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Alpha-Fetoprotein and Gamma-Glutamyltranspeptidase Seromarkers in Patients with Liver Cirrhosis & Hepatocellular Carcinoma

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ABSTRACT

OBJECTIVES: To identify the Alpha-fetoprotein and Gamma-glutamyltranspeptidase seromarkers in patients with liver cirrhosis (LC) and / or hepatocellular carcinoma (HCC) patients.

METHODOLOGY: A retrospective study was conducted at District Headquarter Hospital Jamshoro, Kotri from September 2019 to March 2020. A total of 100 patients included in the study who were diagnosed with liver cirrhosis and/or hepatocellular carcinoma. All the subjects were selected using non-probability convenience sampling technique. Measurement of AFP was conducted using the immunoassay AXSYM AFP (Abbott, USA), the most adequate cut-off point is the value of 20 ng/mL for the identification of hepatocellular carcinoma patients. GGT measurements for the Architect/Aeroset system (Abbott, USA) were made on a specific test; the upper limit of normality for the latter is 64 IU/L. The study excluded all subjects not diagnosed with liver cirrhosis (LC) and/or hepatocellular carcinoma (HCC) patients. SPSS version 22.0 was used for the analyses of data.

RESULTS: Patients were diagnosed with liver cirrhosis in 60 (60%) and hepatocellular carcinoma in 40 patients (40%). Out of which 29 (72.5%) patients had liver cirrhosis with hepatocellular carcinoma, while 11 patients (27.5%) had hepatocellular carcinoma without liver cirrhosis. The liver cirrhosis-associated hepatocellular carcinoma showed higher levels of AFP compared with liver cirrhosis not associated hepatocellular carcinoma (20 and 2.93 ng / mL, $p<0.05$) and GGT in hepatocellular carcinoma associated liver cirrhosis patients (208 and 109 IU / L, $p<0.05$) as well. Not a single patient with hepatocellular carcinoma had both normal AFP and GGT levels simultaneously.

CONCLUSION: In HCC patients, the AFP and GGT levels were significantly higher than in LC patients alone.

KEYWORDS: Alpha-Fetoproteins, Gamma-glutamyltransferase, Hepatocellular Carcinoma, Liver cirrhosis

INTRODUCTION

Biomarkers which can be detected in the blood, urine or tissue as molecular indicators of biological state can be useful for the clinical handling of different conditions. The presence of various disorders can be detected through threshold concentrations. Concentration fluctuations can guide disease development therapy. For different disease states, numerous biomarkers have been identified. Research on the clinical significance of the use of biomarkers is ongoing and evaluated. Cancer is the world's prominent cause of death, which in 2012 accounted for 8.2 million deaths. At 745,000 deaths in 2012, hepatocellular carcinoma (HCC) is the second-most frequent cause of cancer death in the world[1] Pakistan is at the juncture of socio-economic instability and a strong desire for reforms[2]. With an estimated population of 182,142,594, it is the sixth most populated nation in the world. As a low-income country, when compared with peer nations, we fall behind in various significant healthcare determinants.

In the developing world, occurrence of cancer and death are increasing. Pakistan faces serious cancer burden that have a detrimental effect on the outcome of patients[3]. There has been a steady spike in hepatobiliary cancer. The most common malignancies in adult males are hepatobiliary cancers and account for 10.7 percent of all cancers, based on findings from a reputable hospital registry in Pakistan[4].

The age standardized rate for hepatocellular carcinoma in Pakistan is 7.6 for male and 2.8 for female subjects per 100,000 per year[5]. The hepatocellular carcinoma knowledge in Pakistan's population is minimal and mainly reflects the experiences of a single centre. Hepatocellular carcinoma data are collected and we do not know what the history of non-Hepatitis B / Hepatitis C hepatocellular carcinoma is prevalent in our population in patients suffering from Hepatitis B and Hepatitis C. In Pakistan, 60-70% of hepatocellular carcinoma is caused by Hepatitis C. In contrast Hepatitis B remain upper most cause , different from many other Asian Pacific countries[6].

Hepatocellular carcinoma production is one of the possible chronic complications that people with LC may encounter, with a frequency ranging from 3 to 4 percent per year[7]. Gamma-glutamyltranspeptidase (GGT) has been proposed as a potential additional marker for early diagnosis of carcinoma in cirrhotic patients and is used to measure serum levels of certain markers in patients with liver cirrhosis to predict the presence of neoplasm at early stage. As the most useful, alpha-fetoprotein (AFP) has been suggested[8]. Therefore, these both seromarkers, alpha-fetoprotein and GGT can early point out towards development of hepatocellular carcinoma and suggest for early biopsy and therapeutic measures to be taken as curative intent.

METHODOLOGY

A retrospective study was conducted at District Headquarter Hospital Jamshoro, Kotri from September 2019 to March 2020. A total of 100 patients included in the study who were diagnosed with hepatocellular carcinoma and/or liver cirrhosis. All the subjects were selected using non-probability convenience sampling technique. The ethical approval for research project was taken from the research ethics committee of District Headquarter Hospital Jamshoro at Kotri.

The inclusion criteria for liver cirrhosis were determined by the occurrence, with or without evidence of portal hypertension in imaging research, of any of the major clinical complications such as hepatic encephalopathy, esophageal varicose veins, spontaneous bacterial peritonitis hepatorenal syndrome, or ascites plus reports of nodularity. In cases where histopathological information was available when evidence of fibrosis and nodule regeneration was present, the diagnosis was identified. The inclusion of hepatocellular carcinoma patients was based on the guidelines of the European Association for the study of the liver, which included a mixture of diagnostic photographic findings, elevation of the tumor marker and, where appropriate, histopathological characteristics. The study excluded all the subjects who were not diagnosed with hepatocellular carcinoma or liver cirrhosis. All patients / representatives have obtained written approval.

Measurements of alpha-fetoprotein were performed using AxSYM an immunochemical automated analyzer (Abbott, USA), in which for AFP value of 20 ng / mL is considered the most appropriate cut-off point for the identification of hepatocellular carcinoma patients. The specific test for the Architect system (Abbott, USA) was used to perform the GGT measurements; the upper limit of normality for the latter is 64 IU/L. All seromarkers done by private laboratory and funded by researchers themselves.

The data of the various clinical manifestations of the patients were obtained from the medical records. Coagulopathy was described as prolonging prothrombin time by 06 seconds.

The first measurement of the levels of AFP and GGT recorded and clinical history was used for statistical analysis after the patient was admitted through hepatitis clinic (OPD) and admitted at medical Department. Some of the features associated with neoplastic lesions, such as maximum diameter, number and presence of metastatic involvement, were evaluated by computerized tomography or patient magnetic resonance imaging.

Continuous variables were presented as medians, with their respective interquartile range, or with mean and standard deviation, depending on the distribution of the results. The patient was admitted to the medicine Department. Categorical variables were given as absolute numbers and proportions. The two-tailed Mann Whitney U test with a significance level of 0.05 has been used for comparisons between groups.

Comparisons were made between the LC-associated group of patients with HCC and the isolated LC group of patients as defined in the study objectives. While eleven patients with an isolated diagnosis of HCC were identified, comparisons were not considered. With the information gathered, a database was built and then analyzed in the statistical package for social science version 22.0 (IBM, Chicago Inc., USA).

RESULTS

A total of 100 liver cirrhosis and/or hepatocellular carcinoma patients were included in this study. The majority were male (62.6%), while the average age was 57.4 (SD ± 12.06) years. Patients were diagnosed with liver cirrhosis (LC) in 60 (60%) and hepatocellular carcinoma (HCC) in 40 patients (40%). Out of which 29 (72.5%) patients had liver cirrhosis with hepatocellular carcinoma, while 11 patients (27.5%) had hepatocellular carcinoma without liver cirrhosis.

Viral etiology, which was found in 83% of cases. It was the most recurrent liver cirrhosis etiology in the patients studied, leaving alcoholic liver disease in second place (Figure I). The most common clinical feature found in those individuals with liver cirrhosis (33.7%) was the presence of esophageal varicose veins. Other frequent manifestations were Ascites 15 (16.9%) and coagulopathy 8 (9%) (Figure II).

Of all the hepatocellular carcinoma patients 40 (100%) had tumors with more than or equivalent to 2 cm, in which 22 (55%) had multiple lesions of the liver and 11 (27.5%) were classified as metastatic at the time of presentation; 7 (17.5%) patients had a vascular tumor invasion.

Significantly higher levels of Alpha-fetoprotein (AFP) and Gamma-glutamyltranspeptidase (GGT) than with isolated liver cirrhosis were identified in patients with liver cirrhosis and hepatocellular carcinoma. (Table I). There was no substantial difference in AFPs or GGT depending on the age of the subjects being tested, whereas significantly higher GGT levels were found in males (153 IU / L and 64 IU / L, p=0.012); the later finding is not explained by the higher frequency of alcoholic disease in the former. In patients with hepatocellular carcinoma, AFP and GGT above the cut-off point, 61% and 82%, respectively, were found; in patients with isolated liver cirrhosis, 6% and 59%, respectively, were found to be elevated. Each individual diagnosed with hepatocellular carcinoma had at least one of the two markers above the normal range, whether or not linked to liver cirrhosis. (Table II).

FIGURE I: ETIOLOGY OF CIRRHOSIS OF THE LIVER IN PATIENTS OF STUDY

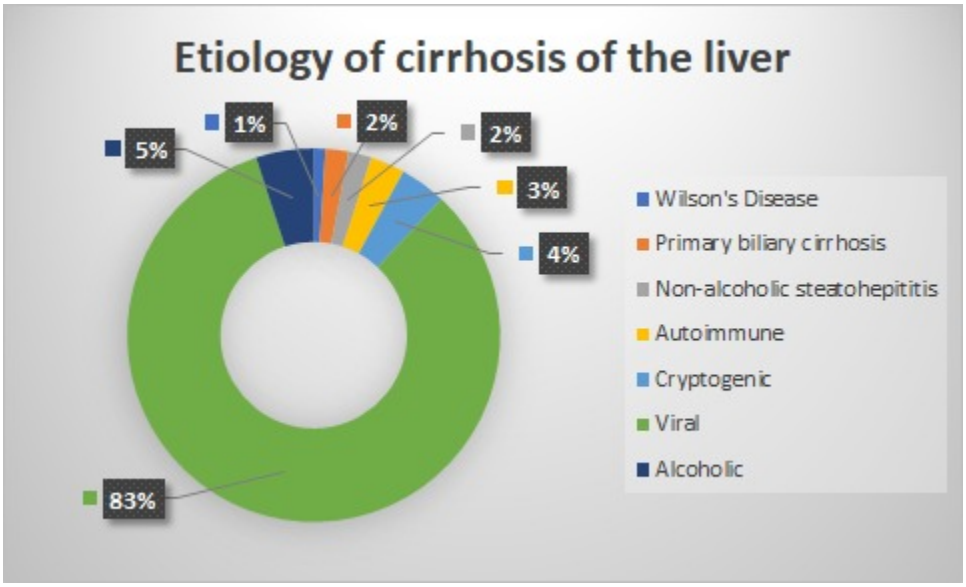

FIGURE II: STUDY PATIENTS CLINICAL CHARACTERISTICS

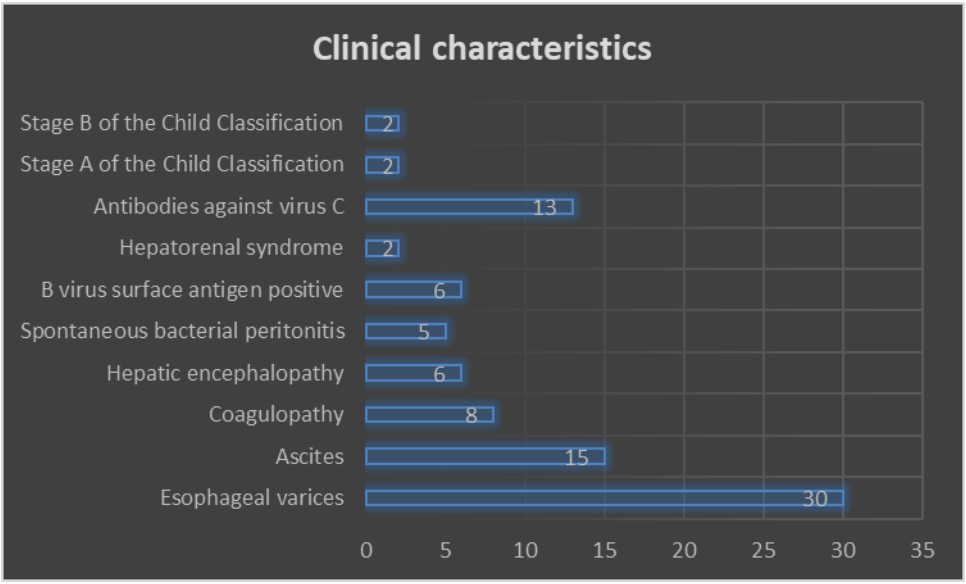

TABLE I: ACCORDING TO THE DIAGNOSIS THE LEVELS OF ALPHA-FETOPROTEIN AND GAMMA-GLUTAMYLTRANSPEPTIDASE

	Liver Cirrhosis N = 60	**Cirrhosis and carcinoma N = 40**	**P value**
AFP Median (25-75 percentile)	2.93 ng/mL (2.03-5.88)	20 ng/mL (4.95-779.00)	P = 0.01
GGT Median (25-75 percentile)	109 UI/L (47.25-191.50)	208 UI/L (100.00 – 355.00)	P = 0.04

TABLE II: AFP VALUES FOR PATIENTS WITH HEPATOCELLULAR CARCINOMA ACCORDING TO OTHER TUMOR CHARACTERISTICS

Characteristic	**Median (ng/dL) (Percentile 25-75)**	**P value**
Multiple liver lesions	339 (4-4584)	0.74
Single liver injury	270 (9.5-4729)	
Vascular invasion	3630 (181-31161)	0.09
Without vascular invasion	60 (4.8-3111)	
Extrahepatic metastases	273 (29-3731)	0.53
Without extrahepatic metastases	56 (4.5-6861)	
Lesions greater than 2 cm	271 (5.8-4230)	0.85
Lesions less than or equal to 2 cm	6 (*)	

* Not calculable because of the limited number of patients

DISCUSSION

Latest data indicates that the biological characteristics of the tumor are likely to be linked to different compositions and activities, resulting in very different clinical results with regard to proliferation and invasive behavior. The chronic liver disease causes one in forty deaths per year in the world[9], including people with both chronic liver disease types liver cirrhosis and hepatocellular carcinoma was diagnosed in two thirds of the cases, while hepatocellular carcinoma was diagnosed in one third. Many of these carcinomas grow in liver cirrhosis in a similar way to those identified by other researchers[10,11].

In the study population, alcoholic liver disease was the leading cause of liver cirrhosis; chronic hepatotropic virus infection was reported as its key cause in less than 20 per cent of all cirrhotics. This activity is generally seen in developing countries, where hepatitis B virus and hepatitis C virus have been able to partly control the spread of the infection.

In Pakistan, most hepatocellular carcinomas are caused by hepatitis C virus. We still do not know today the unique hepatitis-C prevalence in Pakistan. It is estimated that about 10 million people in Pakistan suffer from hepatitis-C infection[12–14]. In this population, the prevalence of cryptogenic cirrhosis (11.2%) and autoimmune cirrhosis (3.4%) is comparable to those previously described in the literature.

Ascites is the most frequently identified complication in individuals with liver cirrhosis; after 10 years of follow-up, it is present in around 60 per cent of them[15,16]. In this study, a similar percentage (16.9%) was observed in patients. On the other hand, the high frequency (33.7%) of oesophageal varicose veins in our patients is striking; this condition has been reported in 50 percent of people with portal hypertension regardless of the disease stage[17].

The vast majority (91%) of the tumors found in the research patients had a diameter greater than 2 cm; a higher percentage than that reported by Mazzaferro et al, who documented neoplastic lesions greater than 3 cm in a sample of therapeutic intervention of individuals with hepatocellular carcinoma [18,19]. This disparity may be explained by the difficulty of tracking patients of our setting, supported by certain patients' repeated delays in accessing the health system and lack of adherence to the medical treatment.

In order to detect the presence of hepatocellular carcinoma early, AFP and GGT are used as useful markers to detect patients with liver cirrhosis [20]. Many patients with liver cirrhosis without hepatocellular carcinoma have normal AFP levels and slightly higher GGT, regardless of the etiology underlying them. according to one study 70% of hepatocellular carcinoma patients had high AFP levels, while another recorded a figure of 54%, similar to that found in this study[21].

The GGT's usefulness as a specific marker for hepatocellular carcinoma detection is very poor; however, it has been defined that the utility is greater when used in combination with AFP. Individuals with hepatocellular carcinoma associated with liver cirrhosis also have substantially higher rates of both markers relative to those with liver cirrhosis alone[22,23]. In the present study, serum AFP and GGT levels in 61 per cent and 82 per cent of patients diagnosed with hepatocellular carcinoma, respectively, were above the upper limit of normal. It is important to note that in patients with chronic liver disease, none of the hepatocellular carcinoma patients had both markers within the normal range; this result may be useful for screening purposes. While several research studies[24] show an opposite results, hepatocellular carcinoma cells propagate in circulation into the bloodstream and are a recurrence source after operation, which may be the primary reason for an unsatisfactory, long-term survival after surgery. GGT mrNA can be found

in healthy adults and hepatocellular carcinoma patients' serum and liver tissues, nonmalignant hepatopathy, benign hepatic tumor, and secondary liver carcinomas.
Under the limitations of this research, we must assume that a bias resulting from data collection may have existed, because it was a retrospective analysis. In addition, higher elevations in AFP values were identified in those individuals with tumors larger than 2 cm or with tumor vascular invasion within the community of hepatocellular carcinoma patients. In our research, however, they did not achieve statistical significance despite finding these differences; this behavior is likely due to the sample size.

CONCLUSION

This study shows that the levels of AFP and GGT were significantly higher in hepatocellular carcinoma patients than in individuals with isolated liver cirrhosis; however, no hepatocellular carcinoma patients reported normal values simultaneously in the AFP and GGT tests. It will be important to consider further research into population with chronic liver disease with long-term follow-up using those markers in the future.

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AUTHOR CONTRIBUTIONS
Kumar K: Concept of project
Khokhar NA: Data collection
Kalhoro MA: Literature search
Luhano MK: Statistical analysis
Memon SM: Revision, draft finalizing

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