RESEARCH COMMUNICATION

Diagnostic Significance of Alpha Fetoprotein in Carcinomas of Liver and Biliary Tract - A Comparative Study from Western Region of Nepal

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Abstract

Objective: To assess the diagnostic significance of α-fetoprotein in carcinomas of liver and biliary tract with the overall goal of reducing morbidity and mortality in Pokhara valley. Materials and Methods: It was a hospital based comparative study carried out in the Department of Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal between 1st January 2009 and 31st December 2010. The variables collected were age, gender, serum alpha feto protein. Approval for the study was obtained from the institutional research ethical committee. Estimation of AFP was performed by ELISA reader for all cases. The standard procedure was followed as per manufacturer's instructions for ELISA. All these laboratory parameters were analyzed using Human reagent kits and with the help of ELISA and semi autoanalyser (Humalyser 3500, Germany). Results: Out of 1200 patients, there were 348(29%) cases of HCC. Out of that, 285 cases were found to be AFP positive with significant elevation. Furthermore, diagnosed cases were of cholangiocarcinomas (96, 8%) and secondary carcinomas of liver (216, 18%). In both of these clinical conditions, there was insignificant elevation of AFP. Another commonly diagnosed condition was cirrhosis (480, 40%) and in 90 cases, AFP values were moderately raised from the upper limit of normal reference range. The last diagnosed cases were of either Hepatitis A/E(60, 5%) and did not show any rise in levels of AFP.Conclusion: Serological markers for hepatocellular carcinoma (HCC) are imperative for early identification, as well as scrutinizing of tumour aggressiveness, treatment responsiveness, reappearance and endurance. It is consequently justifiable to carry out the test for serum AFP to detect and differentiate at early stage of liver cell carcinomas.

Key words: Alpha fetoprotein - hepatocellular carcinoma - biliary tract cancer - prognosis - Nepal

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Introduction

Hepatocellular carcinoma (HCC) represents a disease of crucial importance worldwide, accounting for more than 80% of primary liver tumors and approximately nearly 600,000 deaths each year (Plymoth et al., 2009). Because of poor curability, the number of HCC deaths occurring each year is roughly equivalent to the number of new cases. Hepatocellular carcinoma is the seventh and ninth most common form of cancer worldwide in men and women respectively (Rustgi, 1987).

The global epidemiology of HCC is striking, with both geographic and temporal patterns of incidence paralleling exposure to viral etiological factors. Over 80% of HCCs occur in developing countries, mainly in Southeast Asia, sub-Saharan Africa and East Asia. By contrast, the incidence of HCC is much lower in developed countries in North America (6.8 cases per 100,000 person-years for men; 2.2 cases per 100,000 person-years for women), Europe, Central and South America, Australia and New Zealand (Ferlay et al., 2008). Global variations in incidence rates of this cancer closely reflect the variation in risk factors for HCC; thus, countries with a high prevalence of HBV or HCV infections usually have a high incidence of HCC. It is among the top three causes of cancer death in the Asia Pacific region because of the escalating incidence of its leading etiological agents, chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections (Zhihua et al., 2006). HCC shows a strong male predilection in high prevalence regions (Leong et al., 2005). The major risk factors for HCC include previous hepatitis B infection, heavy alcohol consumption, cirrhosis, aflatoxin exposure, old age, nutritional factors, toxins, metabolic diseases,

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hepatolithiasis and liver fluke infection.

Cholangiocarcinoma is the second most frequent primary malignant epithelial liver tumor accounting for 5% of all primary hepatic malignant tumors (Blechacz and Gores, 2008). It is a neoplasm originating from the intrahepatic or extra bile duct epithelium. The extrahepatic type, including cancers involving the confluence of the right and left hepatic ducts, accounts for 80% to 90%, and the intrahepatic type for 5% to 10% of all cholangiocarcinomas (de Groen et al., 1999). Serum α-fetoprotein (AFP) as a tumor marker is elevated in a considerable fraction in germ cell tumours (GCTs), hepatoblastoma and hepatocellular carcinoma (HCC). Carbohydrate antigen(CA19-9) have been widely used in clinical practice as a tumor marker of intrahepatic cholangiocarcinomas (Tao et al., 2010). Alpha-fetoprotein (α-fetoprotein, AFP) is a large 70 kD serum glycoprotein encoded by a gene on chromosome 4q11-q13, composed of 591 amino acids and 4% carbohydrate residues. The fetal yolk sac and liver generate high levels of AFP during gestation, which decline over the next 12 months of birth to 10-20 ug/Lor ng/ml. (Kashyap et al., 2001). Likewise, elevation of its level up to pathological range in adults correlates with the appearance of severe malignant and chronic conditions. In Nepal, as with many other diseases, there is inadequate prevention, diminutive of surveillance of individuals at high risk of developing HCC, and insufficient deployment of advances in imaging, hepatobiliary surgery, targeted therapies, and liver transplantation due to intermediate or low medical resources (Yang et al., 2010). Early detection is highly enviable as patients with initial stages are often asymptomatic, and consequently HCC is frequently diagnosed late, by which time it is often untreatable. The surveillance of HCC is generally done primarily by AFP and ultrasound of liver (Zhang et al., 2004). α-Fetoprotein (AFP) measurements have clinical implications in detection, differential diagnosis and monitoring of malignant disease. Therefore, the objective of our present study is to assess the diagnostic significance of α-fetoprotein in carcinomas of liver and biliary tract with the overall goal of reducing morbidity and mortality in Pokhara valley.

Materials and Methods

The It was a hospital based comparative study carried out in the Department of Biochemistry of Manipal Teaching Hospital, Pokhara, Nepal between 1st January 2009 and 31st December 2010. The variables collected were age, gender, serum alpha feto protein. Approval for the study was obtained from the institutional research ethical committee. Estimation of AFP was performed by ELISA reader for all cases. The standard procedure was followed as per manufacturer's instructions for ELISA (Sell, 1990). All these laboratory parameters were analyzed using Human reagent kits and with the help of ELISA and semi autoanalyser (Humalyser 3500, Germany). Analysis was done using descriptive statistics and Confidence Interval (CI). The data was analyzed using Excel 2003, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version.

Inclusion Criteria

A brief history of patients was taken with clinical symptoms and signs and initial diagnosis. Patients admitted in wards or visiting OPDs with diagnosis or suspicions of HCC and additional (HCV) and hepatitis B viral (HBV) infections, were selected .When histologically confirmed, HCC patients were evaluated and classified according to the underlying cause. The diagnosis of 348 cases of HCC was established based on the 1) presence of cirrhosis and a newly developed enhancing lesion >2 cm found by two or more imaging modalities (including ultrasound, contrast CT, contrast MRI, and angiography); 2) presence of cirrhosis and a newly developed enhancing lesion >2 cm found by one imaging modality (including contrast CT, contrast MRI, and angiography) and a total AFP >100 ng/ml. 480 patients had cirrhosis whose diagnosis was confirmed histologically or patients with clinically significant portal hypertension with splenomegaly, thrombocytopenia, ascites, esophageal or gastric varices, or portal hypertensive gastropathy.

Exclusion criteria

Patients suffering from any other cancer was excluded from our study.

Sample Size Determination

Sample size was calculated using the formula n= Z2PQ/E2. Where n= sample size, from the pilot study done in Manipal Teaching Hospital, Pokhara. We got 30% of them were HCC. So P=30 %, Q= 1-P=70%, E= allowable error= 10% of P, Z= 1.96. Total sample size needed for this study is 896 at 5% significant level (Sathian et al., 2010).

Results

Of the 1200 subjects, 348 (29%) cases were of HCC and further majority of HCC cases showed elevated levels of AFP. Table 1 depicts that out of 1200 patients, there were 348(29%) cases of HCC. Out of that, 285 cases were found to be AFP positive with significant elevation. Furthermore, diagnosed cases were of cholangiocarcinomas (96, 8%) and secondary carcinomas of liver (216, 18%). In both of these clinical conditions, there was insignificant elevation of AFP. Another commonly diagnosed condition was cirrhosis (480, 40%) and in 90 cases, AFP values were moderately raised from the upper limit of normal reference range. The last diagnosed cases were of either Hepatitis A/E(60, 5%) and did not show any rise in levels of AFP.

Table1. Details of Patients Assessed for AFP in Various Clinical Conditions

Diagnosis	Total number of cases (1200)	AFP Positive	AFP Negative
Heptocellular carcinoma	348(29%)	285	63
Cholangiocellular Carcinoma	96 (8%)	2	94
Secondary carcinoma in liver	216(18%)	2	214
Cirrhosis	480(40%)	90	390
Hepatitis A/E	60 (5%)	---	60

The upper limit of normal reference range for AFP is < 10 ng/ml

Table 2. Distributions of HCC Patients with Respect to AFP Values and Clinical Conditions in Males and Females

Variable (285)	Total	%	Mean Values of AFP(ng/ml)	Confidence Interval
Male				
HCC	180	63%	164.71± 123.84	(146.49,182.92)
Subgroup				
HBV	84	47%	189.04±134.38	(159.87, 218.20)
HCV	60	33%	187.45±134.70	(152.65, 222.25)
No infection	36	20%	117.69± 80.79	(90.36, 145.03)
Females				
HCC	105	37%	158.09± 129.17	(133.09, 183.08)
Subgroup				
HBV	48	45%	177.98 ± 135.78	(138.55,217.41)
HCV	21	20%	174.71 ± 128.49	(116.23, 233.20)
No infection	36	35%	121.50 ± 88.68	(91.49, 151.51)

Table 2 depicts that patients suffering from hepatocellular carcinomas with AFP positive were of age in range of 22 years to 88 years. The gender did not show any significant variation with respect to age. The mean values of AFP in HCC were found to be higher in males (164.71 ± SD123.84) in comparison to females (158.09 ± SD129.17).

In males, the total number of AFP positive with chronic hepatitis B and C infection was 84(47%) and 60(33%) respectively. Further, the mean values of AFP with chronic hepatitis B and C infection were 189.04±SD134.38 and 187.45± SD134.70 respectively. The number and percentage of patients having no infection but suffering from HCC were 36 and 20%. The mean value of AFP in patients having no infection was 117.69 ± SD80.79. In females, the total number of AFP positive with chronic hepatitis B and C infection was 48(45%) and 21(20%) respectively. Further, the mean values of AFP with chronic hepatitis B and C infection was 177.98 ± SD135.78 and 174.71 ± SD128.49 respectively. The number and percentage of patients having no infection but suffering from HCC were 36 and 35%. The mean value of AFP in patients having no infection was 121.50 ± SD88.68.

Discussion

The morbidity and mortality due to liver cell carcinomas is ever-increasing in developing countries due to unawareness of tumor markers and lack of prior diagnosis of chronic liver disease which could lead to development of liver tumors. AFP has been widely used for screen examination and clinical diagnosis as an HCC tumor marker (Taketa, 1990). In this study, we document the presence of raised AFP particularly in cases of HCC and cirrhosis. In 60%-70% HCC patients, serum AFP is higher than the normal range. Our findings concurred with the study done in primary liver cancer in Japan (Anonymous, 1990). Males were more affected with hepatocellular carcinomas than females and age range was somewhat similar in pretext to gender. The present study revealed that there was insignificant increase in levels of AFP in secondary tumors of liver, cholangiocarcinomas and in cases of hepatitis A/E. In countries like Nepal with scarce medical resources, the most common underlying cause of hepatocellular carcinomas was chronic HBV infection. The mean value of AFP was found to be significantly elevated in HBV infected cases of hepatocellular carcinomas. In fact, more than half of all HCCs in the world are attributed to chronic hepatitis B (Parkin, 2006). The pro-oncogenic activity of HBV seems to be multifactorial and it might act through direct and indirect mechanisms, the latter represented by hepatic necroinflammation. These inflammatory cells release cytokines and chemokines capable of favouring cellular transformation and tumour growth (Chemin et al., 2009). The ability of HBV to integrate into the genome of the infected host hepatocytes is also considered one of the most important confirmations of its direct pro-oncogenic role (Bréchot et al., 2000). HBV might generate genomic instability including chromosomal deletions and rearrangements, gain and loss of alleles with loss of heterozygosity (LOH), gene amplifications and mutations frequently involving oncogenes and tumour-suppressor genes, aneuploidy as well as epigenetic alterations., either through viral DNA integration or through the activity of its proteins.Numerous genetic abnormalities have been described in HCC. The other epidemiological and molecular studies also suggested that Hepatitis B virus (HBV) infection is the main risk factor for hepatocellular carcinoma (HCC) development (Szmuness, 1978). HCV infected cases of hepatocellular carcinomas also showed the significant rise in AFP levels. The pathogenesis of hepatocellular carcinoma in chronic HCV infection is due to chronic inflammation and injury which leads to fibrosis with eventual progression to cirrhosis and the subsequent development of hepatocellular carcinoma (Sherman M 2005). In our present study also, there were 480 cases of cirrhosis, and out of that 90 cases were AFP positive and most of the cases having AFP positive with cirrhotic liver were suffering from hepatocellular carcinomas. Therefore, HBV and HCV infected cases having high mean values of AFP mostly develop cirrhosis and further progress to HCC. In our current study , patients of cholangiocarcinomas and secondaries of liver were

mainly due to gastric carcinoma, pancreatic carcinoma and gallbladder carcinoma. Elevation of AFP above the normal limits was rarely observed. Consequently, the assessment of serum tumor marker α-fetoprotein in combination with ultrasonography ought to be there for early stage detection of liver tumors in clinical practice. Further, in Nepal, most of the patients suffering from liver tumors present when they are already symptomatic and are beyond the scope of effective treatment. Therefore, in intermediate-resource or low-resource environments, the fundamental focus should be on primary prevention of HCC, through universal HBV vaccination, taking appropriate precautions and antiviral treatments. Further, regular screening with non invasive methods mainly AFP levels of cirrhotic, HBV, HCV patients could reduce the mortality from liver tumors to a major extent in developing countries.

Hepatocellular carcinoma is one of the most frequent cancers of the liver and is recurrently preceded by chronic viral hepatitis B or C or cirrhosis. Serological markers for hepatocellular carcinoma (HCC) are imperative for early identification, as well as scrutinizing of tumour aggressiveness, treatment responsiveness, reappearance and endurance. It is consequently justifiable to carry out the test for serum AFP to detect and differentiate at early stage of liver cell carcinomas, thus facilitate the clinician to institute early chemotherapy which may offer a longer span of life even despite the fact that in due course, prognosis will remain unaffected.

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