Iran J Public Health, Vol. 45, No.12, Dec 2016, pp.1645-1651

Original Article

Combined Evaluation of AFP, CA15-3, CA125, CA19-9, and CEA Tumor Markers in Patients with Hepatitis B and C

Mehdi ASSMAR [1], Sara YEGANEH [1], Fariborz MANSOURGHANAEI [2], Nour AMIR-MOZAFARI [3]

1. *Dept. of Microbiology, Islamic Azad University, Lahijan, Iran*
2. *Gastrointestinal and Liver Diseases Research Center, Guilan University of Medical Sciences, Rasht, Iran*
3. *Dept. of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*

***Corresponding Author:** Email: mehdiAssmar@yahoo.com

(Received 07 Feb 2016; accepted 16 Jul 2016)

Abstract

Background: This study aimed to determine the role of tumor markers AFP, CA15-3, CA125, CA19-9 and CEA in patients with hepatitis B and C.

Methods: This descriptive cross-sectional study was performed from Oct 2012 to Oct 2014. Serum samples of 129 patients with hepatitis B and C referred to Guilan Liver and Digestive Disease Research Center in Rasht, Iran were collected and checked for the existence of the listed tumor markers by ELISA.

Results: No increase in serum levels of tumor marker CA19-9, CEA and CA15-3 were seen in patients with hepatitis ($P>0.05$). In patients with hepatitis B, increase in CA125 were observed ($P=0.03$). In hepatitis C patients, there was an increase in AFP levels ($P=0.03$).

Conclusion: The levels of AFP and CA125 markers were high in hepatitis C and hepatitis B, respectively. However, the increased levels were not seen is malignancy. Due to the small sample size, further study is necessary to find the reasons of the increase.

Keywords: Tumor markers, Hepatitis B, Hepatitis C, ELISA

Introduction

Hepatitis B is a liver infection caused by hepatitis B virus (HBV). People can catch hepatitis B through contact with body fluid, blood, and sexual intercourse. Hepatitis B may have no symptoms or feelings of sickness for a period. Symptoms include low fever, yellow skin, dark urine etc. (1, 2). If people do not know they are affected, they may spread the virus to other people. From 100 people who get hepatitis B, 1 person dies from this virus. They may also suffer from liver cancer and cirrhosis (3).

Prevalence of this disease in Middle East countries like Iran is in medium range, was 1.7% and 5% in Fars and Sistan-Balochastan provinces, respectively (4, 5). The prevalence of hepatitis B in Gonbad Kavoos City area was 4.5% (6). In city of Shiraz, the prevalence rate was rather low (1.07%); however, the highest prevalence was reported in Tuserkan City (8.96%) (7, 8). Worldwide HCV infection rate is around 3%, which correspond to 170 million people (1).

Infection with HBV or HCV leads to formation of immune complex by virus antigen and antibody in the glomerular basement membrane resulting in production of membranous glomerulonephritis and membrane proliferation. In extreme cases, severity of hepatic disease will surpass the symptoms of hepatic complication. Mixed Cryog-

lobulinemia, due to production of antibody with particular physical properties leading to precipitation in low temperature was reported in hepatitis B and currently in HCV (9). These infections are highly prevalent in prison population. Preventive measures may significantly reduce the rate of infection and infectivity (10).

Tumor markers are biomolecules that share the structure of proteins and hormones found in abnormal blood, urine and tissue of patients with all kinds of cancer. Measurement of a tumor marker in most cases is limited to the diagnosis, since most tumor markers do not act specifically and will increase in number within the patients without cancer. In most cancers, the levels of several tumor markers increase; therefore, identification of their concentration has diagnostic value. They have less than 100% sensitivity; therefore, no tumor marker is specific to an organ or to a specific cancer (9). Tumor markers can be used for one of five purposes; 1) screening for the presence of cancer, 2) monitoring the course of cancer in patients, 3) diagnosis of cancer or of a specific type of cancer, 4) determining the prognosis in patients, 5) determining stage of cancer. High levels of tumor markers in cancer patients can be used to help determine the extent at which the cancer has spread to other tissues and organ (11).

α-fetoprotein: AFP is a 70,000 Da glycoprotein consisting of 591 amino acids and 0.04 carbohydrate residues, encoded by a gene on chromosome 4q11-q13. It is normally produced during gestation by the fetal liver and yolk sac. AFP can be elevated in other neoplasm, including pancreatic cancer (23%), gastric cancer (20%), colorectal cancer (5%) and bronchial cancer (7%). The sensitivity of AFP for liver cancer is about 60%. An AFP level greater than 500 ng/ml is very suggestive of liver cancer (12-14).

CA15-3: Considered as the biomarker of breast cancer. It has a glycoprotein structure. An increased CA15-3 level is indicative of lung, ovarian, liver, or stomach cancer (12-14).

CA125: Is a modified antigen with Mullerian fetus cells. This antigen has a semi-mucin glycoprotein structure and is considered as a major marker in ovary non-mucin tumors and endometriosis (12, 13).

CA19-9: Is considered as a marker of pancreatic cancer, hepatocellular carcinoma, colon and rectum cancers. Identification of its levels helps in identification and prognosis of pancreatic, stomach, colorectal cancer (13, 14).

CEA: Is a glycoprotein that is not specific for a particular organ. It is widely used for diagnosis, follow-up, and prognosis of adenocarcinoma, especially for colorectal cancer (15).

We aimed to determine the role of tumor markers AFP, CA15-3, CA125, CA19-9 and CEA in patients with hepatitis B and C.

Methods

Patients

In a descriptive cross-sectional study performed from Oct 2012 to Oct 2014, 320 patients with hepatitis B and C referred to Guilan Research Center for Digestion and Liver were enrolled. Of the 320 patients, 191 of them who were receiving drugs related to hepatitis including interferon α, ribavirin, lamivudine, etc. were excluded. The study was conducted on 129 untreated patients with hepatitis B and C, 92 (71.31%) of them were male with ages ranging from 18 to 57 yr. Patients were informed of the plan and the fact that their medical record will be kept confidential.

Firstly, patients were introduced to the project after giving consent. Questionnaire included demographic, endoscopy, liver biopsy information and the latest laboratory findings, which was completed through interview. Patients' history included their age, gender and BMI (Body Mass Index).

Inclusion criteria: 1) hepatitis C virus antibody (HCV Ab) by AxSYM and confirmed by real-time polymerase chain reaction (RT-PCR) positive, 2) positive HBsAg, 3) no history of antiviral therapy, 4) no evidence of HCC with regular abdominal sonography or Magnetic Resonance Imaging (MRI) or other imaging.

Exclusion criteria: 1) Patients receiving hepatitis medication (Subcutaneous injection of interfe-

ron-alpha in combination with diet and oral Ribavirin, lamivudine, 2) patients who did not consent to continue.

Laboratory investigations

Blood samples were taken before starting any medication. Blood samples were placed at 4 °C until clotting occurred. Sera were separated by centrifuge and stored at -20°C before analyzing. A Complete Blood Count (CBC), using a SYSMEX K1000 device, of each patient was taken as well. Prothrombin time and concentration were measured using a Bench Electronic coagulator. In addition, serum levels of bilirubin, Alanine aminotransferase (ALT), Asparagine aminotransferase (AST), and Alkaline phosphatase (ALP) were measured using a BM Hitachi 711 Chemistry Analyzer. A serum sample of each patient was tested for hepatitis B and C. Measurement of HBsAg, IgM antibodies to hepatitis B core antigen (Cab IgM), HBcAb, hepatitis B surface antigen (HBsAb) and HCVAb were done by IM.E.SA BT 3000 automated immunoassay analyzer, based on quantitative enzyme micro particle immunoassay. After defining the positive cases of hepatitis, they were analyzed in respect to tumor markers AFP, CA15-3, CA19-9, CA125, and CEA using quantitative ELISA (Xema kits from Russia).

Statistical analysis

Data collected in the questionnaire were analyzed using SPSS v.16 (Chicago, IL, USA) with fisher test. *P*-values less than 0.05 were considered as significant.

Results

Of the 129 patients, 72 cases were infected with hepatitis B (55.8%) and 57 patients were infected with hepatitis C (44.2%). In hepatitis B patients, 29 (40.27%) were female. In patients with hepatitis C, 8 (14.3%) were female. Of these patients, 89.7% were self-employed (38.9% hepatitis B and 50.8% of hepatitis C) (Fig. 1). The patients were in the age range of 18-57 yr. The most prevalent hepatitis B age group was that of 48-57 yr of age with a frequency of 24 persons (33/33%). Whereas, the highest prevalent hepatitis C was in the 28-37 age groups with a frequency of 20 persons (35.08%) (Fig. 2).

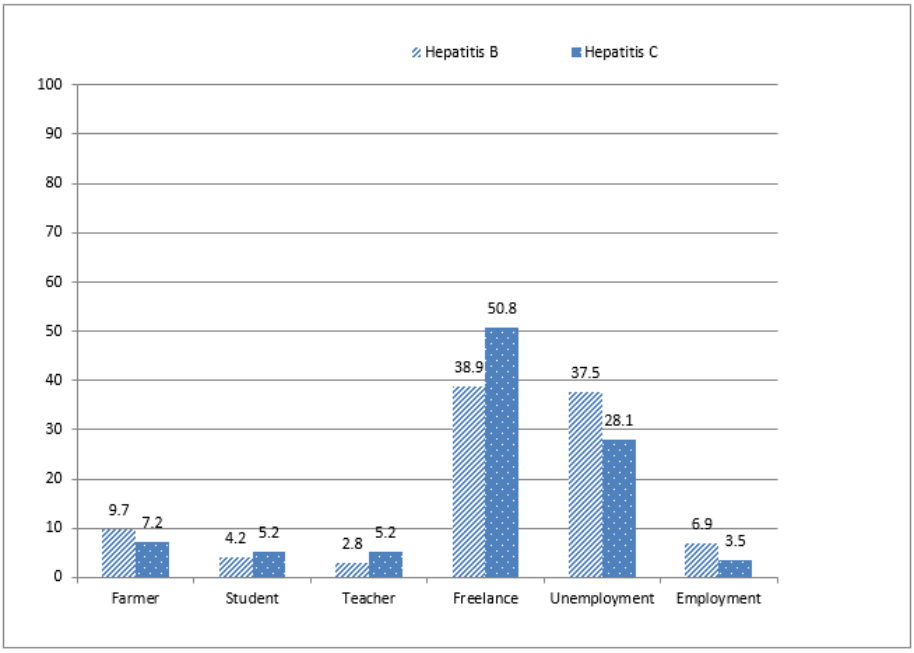

Fig.1: Frequency vs. occupation for two groups of patients infected by hepatitis B and C

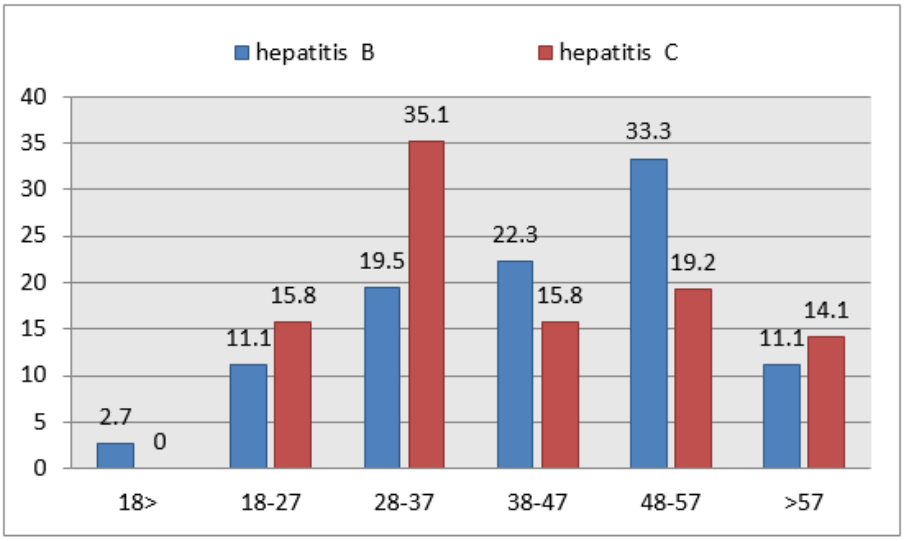

Fig.2: Frequency vs. age for two groups of patients infected by hepatitis B and C

In terms of the relationship between tumor markers under investigation and hepatitis, the following results were obtained:

1. The tumor marker CA19-9 with 95% confidence (P>0.05) in serum of patients with hepatic disease did not increase significantly (Table 1).
2. The tumor marker CA125 with 97% confidence (P=0.03) in the serum of patients with hepatitis B and tumor markers AFP, with 97% confidence (P=0.03) in the serum of patients with hepatitis C had a significant increase (Table 1).
3. Tumor marker CA15-3 and CEA in serum of patients with hepatitis B and hepatitis C had no significant association (Table 1).

There was no significant correlation between levels of the tumor markers and gender, age and serum parameters including albumin, ferritin, and ALP, PT (Table1).

Discussion

The tumor markers AFP, CA19-9 and CEA were studied as non-invasive determinants of patients with hepatitis B and C. Moreover, a significant relationship between AFP level and hepatitis infection was observed. No relationship between CA19-9 and CEA levels and hepatitis B and C were seen. A high AST, ALT, AST/ALT ratio, low platelet count and elevated CA15-3 and CA125 levels correlated with severe liver damage. In damaged or inflamed liver cells, an increased natural range of chemicals such as enzymes are released into the circulation. Low platelet count was correlated with elevated CA125 and CA15-3 levels.

The results of this investigation regarding AFP levels in hepatitis C are consistent with other study (16). Our findings regarding AFP levels are not consistent with other results (17).

We found significant relationship between age 47-58 in hepatitis C and the AFP marker levels. Therefore, it seems that measurement of AFP can be an important tool in care and management of patients with benign and malignant hepatic disorders (18). Furthermore, deliberation of serum AFP levels has also been utilized in screening of patients with viral hepatitis (19). However, further studying seemed to be necessary with a larger sample size.

Elevated serum AFP levels were correlated positively with serum ALT in this study (P=0.04). This was consistent with the result of another investigation, which reported a P value of < 0/001 (20).

Table 1: Frequency distribution of patients infected by hepatitis B and C vs. tumor markers

Hepatitis / Frequency / Tumor marker		B %	C %	*P*-Value
CA15-3	Normal	54(75)	49(85.94)	Ns(0.06)
	Abnormal	18(25)	8(14.03)	
	Total	72(100)	57(100)	
CA125	Normal	61(84.72)	34(59.64)	P=0.03
	Abnormal	11(15.27)	23(40.35)	
	Total	72(100)	57(100)	
CA19-9	Normal	64(88.88)	43(75.43)	Ns(0.1)
	Abnormal	8(11.11)	14(24.56)	
	Total	72(100)	57(100)	
AFP	Normal	61(84.72)	41(71.92)	P=0.03
	Abnormal	11(15.27)	16(28.07)	
	Total	72(100)	57(100)	
	Normal	67(93.05)	47(82.45)	NS(0.1)
CEA	Abnormal	5(6.94)	10(17.54)	
	Total	72(100)	57(100)	

Also in this study a significant relationship between AFP level and serum AST (*P*=0.03) was observed. This is compatible with the suggestion that AFP is a non-invasive predictive marker in patients infected with HCV (21). We saw no relationship between CA19-9 levels and hepatitis. Other investigators also did not report any meaningful correlation between CA19-9 levels with chronic hepatitis and related cirrhosis (22). A number of studies showed small level correlation between CA19-9 and some standard parameters of hepatic function.

Serum CA19-9 levels elevated in hepatocellular, colorectal, esophageal, lung and ovarian carcinomas. The use of CA19-9 as a non-invasive marker for disease activity in patients with hepatitis is not useful. Elevated CA19-9 is not specific for viral hepatitis. Further study seemed to be necessary because a high percentage of false positive and false negative results can occur. There was no relationship between CEA levels and hepatitis which was consistent with the results reported previously (23). Hepatitis patients could have elevated CEA levels without the presence of cancer. CEA levels are used for management purposes. The results of the present study about tumor marker CA15-3 has not been consistent with another study (16). Our data indicates that serum CA15-3 levels may be elevated in patients with hepatitis. The serum concentration of CA15-3 should be considered when severe liver fibrosis is suspected.

There has been a significant correlation between CA125 and infection with hepatitis (P=0.01) which was in line with other studies (24, 25). CA125 is not a tissue or tumor specific antigen, thus it can be found in patients with benign and neoplastic pathologies. Levels of tumor markers can increase in patients without malignant transformation. The causes for the association of tumor markers with liver diseases vary. Elevated CA125 levels in hepatitis maybe are independent of etiology (26).

This study suggests that increase in serum CA125 levels in various benign liver diseases indicates the presence of cirrhosis. This protein can arise in both malignant and benign tumors (27). This study found no significant relationship between location, job, sex, albumin, ferritin, ALP, PT and levels of the tumor markers. The results are consistent with those from another study (28). . Determination of combined tumor markers, CA19-9

and CA125, in liver diseases is useful for identifying patients with advanced disorders with high specificity. Patients without combined elevation still require a histological examination to identify severe liver fibrosis (29).
Limitation of the study was that patients were not very comfortable in releasing their medical history to us. Some people were upset and angry

Conclusion

Tumor marker increase is certainly not a definite sign of malignancy. Survival of these patients strongly depends on the degree of damage due to liver cancer and the possible presence of HBV and HCV infection. Considering the limited number of studies on tumor markers and infection of people with hepatitis B and C worldwide, future studies should take account of a greater number of patients and a longer period in order to reach more results that are desirable.

Ethical considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgments

Authors wish to thank the cooperation of Islamic Azad University of Lahijan, the Liver and Gastroenterology Research Center personnel, and the personnel of research and production complex of ZistFaravard Pars Company. In addition, we acknowledge all people who provided help in this research. The expense of this project was paid by the Islamic Azad University of Lahijan. The authors declare that there is no conflict of interests.

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