British Journal of Medicine & Medical Research
9(6): 1-6, 2015, Article no.BJMMR.18302
ISSN: 2231-0614

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Can We Use Osteopontin as a Reliable Marker for Diagnosis of Early HCC in Chronic HBV Monoinfected Saudi Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEA made substantial contributions to study conception, design, protocol writing and interpretation of data and drafted the article. Author LD performed the statistical analysis and was involved in study conception, design, interpretation of data, protocol writing and drafting the article. Authors BE and TA participated in the study conception and design and was involved in the acquisition of data, design and conduct of the protocol and interpretation of data. All authors approved the final version of the manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/18302
Editor(s):
(1) Shashank Kumar, Department of Biochemistry, University of Allahabad, Allahabad, India.
Reviewers:
(1) Anonymous, Indian Institute of Technology, Bombay, India.
(2) Ramesh gurunathan, Department of Surgery, Sunway medical center, Malaysia.
(3) Anonymous, Ain Shams University, Egypt.
(4) Lau Wan Yee, Joseph, The Chinese University of Hong Kong, Hong Kong.
(5) Anonymous, Alexandria University, Egypt.
(6) Shamim Ahmad, Cancer Research Center, Georgia Regents University Augusta, USA.
Complete Peer review History: http://sciencedomain.org/review-history/10048

Original Research Article

Received 14th April 2015
Accepted 20th June 2015
Published 6th July 2015

ABSTRACT

Background: Chronic hepatitis is a worldwide disease with a catastrophing end as hepatocellular carcinoma (HCC). The objective of the current study was to assess performance of biomarkers for early detection of HCC among Chronic Monoinfected HBV Saudi patients. We selected alpha feto protein (AFP) and serum Osteopontin (OPN) as biomarkers to investigate their performance as

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early diagnostic biomarkers. Subjects and methods: 250 Saudi subjects were included in this study and classified into three groups. First group included 50 healthy subjects serving as control group. Second group consisted of 100 patients suffering from liver cirrhosis. The third group included 100 patients suffering from Early HCC. The condition of liver pathology (in early HCC patients) was confirmed by liver biopsy or spiral CT scan. Demographic and biochemical investigations were recorded and both alpha-feto protein (AFP) and osteopontin (OPN) were measured. Results: Performance of markers included in diagnosis of early HCC AUC was 0.78 for AFP and 0.87 for osteopontin; the best cutoff value for AFP was ≥25 ng/ml; while for OPN it was ≥135 ng/m. At the best cutoff value OPN had sensitivity of 100%, specificity 65%, PPV 66.7% , NPV 84.6% and overall accuracy 93% (CI: 0.621-0.931 and P value: 0.003), AFP showed 90% sensitivity, 55% specificity, PPV 74%, NPV 89% and overall accuracy 80% (CI: 0.765-0.980 and P value: 0.001). **Conclusion:** OPN showed better sensitivity in diagnosis of early HCC than AFP and plasma OPN estimation can be considered as a reliable marker for diagnosis of early HCC.

Keywords: Osteopontin; alpha-feto protein; hepatocellular carcinoma; Saudi.

1. INTRODUCTION

Viral hepatitis B was expected to affect about 400 million subjects worldwide with significant prevalence in the middle east region [1]. In the year 2007, the Saudi Ministry of Health (MOH) classified viral hepatitis as the second most common viral disease after chickenpox, and about 9000 new cases diagnosed in that year. Viral B hepatitis was recognized as the most prevalent type (52% HBV, 32% HCV, and 16% HAV) [2].

Early diagnosis of HCC is the cornerstone for improving the outcome of this type of cancer [3]. Many reports documented that 80% of HCC cases can be due to chronic hepatitis B virus (HBV) infection [4]. The current biomarkers for early diagnosis of HCC are not sufficient as many cases of HCC are diagnosed at late stages wich gives poor prognosis and survival rate for these cases.

α-fetoprotein (AFP), the routinely used biomarker for screening of early HCC remains unsatisfactory as the sensitivity and specificity of serum AFP levels for HCC have been reported to range from 39–64% and 76–91%, respectively, which means that elevated serum AFP levels are not a sufficient marker of HCC [5,6].

The American Associations for the Study of Liver Diseases (ASLD) guidelines have identified AFP as a poor marker for HCC screening and excluded it from the screening recommendations [7]. Hence, the need for a reliable new biomarker for diagnosis of early HCC remains a major demand.

OPN is acidic glycophosphoprotein that has been reported to play an important role in oncogenesis and can be considered as a potential biomarker in a variety of human cancers [8,9,10,11].

The validity of OPN as a diagnostic biomarker for early HCC remains questionable and needs more investigations [12].

The performance of OPN as a biomarker for diagnosis of early HCC in chronic HBV Saudi patients was analyzed in the present study.

2. PATIENTS AND METHODS

The present study included 250 Saudi subjects classified into 3 groups: group I or control group which included 50 healthy individuals, group II comprised of 100 chronic HBV patients complicated with cirrhosis and group III comprised of 100 patients complicated with cirrhosis and early HCC. All the patients were naive patients with average general condition (Child A score) and not previously treated. The patients were collected during the period from Decemer 2013 to December 2014 from hospitals of Taif governorate, KSA.

Before participating in this study, an informed consent was obtained from each patient according to the ethical standards given in the declaration of Helsinki of 1975. Each subject was submitted to full history taking, complete general and abdominal examination, imaging investigation (abdominal ultrasonography, chest x-ray). Healthy control was evaluated by clinical examination, liver biochemistry and abdominal Ultrasound to exclude any hepatic lesion. The condition of liver disease in groups II and III was screened by ultrasonography and the diagnosis

of early HCC was confirmed by ultrasound guided liver biopsy to confirm the diagnosis, the size of liver tumour in group III was assessed by spiral CT scan (only early HCC patients were selected with tumour size less than 5 mm). Laboratory investigations were performed for analysis of liver functions using routine commercially available kit by spinreact for measurement of: Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline phosphatase (ALP), Total bilirubin ((T. Bil.), Direct bilirubin (D. Bil.), serum albumin (Alb.), and prothrombin time (PT). Viral hepatitis markers including HCV antibody, HBV surface antigen and HBV core antibody were done to select monoinfected HBV patients.

2.1 Measurement of Serum OPN

OPN levels were measured by enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, Human OPN Immunoassay Kit, R&D Systems, DOST00, Minneapolis, MN) according to the manufacturer's instructions.

2.2 Measurement of Serum AFP

The quantitative determination of the cancer antigen AFP concentration in the human serum was determined using AFP immunoassay test kit (No: 4S00068), supplied by Medical Technology Promedt Consulting GmbH, D-66386 St. Ingbert, Germany.

2.3 Statistical Analysis of Data

Results were expressed as mean ± standard deviation (SD) for continuous variables and relative frequency and percent distribution for categorical variables. Comparisons between groups were made using one way analysis of variance and Chi square tests for both types of data respectively. Receiver operating characteristics (ROC) analysis was used to evaluate the diagnostic value of OPN. Sensitivity and specificity were profiled by curves; comparison between the 2 AUC was done using Pearson Chi Square test. P value <0.05 was considered statistically significant.

3. RESULTS

Demographic and biochemical investigations for the patients and control groups were analyzed and tabulated as shown in Table 1. There was no significant difference regarding age and sex distribution between the three studied groups. While, significant difference was found between the three groups regarding liver function tests.

Both Table 2 and Fig. 1 demonstrated the Performance of AFP and OPN as diagnostic markers of early HCC. AUC was 0.78 for AFP and 0.87 for osteopontin; the best cuttoff value for AFP was ≥25 ng/ml; while for OPN it was ≥135 ng/m. At the best cutoff value OPN had sensitivity of 100%, specificity 65%, PPV 74%, NPV 89% and overall accuracy 93% (CI: 0.765-0.980) and P value: 0.001), AFP showed 90% sensitivity, 55% specificity, PPV 66.7%, NPV 84.6% and overall accuracy 80% (95% CI: 0.0.621-0.931) and P value: 0.003). Combination of both markers showed that sensitivity was 92%, specificity 59%, PPV 69% , NPV 72% and overall accuracy 84% (CI: 0.695-0.911 and P value: 0.02).

4. DISCUSSION

It is of great importance to diagnose early HCC in chronic HBV patients as that will improve the survival of these patients [13,14]. However the current screening methods may not be helpful.

Ayoola and Gadour, conducted a study about hepatocellular carcinoma in Saudi Arabia and reached a conclusion that "Hepatitis B virus constitutes a major risk factor and HCV contributes a less significant role in the development of HCC" [15].

OPN is a protein involved in TGF-β signaling pathways. TGF-β regulates cell differentiation, and is commonly deregulated in HCC. The suppression of TGF-β occurs early in hepatocarcinogenesis. Hepatitis B virus X proteins shift hepatic TGF-β signaling from tumor suppression to oncogenesis in patients with chronic HBV infection [16]. Thus, plasma biomarker candidates associated with TGF-β will likely help detects HCC in the early stages. OPN has been reported to promote oncogenesis and tumor cell invasion in HCC [17].

In the current study, OPN was demonstrated to be a potential biomarker for detection of early HCC. This finding is consistent with a previous report suggesting that OPN was more sensitive than AFP as a biomarker, and was a marker of early-stage HCC [18]. In addition, up-regulated OPN has been shown to be involved in poor prognosis for HCC [19].

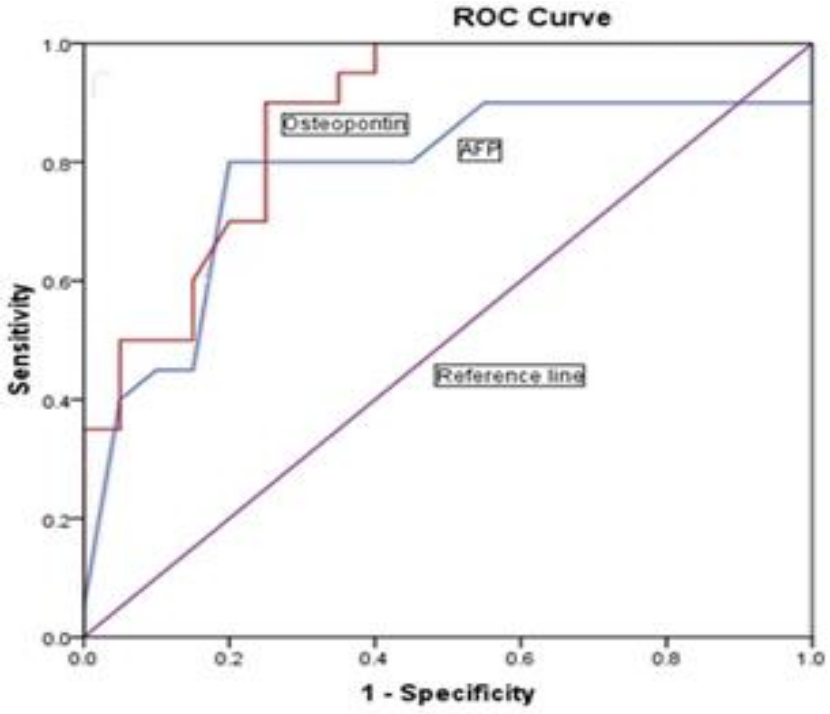

Fig. 1. ROC curve for performance of Osteopontin (OPN) and AFP in diagnosis of early HCC

Table 1. Demographic and biochemical tests in the three studied groups

Variable	Groups			P value
	Control (n=50)	Cirrhosis with HBV (n=100)	Early HCC (n=100)	
Age (years)	42.3±6.2	45.6± 8.9	48.5±9.4	0.43
Sex				
Male	22 (55%)	26 (52%)	32 (53%)	0.53
Female	18 (45%)	24 (48%)	25 (47%)	
ALT (U/L)	30±6.0	103.0±44.3	146.50±23.8 [a,b]	<0.001
AST (U/L)	30.2±6.1	116.7±85.2	189.75±42.3 [a,b]	<0.001
T.Bil (mg/dl)	0.75±0.2	1.2±0.49	1.70±3.42 [a,b]	<0.001
D.Bil (mg/dl)	0.15±0.060	0.34±0.27	1.10±0.69 [a,b]	<0.001
ALP (U/L)	43.65±7.56	133.10±35.06	213. 50±72.18 [a,b]	<0.001
ALB (gm/L)	3.85±0.21	3.40±0.38	2.9±0.9 [a,b]	<0.001
PT (seconds)	11.19±0.62	12.50±3.48	13.8±5.96 [a,b]	<0.001
AFP (ng/ml)	5.86±2.02	8.3±2.86	45.55±23 [a,b]	<0.001
OPN (ng/ml)	24.5±5.6	59.70±17.72	652±166.43515 [a,b]	<0.001

a Significant difference from control group b Significant difference from cirrhosis group

The present study found a statistically significant difference ($P < 0.05$) between early HCC group in comparison to both control and cirrhosis groups regarding all biochemical liver functions measured: ALT, AST, ALP, Bil (Total and Direct), ALB and PT. The means of ALT, AST were higher significantly in early HCC group than both control or early cirrhotic HBV groups (P=0.001), this may be due to the destructive effect of tumour cells on liver tissues. Also, means of T. Bil., D. Bil., ALP, AFP, PT and OPN in early HCC group showed higher values significantly than both control or cirrhotic HBV groups (P=0.001) while the mean level of seum albumin was significantly low in early HCC group in camparison to the other groups. These results may be due to the deterioration of liver functions in early HCC than other groups.

Table 2. Performance of diagnostic markers of early HCC

Variable	AFP	OPN	AFP & OPN delet
AUC	0.78	0.87	--
Cutoff	≥25	≥135	--
Sensitivity	90%	100%	92%
Specificity	55%	65%	59%
PPV	66.7%	74.0%	69%
NPV	84.6%	89%	72%
Accuracy	80%	93%	84%
95% CI	0.621-0.931	0.765-0.980	0.695-0.911
P	0.003	0.001	0.02

Both Table 2 and Fig. 1 demonstrated the Performance of AFP and OPN as diagnostic markers of early HCC. AUC was 0.78 for AFP and 0.87 for osteopontin; the best cuttoff value for AFP was ≥ 25 ng/ml; while for OPN it was ≥ 135 ng/m. At the best cutoff value OPN had sensitivity of 100%, specificity 65%, PPV 74% , NPV 89% and overall accuracy 93% (CI: 0.765-0.980) and P value: 0.001), AFP showed 90% sensitivity, 55% specificity, PPV 66.7%, NPV 84.6% and overall accuracy 80% (95% CI: 0.0.621-0.931) and P value: 0.003). Combination of both markers showed that sensitivity was 92%, specificity 59%, PPV 69% , NPV 72% and overall accuracy 84% (CI: 0.695-0.911 and P value: 0.02). Roc Curve Area Comparison between AFP and OPN =0.09 with p = 0.18 NS.

Diagnostic markers for early HCC (AFP and OPN) were also elevated with statistically significant difference (P < 0.05) in early HCC group in comparison to the other groups. Comparison of performance of both markers was held using ROC curve and the overall accuracy of OPN was better than AFP (93% vs 80%). These results suggested that OPN can replace AFP as a screening test for diagnosis of early HCC but more studies on large scale of patients may be needed for validation of OPN as a screening tool. Combination of both markers in diagnosis of early HCC didn't improve the performance of OPN alone.

Yang et al., studied the performance of OPN and AFP as a diagnostic biomarker for early HCC in chronic HBV Chinese patients, their study reported that the performance of OPN in diagnosis of early HCC at cutoff 84.4 ng/ml was as follow (AUC:0.911, sensitivity 87.15% and specificity 85%) while for AFP at cutoff 20 ng/ml was (AUC: 0.642, sensitivity 36.87% and specificity 84.81%) [20]. The results reported by Yang et al. were in agreement with our reported results and support the idea of validation of OPN as a diagnostic biomarker for early HCC.

5. CONCLUSION

OPN showed better sensitivity in monitoring patients with early HCC than AFP and serum OPN estimation can be considered as a reliable marker for screening of early HCC.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/10048