The safety of alpha fetoprotein in diagnosis of hepatocellular carcinoma in patients with type 2 diabetes mellitus

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Abstract

Aim: Diabetes mellitus is closely associated with many types of cancer including hepatocellular carcinoma (HCC). Alpha feto protein is still used as a biomarker for HCC worldwide but relation between type 2 diabetes mellitus (T2DM) and AFP is unclear. We aimed to investigate relations between AFP, T2DM and metabolic markers in this study.

Material and Methods: 208 HCC patients were enrolled to study. 50 patients had T2DM (T2DM+HCC) and 158 patients were non-diabetic (NDM+HCC). 50 healthy people enrolled to the study as control group. Serum AFP levels were compared between healthy control group, T2DM+HCC group and NDM+HCC group. Serum AFP levels were compared with age, BMI, HgbA1c, fasting plasma glucose level (FPG), serum insulin level and insulin resistance (HOMA-IR).

Results: AFP levels were higher in T2DM + HCC group patients according to healthy control group (p<0,01). Mean AFP level was 201.97 ± 1093,89 in NDM + HCC group and 43.73 ± 85.67 in T2DM + HCC group. AFP levels were significantly higher in NDM + HCC group according to T2DM + HCC group (p<0.05). Serum AFP levels were negatively correlated with increasing age and FPG (p<0.05).

Conclusion: HCC is an aggressive tumour that usually develops on cirrhotic liver and AFP levels are important for the diagnosis of HCC. Our study showed AFP levels could be significantly lower in the presence of T2DM. This study showed AFP could not be a reliable marker in cirrhosis for screening or diagnosis of the HCC in patients with T2DM.

Keywords: Hepatocellular carcinoma; AFP; HCC; diabetes mellitus

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the ninth in women worldwide. HCC is the second most common cause of cancer-related deaths. Around eight hundred thousand new HCC cases are diagnosed in the world every year (1). HCC usually develops in cirrhotic liver parenchyma (2). Several risk factors have been identified for HCC development such as chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), alcoholic liver disease, nonalcoholic steatohepatitis (NASH), diabetes mellitus (DM), obesity, intake of aflatoxins-contaminated food, tobacco smoking and genetically inherited disorders such as hemochromatosis, α-1 antitrypsin deficiency, porphyrias (3).

Type II diabetes mellitus (T2DM) is a global health problem. There were 380 million patients with T2DM worldwide in 2013 according to International Diabetes Federation data (4). T2DM is an independent risk factor for HCC development, with higher incidence of HCC and poorer survival rates among diabetic patients (5). In a recent meta-analysis, it was found that the risk of developing HCC was 2.5-fold higher in patients with T2DM than in the normal population (6). Obesity and obesity induced NASH is also an independent risk factor for HCC development. According to United States data, HCC mortality rates were 4.5 times higher in men with BMI> 35 and 1.7 times higher in women with BMI> 35 compared to normal weight population (7). In European studies, HCC development risk also was found to be 2-3 folds higher in obese patients (8-9).

Alpha feto protein (AFP) is still the main serological marker used and recommended in HCC early diagnosis and screening worldwide (10). AFP is still used as a tumor marker for HCC but high AFP levels are found in 33-65% of HCC cases. Non-specific serum AFP elevation is seen in 15 to 58 % of patients with chronic hepatitis and in 11% to 47% of cirrhotic cases (11).

Although there are many studies examining the relationship between HCC and diabetes mellitus in the literature, the number of studies focusing on the relationship between AFP and diabetes mellitus is quite rare. The main aim of this study was to investigate whether AFP is a reliable biomarker in the diagnosis and screening of HCC in diabetic cirrhosis patients. The secondary aim of the study was to evaluate the relationships between AFP levels and metabolic markers in patients with HCC.

MATERIAL and METHODS

With the review and approval of the ethics committee of our university, the files of 1077 patients with liver cirrhosis who admitted to the gastroenterology outpatient clinic of Demiroğlu Bilim University between January 2004 and February 2019 were retrospectively reviewed. 237 patients with HCC were identified. Patients under the age of 18 or whose laboratory and demographic data were not available or who had a chronic disease other than diabetes mellitus were excluded. A total of 208 HCC patients were included in the study. Patients with HCC were screened among themselves and 50 patients with diabetes mellitus and HCC were identified. 50 patients who applied to the internal medicine check up department for health control were included in the study as control group. The control group were consisted of individuals over 18 years of age with no systemic disease. The patients were divided into three groups: 50 healthy control, 50 HCC patients with T2DM and 158 HCC patients without T2DM. The entire HCC patients group consisted of cases with HCC on the background of cirrhosis.

The following parameters of study population were collected; age, gender, height, weight, body mass index (BMI), diabetes history, fasting plasma glucose level (FPG), glycosylated hemoglobin A1c (HbA1c), AFP (alpha feto protein), serum fasting insulin level and insulin resistance (HOMA-IR).

The diagnosis of HCC was made by histopathological examinations or using two imaging modalities (magnetic resonance imaging or computed tomography). AFP was tested using commercially available immunoassays utilizing enhanced chemiluminescence at our hospital laboratory and the upper limit of the normal level was 13 ng/ml.

This study approved by ethics commitee of Demiroglu Bilim University. Informed consent form was obtained from all study patients and control group members.

Statistical Analysis

Statistical analysis was performed with the help of SPSS version 17.0 program. The normal distribution of the variables was examined by histogram graphs and Kolmogorov-Smirnov test. Mean, standard deviation, median and minimum-maximum values were used to present descriptive analyzes. Pearson Chi-Square and Fisher's Exact Tests were compared with 2x2 eyes. Nonparametric variables were evaluated among the two groups while Mann Whitney U Test was evaluated among more than two groups and Kruskal Wallis Test was used. The Spearman Correlation Test was used to analyze the measurement data. P-values below 0.05 were evaluated as statistically significant results.

RESULTS

A total of 208 HCC patients, 177 male (85.1%) and 31 female (14.9%) were enrolled to the study. Fifty patients had T2DM-HCC (24 %) and 158 patients (76 %) were non-diabetic-HCC (NDM-HCC). Mean age was 35 ± 12.3 in control group , 59±7.1 in T2DM-HCC group, 55.3±11.4 NDM-HCC group. Mean age of control group was lower than HCC and non-HCC cirrhosis group (p<0,001). Mean BMI was 24.4±3.9 in control group, 28.1±3.4 in T2DM-HCC group,and 27.1±4.3 in NDM-HCC group patients. Mean AFP levels in T2DM-HCC group and NDM-HCC group were significantly higher than those in the control group (T2DM-HCC, 43.73±85.67; NDM-HCC, 201.97±1093.89; Control group, 3.23 ±1.92). When HCC patients were compared according to AFP levels, AFP levels were significantly higher in NDM-HCC group than T2DM-HCC group. (p<0.05) (Table 1).

Table 1. Characteristics and AFP levels of study population				
Patiet characteristics	Control group (n=50)	T2DM-HCC (n=50)	HCC-NDM (n: 158)	P
	Median	Median	Median	
Male / Female	25 / 25	32 / 18 [a]	97/ 61	< 0.05
(%)	(50 / 50)	(64 / 32)	(61.3 / 38.7)	
Age (years)	35 ± 12.3	59.0 ± 7.1 [a]	55.3 ± 11.4	< 0.01
BMI (kg/m2)	24.4 ± 3.9	28.1 ± 3.4 [a]	27.1 ± 4.3	< 0.01
AFP (ng/mL)	3.23 ± 1.92	43.73 ± 85.67 [a]	201.97 ± 1093.89	< 0.05

T2DM: Type 2 Diabetes mellitus, HCC: Hepatocellular carcinoma, BMI: Body mass index, AFP. Alpha feto protein,
[a] p< 0.05 as compared with control,
[b] p< 0.05 as compared with T2DM+HCC group

AFP levels in HCC group patients evaluated according to age and metabolic markers such as fasting plasma glucose, insulin level, Hgb A1C and HOMA –IR. AFP levels were tended to decrease with increasing age (p < 0.05). When patients evaluated according to fasting plasma glucose (FPG) levels, serum AFP levels decreased significantly as fasting blood glucose levels increased (p<0.05). Table 2 summarized relations between AFP and metabolic markers.

Serum AFP levels showed negative correlation with increasing age and fasting plasma glucose levels in Spearman correlation analysis (p<0.05). There was no correlation between serum AFP levels and Hgb A1c, serum fasting insulin levels, HOMA-IR and BMI in HCC patients (Table 3).

Table 2. Comparison of AFP levels and serum diabetes markers in the study population				
Serum metabolic markers		**AFP**		**p¹**
		Mean	**S.D.**	**Median**	
Hgb A1c (%)	≤6	191.99	±171.32	20.00	0.723
	>6	56.86	±87.24	22.72	
FPG	<100 (Normal)	271.03	±130.46	27.24	**0.043²**
	100-125 (IFG)	57.38	±93.49	18.86	
	≥126 (DM)	47.23	±88.29	10.10	
Insulin	<25	202.31	±113.28	19.40	0.489
	≥25	70.14	±103.14	23.40	
HOMA-IR	≤2.5	317.86	±170.16	27.24	
	>2.5	105.23	±386.02	18.57	0.277

Hgb A1 c: Glycosylated hemoglobin A1c , FPG: Fasting plasma glucose, HOMA-IR: Insulin resistance
[1]Mann Whitney U Test
[2]Kruskal Wallis Test

Table 3. Correlation analyses of AFP with age and metabolic markers in HCC patients		
Metabolic and demographic markers		**AFP**
Hgb A1c	r	-0.117
	p	0.095
FPG	r	-0.179
	p	**0.010**
Insulin	r	-0.002
	p	0.981
HOMA-IR	r	-0.055
	p	0.434
BMI	r	-0.040
	p	0.569
Age	r	-0.150
	p	**0.032**

FPG: Fasting plasma glucose, BMI: Body mass index,
HOMA-IR: Insulin resistance,
Hgb A1 c: Glycosylated hemoglobin A1c
Spearman Correlation analysis

DISCUSSION

In this study, it was found that HCC patients with T2DM had significantly lower AFP levels in comparison to HCC patients without DM. In addition, serum AFP levels were markedly lower in HCC patients with older age and high serum fasting glucose levels.

Diabetes mellitus is related with the increased risk of many types of cancer such as liver, gallbladder, renal, breast, endometrial, pancreatic, and colorectal cancers according to many studies (12-17). HCC incidence is rapidly increasing worldwide, and this is likely linked to the increasing incidence of T2DM (18-20). The pathophysiological molecular mechanisms between HCC and T2DM are still unclear. In diabetic patients, many molecular pathways such as, PPAR-γ, AMPK , PDGF, JNK, VEGRF, Ret and c-kit are thought to play critical roles in the development of HCC (17). Hyperglycemia, increased serum insulin levels and increased insulin resistance may lead to IGF-I upregulation in diabetic cases. IGF-I increases cellular proliferation and inhibits apoptosis in liver. IGF-I-induced cellular proliferation may increase the production of free oxygen radicals. This increase can cause DNA damage and gene mutations associated with free radicals, leading to the development of HCC in liver tissue (21).

AFP and many other serum markers have been identified in the early diagnosis of HCC, but none are sufficiently reliable. The most commonly used marker for HCC is AFP and it is generally accepted that serum levels greater than 400 mcg/l is diagnostic for HCC especially in high risk cirrhotic patients. However, in many cases of HCC, AFP values are often normal at the time of diagnosis. In the literature several authors have been investigated the association between T2DM and HCC. Interestingly, only one study was evaluated the diagnostic role of AFP levels in HCC patients with T2DM (21). To our knowledge our study is the second study about the diagnostic value of serum AFP levels in HCC patients with T2DM.

Recently Yang et al. showed that serum AFP levels in HCC patients with T2DM were significantly lower than non-diabetic HCC population. They also speculated that low AFP levels in diabetics might delay HCC diagnosis and leading to higher degree of malignant HCC. Similar to their study, we found that serum AFP levels were significantly higher in the non-diabetic HCC group patients than in the T2DM-HCC group. However they also found that serum AFP levels in T2DM group were significantly lower than in healthy control group (21). In our study, serum AFP levels in T2DM-HCC group had higher than in control grouped. The differences between sample size, statistical methods and study designs can be considered as the reasons of the different results between two studies.

The exact pathophysiological mechanism of low levels of AFP in HCC patients with T2DM is unclear. Turgutalap et al. have suggested that increased urinary protein loss due to diabetic nephropathy may lead to low serum AFP levels in diabetic HCC patients (22). Although low AFP levels

seen in T2DM patients may be explained by this theory, the issue is still unclear as there is insufficient evidence in the literature. Due to the retrospective design of our study, proteinuria levels in diabetic HCC patients could not be obtained retrospectively and therefore no statistical evaluation could be made on this subject.

Obesity has been identified as an independent risk factor for HCC development in many studies (23,24). In our study AFP levels were also significantly negatively correlated with increasing age and serum fasting glucose levels in HCC cases. HOMA-IR, BMI, HgA1c and serum fasting insulin levels were not correlated with AFP levels in diabetic or non-diabetic HCC patients. In our study, the degree of BMI was significantly higher both T2DM-HCC group and NDM-HCC group patients compared to control group. However we found no significant relation between AFP levels and BMI in our study.

Insulin resistance (IR) is a critical pathway for hepatocarcinogenesis. Hyperinsulinemia may induce the synthesis of IGF -1 and it may also down regulate the level of IGF binding protein 1 (IGF BP-1). Down regulation of IGF BP-1 also increases total IGF-1 levels in plasma. Increased IGF-1 levels may play a critical role on HCC development. Therefore, hyperinsulinemia and insulin resistance may play an indirect role in hepatocarcinogenesis. Many studies have found a significant relationship between IR and HCC development, but the number of studies investigating the relationship between insulin resistance and AFP is very limited. IR and AFP levels were not correlated in our study.

Elsayed et al. have evaluated 100 HCV related HCC patients and they found positive correlation between HOMA IR, BMI, AFP, insulin, FPG, LDL Cholesterol and Child-Pugh score (25). AFP levels were negatively correlated with FPG and increasing age in our study. AFP levels were not positive correlated with any metabolic marker in our study. The HCC group in our study did not consist of HCV-induced cases. There were almost all etiologic factors in the HCC group, including HCV in this study. Chronic HCV infection is associated with increased insulin resistance and diabetes mellitus through different metabolic pathways (26-28). The fact that this study was performed only in patients with HCV may be the main reason for the positive correlation between AFP and insulin resistance and other metabolic markers. This was considered to be the main reason for the different results between the two studies.

This study had some limitations. First, our study was a retrospective single center study and the number of diabetic and non-diabetic HCC groups was not equal. Since our study was retrospective, drug and treatment history of diabetic patients could not be included in study design and this prevented the investigation of the possible role of antidiabetic drugs in HCC development. In addition, since our study had a retrospective design, it was not possible to determine whether low AFP levels in diabetic HCC patients were due to protein loss through urine due to diabetic nephropathy.

Diabetes mellitus is closely associated with the increased the risk of multiple types of cancer including HCC. HCC is a highly aggressive tumour with poor prognosis and this behavior pattern increases the importance of early diagnosis. AFP is still using as main biomarker for the screening and diagnosis of HCC globally. Lower levels of AFP may lead to a delay in the diagnosis of HCC in cases with cirrhosis and diabetes, which may lead to a significant reduction in treatment success.

CONCLUSION

The results of our study showed that AFP is not a reliable marker in the diagnosis and screening of HCC in diabetic patients with cirrhosis. In the future, researchers may focus on more reliable new biomarkers for HCC screening, especially in diabetic patients with cirrhosis.

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