Original Article

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

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ABSTRACT

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

METHODOLOGY: A retrospective study was conducted at District Headquarters Hospital Jamshoro, Kotri, from September 2019 to March 2020. One hundred patients diagnosed with liver cirrhosis and/or hepatocellular carcinoma were included in the study. All the subjects were selected using a non-probability convenience sampling technique. AFP was measured using the immunoassay AXSYM AFP (Abbott, USA); the most adequate cut-off point is a value of 20 ng/mL for identifying 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 (40%) patients. Of these, 29(72.5%) patients had liver cirrhosis with hepatocellular carcinoma, while 11 (27.5%) patients 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. No 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 a biological state, can be helpful 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 other disease states, numerous biomarkers have been identified. Research on the clinical significance of using 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, the occurrence of cancer and death are increasing. Pakistan faces severe cancer burdens that have a detrimental effect on patient outcomes. There has been a steady spike in hepatobiliary cancer. The most common malignancies in adult males are hepatobiliary cancers, accounting 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 the history of non-Hepatitis B / Hepatitis C hepatocellular carcinoma, which is prevalent in our population in patients suffering from Hepatitis B and Hepatitis C. In

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Pakistan, 60-70% of hepatocellular carcinoma is caused by Hepatitis C. In contrast, Hepatitis B remains the uppermost cause, unlike 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 specific 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 seromarkers, alpha-fetoprotein and GGT can early point out the development of hepatocellular carcinoma and suggest an early biopsy and therapeutic measures to be taken as curative intent.

METHODOLOGY

A retrospective study was conducted at District Headquarters Hospital Jamshoro, Kotri, from September 2019 to March 2020. One hundred patients in the study were diagnosed with hepatocellular carcinoma and/or liver cirrhosis. All the subjects were selected using a non-probability convenience sampling technique. The ethical approval for the research project was taken from the District Headquarters Hospital Jamshoro research ethics committee 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 significant 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 European Association for the Study of the Liver guidelines, which included a mixture of diagnostic photographic findings, the elevation of the tumor marker and, where appropriate, histopathological characteristics. The study excluded all subjects not diagnosed with hepatocellular carcinoma or liver cirrhosis. All patients/ representatives have obtained written approval.

Alpha-fetoprotein measurements were performed using AxSYM, an immunochemical automated analyzer (Abbott, USA), in which the AFP value of 20 ng / mL is considered the most appropriate cut-off point for identifying 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 are done by private laboratories and funded by researchers themselves.

The data on 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 was recorded, and clinical history was used for statistical analysis after the patient was admitted through the hepatitis clinic (OPD) and admitted to the medical Department. Some 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 to compare 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 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(40%) patients. Of these, 29(72.5%) patients had liver cirrhosis with hepatocellular carcinoma, while 11(27.5%) patients had hepatocellular carcinoma without liver cirrhosis.

Viral etiology, found in 83% of cases, 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 2cm, 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 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 future 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

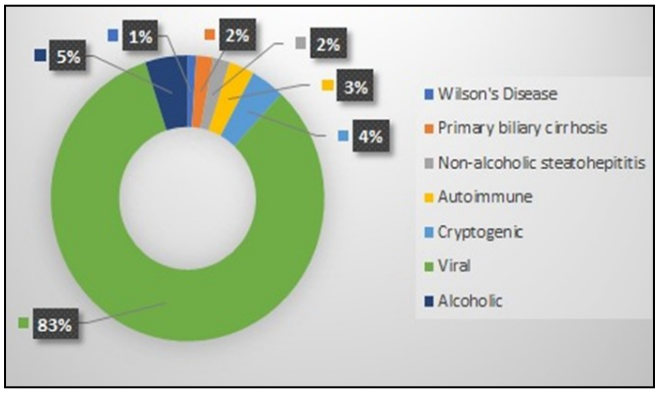

FIGURE II: STUDY PATIENTS CLINICAL CHARACTERISTICS

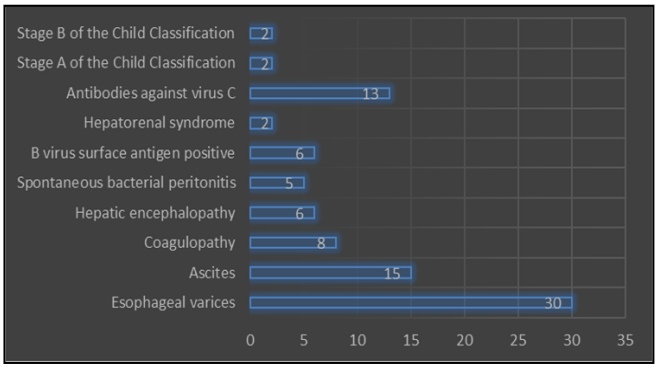

TABLE I: ACCORDING TO THE DIAGNOSIS, THE LEVELS OF ALPHA-FETOPROTEIN AND GAMMA-GLUTAMYLTRANSPEPTIDASE

	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

The 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 concerning proliferation and invasive behavior. 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 were diagnosed in two-thirds of the cases, while hepatocellular carcinoma was diagnosed in one-third. Many of these carcinomas grow in liver cirrhosis, similar 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 vital cause in less than 20 per cent of all cirrhotics. This activity is generally seen in developing countries, where the 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. About 10 million people in Pakistan are estimated to 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 ten years of follow-up, it is present in around 60 per cent of them[15,16]. This study observed a similar percentage (16.9%) 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 in our setting, supported by certain patients' repeated delays in accessing the health system and lack of adherence to medical treatment.

AFP and GGT are valuable markers for detecting the presence of hepatocellular carcinoma early, and they are used 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 their underlying aetiology. 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 more significant when combined with AFP. Individuals with hepatocellular carcinoma associated with liver cirrhosis also have substantially higher rates of both markers than 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 helpful for screening purposes. While research studies[24] show the opposite effects, hepatocellular carcinoma cells propagate in circulation into the bloodstream. They are a recurrence source after operation, which may be the primary reason for 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 tumors, 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 2cm 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 patients reported typical values simultaneously in the AFP and GGT tests. It will be essential to consider further research into the population with chronic liver disease with long-term follow-up using those markers in the future.

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AUTHOR CONTRIBUTION

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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