https://doi.org/10.5272/jimab.2018243.2160

Journal of IMAB
ISSN: 1312-773X
https://www.journal-imab-bg.org

Journal of IMAB - *Annual Proceeding (Scientific Papers)*. 2018 Jul-Sep;24(3)

Original article

ASSOCIATION BETWEEN ALPHA-FETOPROTEIN AND OTHER SEROLOGICAL MARKERS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA: ONE CENTER'S EXPERIENCE

Lejla Mehinovic[1,*], Zora Vukobrat-Bijedic[2], Azra Husic-Selimovic[3], Amina Kurtovic-Kozaric[1,4], Erna Islamagic[1], Damir Suljevic[1,*],

1) Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

2) Polyclinic Al-Tawil, Sarajevo, Bosnia and Herzegovina.

3) Clinic for Gastroenterohepatology, Clinical Centre of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

4) Department of Clinical Pathology, Cytology and Human Genetics, Clinical Centre of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

**-those authors contributed equally.*

ABSTRACT

Purpose: In this study, we analyzed biochemical parameters in the serum of patients with a diagnosis of hepatocellular carcinoma (HCC) C and B viral etiology.

Material/Methods: All patients (31 males and 20 females) with a diagnosis of HCC that were treated at the Clinical Centre of the University of Sarajevo were included in this retrospective-prospective study. Serum alpha-fetoprotein was analyzed as a tumor marker, and hepatitis markers included HBs Ag, anti-HBs, anti-HBc, anti-HCV and anti-HB. Spearman test and Kolmogorov-Smirnov test were used for correlation and normality analysis, respectively.

Results: The largest number of patients (68.62%) had cirrhosis of C viral etiology that developed in cancer. Hepatocellular carcinoma was diagnosed more in men than in women (60.78%). The most patients were middle-aged (41-64 years). HCC was present in the right liver lobe at 82.85% HCV and 87.5% HBV patients. Only 6.25% of HBV patients were both liver lobes affected. All biochemical parameters had very high values, especially AFP and ãGT. Significant differences for AST and ALT were found between men and women. Serum bilirubin levels (total, direct and indirect) and AP are higher in men than women. Hepatitis markers had high values, and the incidence of HBs Ag (78%) and anti HBc (78.72%) was established.

Conclusions: A positive correlation was established between AFP and other parameters, while a significant difference between AFP and γGT (r = 0.372, p = 0.008) was confirmed. In addition to imaging methods for determining liver cirrhosis and hepatocellular carcinoma, high values of AFP and γGT, are a powerful diagnostic marker for these diseases.

Keywords: alpha-fetoprotein, hepatocellular carcinoma, γGT, HBs Ag, anti-HBc

INTRODUCTION

Since the hepatocellular carcinoma (HCC) is cancer with the worst prognosis in terms of survival, early diagnosis and treatment are important for such patients. Serological marker analysis for high risk patients for HCC development can reduce HCC mortality and reduce treatment costs [1, 2]. HCC is a common in males than in females (2.4: 1). High frequency is recorded in East and South Asia, Central and West Africa and Polynesia [3]. Despite recent findings in the treatment of early and advanced hepatocellular carcinoma, the development of serum markers for the prognosis of therapeutic outcomes and treatment monitoring have not been investigated yet. Serological AFP has been very frequently used for better prognosis of disease and monitoring of therapeutic effect. Although serum AFP levels have been shown to increase in several cancers, this parameter is used as tumor marker for HCC only [4, 5]. AFP is used in diagnosis as an adequate marker due to its sensitivity and specificity for the diagnosis of HCC in patients with cirrhosis of the liver. The combination of this test with abdominal ultrasound test increases the sensitivity and specificity for HCC diagnosis [5].

Recent studies emphasize that there is no optimal biomarker for HCC detection - it is suggested to use a combination of early detection biomarkers which include additional biomarkers such as DCP, GP73 and Lens culinaris agglutinin reactive AFP-L3 [4, 5]. Recent studies by El-Garem et al. suggest the use of tissue glypics 3 (GPC3), PAT10 (paternally expressed gene 10), serpine peptidase inhibitors (SERPINI1) and ubiquinol-cytochrome (QP-C)

inhibitors that are also detected in the normal concentration of AFP [6]. Hepatitis B surface antigen (HBsAg) represents a significant risk for HCC development. Patients with positive hepatitis B core antibodies (anti-HBc) that are HBsAg-negative can develop HCC [7].

The aim of the study is to investigate the possible correlation between AFP and specific biochemical in early HCC detection.

MATERIAL AND METHODS

This retrospective-prospective study includes all patients (31 males and 20 females) with a diagnosis of hepatocellular carcinoma that was treated at the Clinic for Gastroenterohepatology**,** Clinical Centre of the University of Sarajevo, in the period January 2010 - March 2013. Among patients, 35 had liver cirrhosis of viral C and 16 patients with B etiology that developed in liver cancer (hepatocellular carcinoma or HCC). The research was conducted at the Clinic for Gastroenteropathology of the Clinical Center of the Univesity of Sarajevo. Approval for research has been given by the Ethics Committee of the Clinical Center.

Biochemical and pathohistological analysis

Following biochemical parameters were collected: bilirubin (total, direct and indirect), enzyme activity (aspartate aminotransferase - AST, alanine aminotransferase - ALT, gamma-glutamyltransferase -γGT and alkaline phosphatase - AP). Immunological tests for hepatitis markers included HBs Ag, anti-HBs, anti-HBc, anti-HCV and anti-HB while a serum alpha-fetoprotein - AFP was analyzed as a tumor marker. Biochemical parameters were analyzed using VITROS 5600 Integrated System analyzer (Ortho Clinical Diagnostics, USA) and Elisa method for detect hepatitis markers was used. For AFP analysis Hemiluminescent Micro-particle Immunoassay ARCHI-TECT AFP assay (CMIA, Ireland) was used. Imaging methods were used to detect morphological and pathohistological liver changes such as computerized tomography (CT) and ultrasonography (US).

Statistical analysis

Data were analyzed using IBM SPSS Statistics ver. 20 (USA). Variance analysis (ANOVA) was used to test differences between groups. The non-parametric Spearman test was used for correlation analysis and for the normality test used the Kolmogorov-Smirnov test.

RESULTS

Table 1 shows age and gender structure of HCC patients which are divided into four groups: young-aged (18-40 years), middle-aged (41-64), old-aged (65-74) and very old-aged (≥75 years). Male patients and (60.78%) and middle-aged group patients (41.17%) were the most represented among the whole HCC patients.

Table 1. Percentage ratio of HCC patients

Patients	Young-aged (18-40)	Middle-aged (41-64)	Old-aged (65-74)	Very old-aged (≥75)	Total
Σ (%)*	1 (1.96%)	29 (56.86%)	13 (25.49%)	8 (15.69%)	51 (100%)
Males	1 (100 %)	16 (55.18%)	8 (61.54%)	6 (75.00%)	31 (60.78%)
Females	-	13 (44.82%)	5 (38.46%)	2 (25.00%)	20 (39.22%)

*percentage ratio of different age categories and total number of HCC patients

Table 2 shows the distribution of tumor lesions in live lobes detected by EHO and CT. Patients were classified according to etiology in two groups: HCV and HBV. Liver cirrhosis has developed in hepatocellular carcinoma in both groups of patients. Among HCC patients 68.62% were HCV patients and the other 31.38% HBV patients. These lesions mostly affected the right liver lobe (over 82%) in both groups. Only 6.25% of HCV patients had affected both lobes. The size of this lesion ranged from 3 to 20 cm. Splenomegaly with many local changes has also been reported in some patients.

Table 2. Imagining methods (EHO, CT) and tumor lesions

Etiology	N (%)	Right lobe	Left lobe	Both lobes
HCV	35 (68.62%)	82.85 %	17.15 %	-
HBV	16 (31.38%)	87.50 %	6.25 %	6.25 %

The data analysis for biochemical parameters of HCC patients is presented in Table 3. All analyzed parameters had increased values as well as a significant deviation of the normal distribution. The most noticeable variations were found for bilirubin (total, direct and indirect) and especially for AFP values.

Table 3. Biochemical parameters of HCC patients (males and females) and test normality

Parameters (n = 51)	Referent values	males&females (n=51)	males&females (n=51)	Kolmogorov-Smirnov test	
		Mean ± SD	Range	Statistic	p values
Total bilirubin (μmol/L)	6.8-20.5	62.40±101.34	4.60-573	.284	.000*
Direct bilirubin (μmol/L)	6.8-20.5	63.19±89.39	3.40-415	.298	.000*
Indirect bilirubin (μmol/L)	6.8-20.5	48.18±40.61	3.50-171.20	.212	.000*
AFP (ng/mL)	≤7	435.54±582.79	2.30-2420.68	.256	.000*
AST (U/L)	≤38	163.35±138.68	20-623	.153	.000*
ALT (U/L)	≤48	78.86±60.42	12-277	.182	.000*
γGT (U/L)	11-55	195.06±187.26	1.07-924	.199	.000*
AP (U/L)	60-142	199.53±147.91	68-758	.239	.000*

* Statistically significant differences

The values of biochemical parameters, as well as statistically significant differences between males and females (ANOVA) are presented in Table 4. Males had higher values of bilirubin (total, direct and indirect) and AP compared to females while others parameters (AFP, AST, ALT, γGT) were higher in females. Only the AST and ALT values showed significant differences between males and females.

Table 4. Gender distribution of biochemical parameters for HCC patients and ANOVA

Parameters (n = 21)	B&B… (n=31)	@&@… (n=20)	ANOVA
	Mean ± SD	Mean ± SD	p-value
Total bilirubin (μmol/L)	67.21±120.97	55.17±63.71	0.324
Direct bilirubin (μmol/L)	65.71±105.36	58.43±52.24	0.408
Indirect bilirubin (μmol/L)	50.27±47.12	44.23±26.19	0.339
AFP (ng/mL)	422.27±657.34	457.77±460.59	0.435
AST (U/L)	129.39±97.71	216±175.31	0.027*
ALT (U/L)	65.29±47.54	99.90±72.60	0.034*
γGT (U/L)	159.03±128.75	253.84±248.90	0.068
AP (U/L)	202.87±165.55	194.26±121.09	0.417

* Statistically significant differences between males and females

Table 5. shows the values of hepatitis markers for HCC patients. The most important diagnostic hepatitis marker present in HCC patients was HBs Ag with a frequency of 78% and anti-HBc (78.72%). All analyzed markers had high values, and the highest variations showed anti-HBs Ag (0.3-4364 S/CO).

Table 5. Hepatitis markers distribution in HCC patients

Parameters	HBs Ag	anti-HBs	anti-HBc	anti-HCV
Reactive	78%	46%	78.72%	40.42%
Nonreactive	22%	54%	21.27%	59.97%
Range	0.3-4364	0.04-1000	0.14-14.47	0.04-15.79
Referent values	0.00-0.99 (S/CO)	0.00-0.99 (mlU/ml)	0.00-0.99 (S/CO)	0.00-0.99 (S/CO)

Correlation between AFP and other parameters is shown in Table 6. A positive correlation is established for all parameters, but only significant differences are found between AFP and γGT (r=0.372, p= 0.008).

Table 6. Nonparametric Spearman's correlations between AFP and biochemical parameters

Parameters	Total Bilirubin (μmol/L)	Direct bilirubin (μmol/L)	Indirect bilirubin (μmol/L)	AST (U/L)	ALT (U/L)	γGT (U/L)	AP (U/L)
r	.141	.003	.213	.265	.129	.372	.179
Sig.	.329	.989	.297	.060	.368	.008**	.218

**Correlation is significant at the 0.01 level

DISCUSSION

HCC etiology varies across the globe, but so far in most studies where AFP was rated, viral hepatitis was the major cause of cirrhosis and HCC [4, 8, 9]. The prevalence of hepatitis B in our country is 1.5% compared to hepatitis C prevalence of 1% [10]. Our research has shown that the hepatitis B virus is the major cause of HCC which is consistent with the results of many epidemiological studies. Most of the HCC patients in our study were men. One reason is estrogen-mediated inhibition of IL-6 production from Kupfer's cells in women which reduces liver damage and prevents tumor spread. On the other hand, the effect of testosterone may increase the activation of androgen receptor in males inducing liver cell proliferation. In addition, the risk of HCC is higher in males due to higher exposure to liver-like carcinogens (such as smoking and alcohol) and higher rates of hepatitis by viral infections [11,12].

Hbs-Ag (surface antigen of hepatitis B virus, also known as Australian antigen) was detected in 78% HCC patients while anti-HCV antibodies were detected in 40.42% of patients. These results are similar to the results of Biliæ-Komarca et al. [13]. Unlike our results Hernandez et al. [14] reported that previous HCV infection is the primary cause of HCC, which is consisted with the results of developed Western countries characterized by low prevalence of HBV compared to other countries such as Southeast Asia and Sub-Saharan Africa, where HBV infection is a major etiological factor [15]. On the other hand, anti-HBc in our study was detected in all HBV patients, which could be a very important prognostic parameter for HCC development because of its highest frequency.

Biochemical markers that suggest a change in the liver metabolism such as bilirubin (direct, indirect, and total) were incresed. However bilirubin is not sufficient for diagnosis, but it is very diagnostically significant parameters in our research. High levels of enzymes activity can be the result of hepatic destruction leading to the release of the enzymes in serum. High ALT values are a liver destruction indicator [16]. According to Bashir et al. there are no significant changes for AP in HCV infection, but a significant increase in γGT in liver cirrhosis as well as in our research has been noted [17].

Also, our results showed that there was no significant difference in the AFP values between males and females for the HCC patient although the average AFP values in males were slightly higher than females. These results are consist with the results of Tangkijvanich et al. and Peng et al. who also reported that average serological AFP levels in males with HCC were somewhat higher than females but this difference was not significant [18, 19].

Our data indicate that the type of viral infection affects serum levels of AFP in HCC patients, which is not consistent with the results of previous research [20]. Namely, our research has shown that patients with HBV-related HCC had significantly higher AFP values compared to HCV-related HCC patients. This result indicates the role of HBV not only in the etiology of hepatocellular carcinoma but also in the reactivation of genes encoding this fetal protein. High levels of serum AFP in HBV positive HCC patients were also demonstrated in a study

by Peng et al. [19].

Biochemical and serological parameters including AFP as tumor marker are a useful tool for the diagnosis of HCC in patients with cirrhosis of the liver. However, the senistivity and specificity of final diagnosis can be incresed using imaging methods. High AFP values in correlation with γGT values as well as serological parameters, especially HBs-Ag and anti-HBc, are a safe indicator of liver damage and very important as prognostic parameters in early detection of liver changes, HCC development and patient therapy.

CONCLUSIONS

In conclusion, a positive correlation was detected between concentration of AFP and other biochemical parameters. We estimated significant difference between concentration of AFP and γGT activity. High values and ratio between AFP and γGT are a powerful diagnostic marker to distinguish liver cirrhosis and hepatocellular carcinoma.

REFERENCES:

1. Aronson JK. Biomarkers and surrogate endpoints. *Br J Clin Pharmacol*. 2005 May;59(5):491-94. [PubMed]

2. Strimbu K, Tavel JA. What are Biomarkers?. *Curr Opin HIV AIDS*. 2010 Nov;5(6):463-66. [PubMed]

3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010 Dec;127(12):2893–971. [PubMed]

4. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, et. al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology*. 2009 Jul;137(1):110-8. [PubMed]

5. Forner A, Reig M, Bruix J. Alfafetoprotein for hepatocellular diagnosis: The demise of briliant star. *Gastroenterology*. 2009 Jul;137(1): 26-9. [PubMed]

6. El-Garem H, Abdel-Hafez H, Foaud A, Al Akel W, Atia Met E, Salah M, et al. Tissue biomarkers in the early detection of hepatocellular carcinoma among egyptian patients with chronic hepatitis C: A possible genetic profile. *Br J Med Med Res*. 2013; 3(4):1858-70. [CrossRef]

7. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Long-term entecavir treatment reduces hepatocellular carcinoma incidence in patients with hepatitis B virus infection. *Hepatology*. 2013 Jul;58(1):98–107. [PubMed]

8. Yamashiki N, Sugawara Y, Tamura S, Kaneko J, Yoshida H, Aoki T, et al. Diagnostic accuracy of β-fetoprotein and des-γ-carboxy prothrombin in screening for hepatocellular carcinoma in liver transplant candidates. *Hepatol Res*. 2011 Dec; 41(12):1199-207. [PubMed] [CrossRef]

9. Sterling RK, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, et al. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am J Gastroenterol*. 2012 Jan;107(1):64-74. [PubMed] [CrossRef]

10. Suljevic D, Mehinovic L, Alijagic A. Hepatitis and biochemical markers in correlation with alpha-fetoprotein as a diagnostic indicator for the HBV and HCV differentiation. *Alban Med J*. 2016;3:13-20.

11. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*. 2007 Jun;132(7): 2557-76. [PubMed] [CrossRef]

12. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology*. 2004 Nov;127(5 Suppl 1):35-50. [PubMed] [CrossRef]

13.Bilic-Komarica E, Vukobrat-Bijedic Z, Dautovic-Krkic S. [Possible predictors of hepatocellular carcinoma in chronic viral hepatitis B and C etiology.] [in Bosnian] *Medical Journal*. 2012; 18(3):159-64.

14. Hernandez JC, Samanda M, Roque A, Cruz Y, Howland I, Fernandez I. Diagnostic value of alpha-fetoprotein for hepatocellular carcinoma. *Biotecnol Apl*. 2011; 28(1):34-39.

15. Seef LB, Hoofnagle JH. Epidemiology of hepatocellular carcinoma in areas of low hepatitis B and hepatitis C endemicity. *Oncogene*. 2006 Jun;25(27):3771-7. [PubMed] [CrossRef]

16. Karin M, Bevanda M, Babic E, Mimica M, Bevanda-Glibo D, Volaric M, et al. Correlation between biochemical and histopathological parameters in patients with hronic hepatitis C treated with pegylated interferon and ribavirin. *Psychiatr Danub*. 2014; 26(Suppl 2):S364-69.

17. Bashir MF, Haider MS, Rashid N, Riaz S. Association of Biochemical Markers, Hepatitis C Virus and Diabetes Mellitus in Pakistani Males. *Trop J Pharm Res*. 2013;12(5):845-50. [CrossRef]

18. Tangkijvanich P, Mahachai V, Suwangool P, PoovorawanY. Gender difference in clinicopathologic features and survival of patients with hepatocellular carcinoma. *World J Gastroenterol*. 2004 Jun;10(11):1547-50. [PubMed] [CrossRef]

19. Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC. High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus

infection, age, p53 and beta-catenin mutations. *Int J Cancer.* 2004 Oct 20; 112(1):44-50. [PubMed] [CrossRef]

20. Cedrone A, Covino M, Caturelli E, Pompili M, Lorenzelli G, Villani MR, et al. Utility of alpha-fetoprotein (AFP) in the screening of patients with virus-related chronic liver disease: does different viral aetiology influence AFP levels in HCC? A study in 350 Western patients. *Hepatogastroenterology.* 2000 Nov-Dec;47(36):1654-8. [PubMed]

Please cite this article as: Mehinovic L, Zora Vukobrat-Bijedic Z, Husic-Selimovic A, Kurtovic-Kozaric A, Islamagic E, Suljevic D. Association between alpha-fetoprotein and other serological markers in patients with hepatocellular carcinoma: one center's experience. *J of IMAB.* 2018 Jul-Sep;24(3):2160-2165.
DOI: https://doi.org/10.5272/jimab.2018243.2160

Received: 15/01/2018; Published online: 07/09/2018

Address for correspondence:
Damir Suljevic
Department of Biology, Faculty of Science, University of Sarajevo,
Zmaja od Bosne, 33-35, 71 000 Sarajevo, Bosnia and Herzegovina.
E-mail: suljevic.damir@gmail.com
E-mail: erna.islamagic@hotmail.com