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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