in chronic liver disease Group (2) patients (56 μmol/l) than in normal control Group (3) (22 μmol/l). The diagnostic performance of nitric oxide in HCC patients at a cut-off of 110 μmol/l showed that the sensitivity, specificity, positive predictive value, and negative predictive value were (68%, 90%, 91.9%, and 62.8%, respectively) and the combined determination of AFP and nitric oxide had a sensitivity of 96.2% in determination of HCC patients. Another study showed similar results where the sensitivity and the specificity of nitric oxide were (79.5% and 72%) in HCC patients and that nitrite/nitrate was positive in 70% of AFP-negative HCC patients and that the simultaneous determinations of serum AFP and plasma nitrite/nitrate concentrations gave significant improvement in HCC detection compared with AFP alone.[35]

5. Conclusion

Nitric oxide is a novel diagnostic marker for hepatocellular carcinoma and the combined estimation of nitric oxide and AFP increases the sensitivity of detection and diagnosis of HCC to 96%.

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