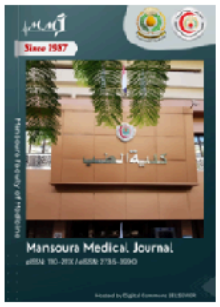
MANSOURA MEDICAL JOURNAL

ISSN - Print: 1110-211X - Online: 2735-3990

journal homepage: mmj.mans.edu.eg



Volume 34 | Issue 1 Article 12

THE DIAGNOSTIC VALUE OF CIRCULATING TUMOR NECROSIS FACTOR ALPHA (TNFa) VERSUS ALPHA FETOPROTEIN (AFP) IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

Hosam Ghanem

Clinical Pathology Departments Faculty of Medicine, Mansoura University.

Salah Agha

Clinical Pathology Departments Faculty of Medicine, Mansoura University.

Mostafa Abu Zeid

General Surgery Departments Faculty of Medicine, Mansoura University.

Iman Fawzy

Clinical Pathology Departments Faculty of Medicine, Mansoura University.

THE DIAGNOSTIC VALUE OF CIRCULATING TUMOR NECROSIS FACTOR ALPHA (TNFα) VERSUS ALPHA FETOPROTEIN (AFP) IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

By

Hosam Ghanem*, Salah Agha*, Mostafa Abu Zeid, & Iman Fawzy***

From

*Clinical Pathology*and General Surgery Departments**, Faculty of Medicine, Mansoura University.*

ABSTRACT

The aim of the present work is to assess the level of serum tumor necrosis factor alpha (TNFα) in patients with hepatocellular carcinoma (HCC) and compare the sensitivity of this marker with conventional used marker, alpha-fetoprotein (AFP). This study was done on sixty five patients attending the Gastroenterology Surgical Center, Mansoura University, 25 patients with HCC, 20 patients with liver cirrhosis, 20 patients with chronic hepatitis in addition to 15 apparently healthy controls (both patients and controls were age and sex matched).

Serum AFP was estimated by an immunoenzymatic assay. Serum TNFα was assayed by a solid phase enzyme amplified sensitivity immunoassay.

Results show that serum AFP and TNFα levels were significantly elevated in hepatocelluar carcinoma, cirrhosis and chronic hepatitis groups in comparison to control group. AFP and TNFα showed no significant difference in cirrhosis group in comparison to chronic hepatitis group. No significant correlation was found between HCC stages and both AFP and TNFα. TNFα had a higher sensitivity (100%) than AFP (80%) and lower specificity (40% for TNFα and 64% for AFP) in patients with HCC.

In conclusions, serum TNFα is nonspecific marker as it increases in different end stage of liver diseases. TNFα could be used in association with APF in diagnosis of HCC cases. TNFα has higher sensitivity than AFP, but lower specificity as it was elevated in benign inflammatory diseases. TNFα could be used as a marker for early detection of HCC by following up of its level in patients with cirrhosis and chronic hepatitis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the ten most common tumors in world, and the most common primary liver malignancy [1]. It is increasing in many countries [2]. Alpha feto-protein (AFP) is produced during fetal development by the liver, yolk sac and gastrointestinal tract[3]. It is the most specific biochemical test for the diagnosis of HCC [4]. Elevated levels of AFP in HCC is explained by increased synthesis of AFP by HCC cells which are analogous to fetal hepatocytes [5].

HCC is increasing in many countries [6]. The genetic basis of hepatocarcinogenesis is poorly understood [7]. Clinical diagnosis which based on modern imaging has improved greatly but still unsatisfactory in some cases. AFP is the most important tumor marker for the diagnosis of HCC. However, a considerable proportion of HCC does not produce AFP or it elevates its serum level minimally, making early diagnosis difficult with this marker alone [7].

Tumor necrosis factor-alpha (TNF α) is a cytokine which is involved in apoptotic cell death, metabolism, inflammation, thrombosis and fibrinolysis [8]. It is mainly produced by monocytes, macrophages and T cells [9].

Liver is an important site for TNFα synthesis and clearance [10]. TNFα is important for liver regeneration [11], proliferation and also several hepatotoxic effects [12].

TNFα is a member of the large family of cytokines [13]. It is implicated in a variety of pathological situations [14], such as inflammation [15], antitumor [16], antiviral effects [17] and immunity [18]. It is involved in a diversity of liver conditions [19] as

viral hepatitis [20], cirrhosis [21] and HCC [11].

The aim of the present work is to evaluate the reliability of the diagnostic value of serum TNFα in HCC. Also, the serum TNFα results will be compared with the traditional and recommended tumor marker of HCC, alpha-fetoprotein.

SUBJECTS AND METHODS

Subjects

This study was done on 25 patients with HCC, aged 52 ± 10 years, 20 patients with liver cirrhosis, aged 46 ± 5 years, 20 patients with chronic hepatitis (CH), aged 43 ± 7.7 years, in addition to 15 healthy controls aged 45 ± 3.3 years. The patients were attending to the Gastroenterology Surgical Center, Mansoura University. Those who were negative for HBs antigen (HBsAg) and Anti-HCV antibodies were excluded from this study. Those with negative HBsAg and positive anti-HCV antibodies were 65 cases (25 HCC, 20 cirrhosis and 20 CH patients). They were 37 males and 28 females, while healthy matched subjects (8 males and 7 females).

The 65 patients with negative HBsAg and positive anti-HCV antibodies as well as the control group were subjected to thorough clinical examination. Abdominal ultrasound and computed tomography were done to all patients and controls to assess the liver. Patients with HCC were assessed by TNM staging [22].

Methods

Fasting blood samples were obtained from patients and controls. The samples were divided into three tubes:

* 3 ml blood in a plain tube and unhaemolyzed sera were used for the determination of liver function tests, hepatitis markers and AFP.
* 1.8 ml blood was added to 0.2 ml of trisodium citrate (9 parts venous blood + 1 part citrate). The samples were centrifuged and the plasma was separated and used for determination of prothrombin time and concentration.
* 3 ml blood in plain tube and sera were stored at -70 ^{o}C until used for measurement of TNFα. Very strict precaution were taken during sampling, to avoid impurities contained in sampling materials that would

stimulate TNFα production by blood cells and thus falsely increased TNFα values. Therefore; the utilized serum was collected on sterile, clean, dry tubes, rapidly separated after coagulation and also haemolysis was avoided.

Serum albumin, bilirubin and aminotransferases (ALT & AST) were determined using bioMerieux Kits, France). Prothrombin time was estimated using Diamed Kits.

HBsAg was detected by a non-competitive enzyme immunoassay (ABBOTT laboratories). Anti-HCV was detected by qualitative enzyme immunoassay (ABBOTT HCV EIA, 3rd generation test).

AFP was assayed by one step immunoenzymatic assay based on formation of a sandwich between the analyte to be detected and two specific monoclonal antibodies directed to different epitopes on the AFP molecule. The captured antibody is conjugated to biotin, while the second antibody, used to reveal the reaction, is labeled with horse radish peroxidase (HRP). The immunological reaction between the analyte and the two monoclonal antibodies occurs in homogenous phases in the presence of streptavedin immbolized on the solid phase, which allows bound separation (Sorin Biomedica).

TNFα was assayed by ELISA which is a solid phase enzyme amplified sensitivity immunoassay. It is based on the oligoclonal system in which several monoclonal antibodies directed against distinct epitopes of TNFα (Medgenix Diagnostics, Brussels, Belgium).

STATISTICAL ANALYSIS

Statistical analysis was carried out with SPSS (statistical package for social science) program version 10. for windows. The qualitative data were presented in the form of number and percentage) The quantitative data were presented in the form of mean and standard deviation.Student (t) test was used to compare between quantitative data of two groups. The significance value was of $p < 0.05$. Correlation was calculated with Pearson's method.

Using these comparisons, sensitiv-

ity (true-positive/[true positive + false negative]), specificity (true-negative/[true-negative + false positive]) were calculated for each test. The accuracy was calculated as true positive + true negative divided by the total number of patients.

Positive predicted value was calculated as true positive divided true positive + false negative while negative predicted value was calculated as true negative divided by false positive + true negative [23].

RESULTS

Serum total bilirubin, ALT, AST were significantly increased while prothrombin concentration and serum albumin were significantly decreased in all studied three patient groups in comparison to control group (Table 1). There were very highly significant ($p<0.001$) increases in AFP levels in all studied groups compared to control group. Also, there were very highly significant ($p < 0.001$) increases in AFP levels in HCC versus cirrhosis and CH groups. There were no significant changes in AFP levels between cirrhosis and CH groups (Table 2). There were very highly significant ($p<0.001$) increases in TNFα levels in all studied groups compared to control group. Also, there were very highly significant ($p < 0.001$) increases in TNFα levels in HCC versus cirrhosis and CH groups. There were no significant differences in TNFα between cirrhosis and CH groups (Table 3).

Serum AFP showed significant positive correlation with total serum bilirubin, ALT, AST and TNFα and negative significant correlation with albumin and prothrombin concentration in HCC. While, in cirrhosis serum AFP showed significant positive correlation with total bilirubin. On the other hand, there was negative significant correlation between AFP levels and prothrombin concentration (Table 4).

Serum TNFα showed significant positive correlation with total serum bilirubin, ALT and AST and negative significant correlation with albumin and prothrombin concentration in HCC (Table 5). Serum TNFα showed significant positive correlation with total serum bilirubin and negative significant correlation with albumin and prothrombin concentration. While there were no significant correlation be-

tween TNFα and AST, ALT and AFP in both cirrhosis and CH.

AFP showed high significant difference ($p<0.01$) between early and advanced stages, on the other hand, there were no significant differences in TNFα levels between early and advanced stages (table 6).

TNFα is more sensitive while AFP is more specific . TNFα is less accurate. AFP has higher positive predictive value, while TNFα has 100% negative predictive value (Table 7).

Table (1) : Liver function test results in HCC, cirrhosis, CH groups versus control group.

Liver function tests		HCC (n=25)	Cirrhosis (n=20)	CH (n=20)	Control (n=15)
T. Bilirubin (mg/dl)	Mean ± SD P	3.6 ± 0.8 < 0.001	2.8 ± 0.9 < 0.001	3.4 ± 1.1 < 0.001	0.6 ± 0.1
ALT (IU/ml)	Mean ± SD P	72.1 ± 20.7 < 0.001	63.3 ± 17.6 < 0.001	76.9 ± 19.1 < 0.001	25.1 ± 1.3
AST (IU/ml)	Mean ± SD P	74.4 ± 22.6 < 0.001	65.1 ± 18.5 < 0.001	79.5 ± 18.9 < 0.001	25.4 ± 1.1
Albumin (g/dl)	Mean ± SD P	2.1 ± 0.7 < 0.001	2.5 ± 0.7 < 0.001	3.0 ± 1.1 < 0.001	4.9 ± 0.2
Prothrombin Concentration%	Mean ± SD P	45.3 ± 11.7 < 0.001	50.7 ± 9.9 < 0.001	63.2 ± 16.7 < 0.001	87.9 ± 10.3

Table (2): AFP levels in HCC, cirrhosis, CH groups versus control group.

AFP (ng/ml)	HCC (n=25)	Cirrhosis (n=20)	CH (n=20)	Control (n=15)
Mean ± SD	442.3 ± 28.9	18.5 ± 7.8	15.2 ± 6.2	7.8 ± 1.1
P P1 P2	< 0.001	< 0.001 < 0.001	< 0.001 < 0.001 >0.05	

AFP: alpha fetoprotein, normal values up to 10 ng/ml.
P: Comparison between the studied groups and control group.
P1: Comparison between HCC versus cirrhosis and CH.
P2: Comparison between cirrhosis versus CH.

Table (3) TNFα levels in HCC, cirrhosis, CH groups versus control group.

TNFα (pg/ml)	HCC (n=25)	Cirrhosis (n=20)	CH (n=20)	Control (n=15)
Mean ± SD	90.6 ± 12.6	64.9 ± 22.1	66.8 ± 21.8	9.1 ± 2.1
P P1 P2	< 0.001	< 0.001 < 0.001	< 0.001 < 0.001 >0.05	

TNFα: Tumor necrosis factor alpha.
P: Comparison between the studied groups and control group.
P1: Comparison between HCC versus cirrhosis and CH.
P2: Comparison between cirrhosis versus CH.

Table (4) : Correlation coefficients between AFP levels and studied parameters in different studied groups.

	HCC (n=25)		Cirrhosis (n=20)		CH (n=20)		Control (n=15)	
	R	p	r	P	R	P	r	p
T. Bilirubin (mg/dl)	0.45	<0.05	0.50	<0.05	0.41	>0.05	-0.14	>0.05
ALT (IU/ml)	0.40	<0.05	-0.39	>0.05	-0.16	>0.05	0.09	>0.05
AST (IU/ml)	0.41	<0.05	-0.38	>0.05	-0.12	>0.05	-0.27	>0.05
Albumin (g/dl)	-0.49	<0.05	-0.27	>0.05	-0.36	>0.05	-0.36	>0.05
Prothro-mbin Concentr-ation%	-0.44	<0.05	-0.09	>0.05	-0.47	<0.05	-0.27	>0.05
TNFα	0.49	<0.05	0.24	>0.05	0.39	>0.05	-0.26	>0.05

Table (5) Correlation coefficients between TNFα levels and studied parameters in different studied groups.

	HCC (n=25)		Cirrhosis (n=20)		CH (n=20)		Control (n=15)	
	R	p	r	P	R	P	r	p
T. Bilirubin (mg/dl)	0.75	<0.001	0.65	<0.01	0.49	<0.05	-0.34	>0.05
ALT (IU/ml)	0.71	<0.001	0.03	>0.05	-0.23	>0.05	0.64	>0.05
AST (IU/ml)	0.69	<0.001	0.01	>0.05	-0.18	>0.05	-0.23	>0.05
Albumin (g/dl)	-0.57	<0.01	-0.65	<0.01	-0.54	<0.05	0.49	>0.05
Prothromb-in Concentra-tion%	-0.44	<0.05	-0.46	<0.05	-0.53	<0.05	0.41	>0.05

Table (6) Comparison of values of AFP and TNFα in early (stage I and II) and advanced (stage III & IV) stages of HCC.

	AFP (ng/ml)	TNFα (pg/ml)
	Mean ± SD	Mean ± SD
Early HCC (I&II)	198.4 ± 28.9	86.2 ± 4.3
Advanced HCC (III & IV)	503.3 ± 146.4	91.7 ± 13.8
P	< 0.01	> 0.05

Table (7) Comparison between AFP and TNF α levels regarding sensitivity, specificity, accuracy and predictive values in patients with hepatocellular carcinoma.

	AFP	TNF α
Sensitivity	80%	100%
Specificity	64%	40%
Accuracy	65%	58.8%
Positive predictive values.	52.6%	43.1%
Negative predictive values.	86.5%	100%

DISCUSSION

Brechot [24] suggested that the implication of HCV and HBV in liver carcinogenesis expands far beyond that predicted by classic serologic assays. A number of epidemiologic studies had shown a high prevalence of anti-HBs and anti-HBc antibodies in HBsAg negative subjects (around 40% to 50% in france), indicating exposure to the virus [25]. Hepatocarcinogenesis appears to be a multifactorial process [26], including in addition to HBV and HCV, other factors such as alcohol, chemical carcinogens and hormonal factors [24]. Bioulac-Sage et al., [27] reported that in 60% of HCC cases, no etiologic factors could be identified. This controversy in results may be due to the low number of our cases, the difference in the environment and the high incidence of HCC and chronic liver diseases in Egypt. In the current study, 25 out of 40 (62.5%) of HCC cases were HBs Ag negative and anti HCV positive.

In the current study, there were highly significant ($p < 0.001$) increases in total serum bilirubin, ALT and AST in all studied groups compared to control group, while there was a highly significant ($p < 0.001$) decreases in albumin and prothrombin concentration in all studied groups compared to control group (Table 1).

Increases in serum hepatocellular enzyme activities (ALT & AST) imply an ongoing ischemic or hepatic process. Prolongation of prothrombin time, which is not correctable with vitamin K is a relatively accurate indicator of poor hepatic reserve. Decrease in serum albumin level may reflect impaired synthesis, nutritional deficiencies, or septic stress [2].

The present study revealed that the mean value of serum AFP was (7.8 ± 1.1 ng/ml) in the normal control group (Table 2). Marrero et al., [28] found that the mean AFP level in control group was 4 ± 2. Butch et al.,[4] reported that it is less than 20 ng/ml.

In this study, the mean value of serum AFP in patients suffering from HCC was (442.3 ± 208.9). There was very high significant increase in AFP levels in HCC versus control group. (Table 2).

Marrero et al., [28] found that the mean AFP levels was (1400 ± 361 versus 4 ± 2 in HCC and control, respectively and $p < 0.001$). Dan et al., (29) found that 40% of their study patients (292 HCC patients) had AFP more than 100 ng/ml. This variation may be due to wide range of AFP levels in HCC and the concomitant presence of other causes which causes increase in AFP levels as benign liver diseases.

AFP levels in our HCC cases were very highly significantly increased when compared to the cirrhotic and chronic hepatitic patients (442.5 ± 28.9, 18.5 ± 7.8, and 15.5 ± 6.2 respectively, $p < 0.001$). Marrero et al., (28) reported that AFP levels were (40 ± 4, 10 ± 4 in cirrhosis and CH, respectively and very high significant differences when compared to HCC group 1400 ± 361, $p< 0.001$).

In this study, cirrhotic patients, the mean value of serum AFP was (18.5 ± 7.8), while in CH group, the mean value of serum AFP was (15.2 ± 6.2). Lamerz, [30] reported that the serum AFP can be minimally elevated in benign liver diseases. Okuda [7] reported that its levels are less than 20 ng/ml in benign liver diseases. Ankoma-Sey et al., [31] reported that 50% of chronic hepatitis C patients in their study had abnormal AFP levels. AFP levels in cirrhosis and CH were highly significantly increased ($p < 0.001$) when compared to the corresponding mean values in the control group (Table 2).

Marrero et al., [28] reported 40 ± 4. 10 ± 2 in cirrhosis, CH versus control, respectively, $p < 0.001$) for both. No significant differences were found between AFP levels in cirrhosis and CH groups ($P > 0.05$). These findings are in agreement with those obtained by Yao et al., [32]. However, Marrero et al., [28] reported significant differences between both groups. This may be attributed to different number of cases, different underlying causes of cirrhosis and CH and variations in the strains of HCV in different localities.

Ankoma-Sey et al., [31] reported that AFP is correlated with AST levels in chronic HCV infection, however, it is not significantly correlated with the extent of necroinflammatory activity or hepatic fibrosis. This difference in our

study may be due to different number of cases or different prevalence of HCV infection.A high positive significant difference ($p< 0.001$) was found between AFP levels in early versus advanced stages in HCC patients (Table 6). Kelsten et al [33] and Yao et al., [32] reported that AFP level correlates with the size of the tumor.

The sensitivity of AFP in HCC cases was 80%, the specificity was 64% and the accuracy was 65% (cut off value = 10 ng/ml). The positive predictive value was 52.6% (table 7). Yao et al., [32] reported that AFP sensitivity was 62.6%, the specificity was 88.8% , the accuracy was 77.3% and the positive predictive value 81.4%. While EL-Shaer et al., [34] found that the sensitivity was 78.9% while the specificity was 79.9%. Johnson [35] found that AFP had a sensitivity of 50% and a specificity of 90% when using 500 ng/ml as a cut-off point.

In the present study, the mean value of serum TNFα was (9.1 ± 2.1 pg/ml) in the control group (table 3). Haung et al., (36) reported that the mean serum TNFα was (10.4 ± 2.4) while Yuan et al., (37) found that the mean value of serum TNFα was (4.3 ± 2.9) in the control group. Kallinowski et al., [38] reported that control liver tissues showed no or very small amount of TNFα on hepatocytes, bile duct epithelium, sinusoidal epithelial cells and lymphocytes. In this work, serum TNFα levels in HCC cases were significantly increased compared to their corresponding values in the normal control group (90.6 ± 12.6 versus 9.1 ± 2.1 respectively, $p < 0.001$) (table 3). This finding agrees with that obtained by Webber et al., [39].

In cirrhotic group of this study, the mean value of serum TNFα was (64.9 ± 22.1 pg/ml). Tilg et al., [40] found that TNFα was (21.5 ± 4.0) in cirrhosis. This difference may be due to different underlying etiology and pathology of cirrhosis. In our cirrhotic group, TNFα was very highly significantly increased when compared to their corresponding mean value in the normal control group ($P< 0.001$) (table 3). Similar results ($P< 0.001$) were obtained by Mammaev et al., [41].

In chronic hepatitis patients of the present study, the mean value of ser-

um TNFα was (66.8 ± 21.8). Kallinowski et al., [38] founded that the mean TNFα in CH was (83.8 ± 19.7), while Nelson et al., [42] founded that the mean TNFα in CH was (9.62 ± 9.01). TNFα varies with histological severity of chronic HCV infection [43]. TNFα levels in CH patients of the present work significantly increased when compared to their corresponding mean value in the control group ($p< 0.001$) (table 3). Toyoda et al., (44) reported a mean level of TNFα of (82.7 ± 70.8 versus 28.2 ± 24.6 for CH versus control, $p < 0.01$), Kallinowski et al., [38] showed (83.8 ± 91.7 versus 18.8 ± 8.4 for CH, control, respectively; $p < 0.001$). Serum TNFα in HCC group in the current study, was also significantly increased when compared to the cirrhosis, $p < 0.001$ for both) (table 3). Haung et al., [36] reported results of $p < 0.05$ when compared between HCC versus cirrhosis and CH. This suggested that TNFα increased in close correlation with liver disease progression [40, 45]. On the other hand, no significant differences were found between TNFα levels in cirrhosis and CH groups of this study ($p > 0.05$) (table 3) and figure 8. Yuan et al., [37] obtained similar results. On the other hand, Haung et al., [36] found that TNFα levels were increased significantly ($P < 0.05$) in CH patients when developed cirrhosis in the follow up of them. This may be referred to that TNFα increases with liver disease progression, and this difference from our results may be due difference in the underlying etiology and pathology of our cirrhosis and CH cases.

In HCC cases, there were significant correlations ($p < 0.001$) between TNFα versus total serum bilirubin, ALT and AST. There was a negative high significant correlation ($p< 0.01$) between TNFα and albumin. Also, there was a negative significant correlation ($p < 0.05$) between TNFα versus prothrombin concentration. In cirrhosis group, there was a high positive significant correlation between TNFα and total serum bilirubin. Also, there was a high negative significant correlation ($p < 0.01$) between TNFα and total serum albumin. There was negative significant correlation between TNFα prothrombin concentration. No significant correlations ($p> 0.05$) were found between TNFα versus ALT and AST. In CH group of this

study, there was a positive significant correlations ($p < 0.05$) between TNFα and total serum bilirubin. Also there were negative significant correlations ($p < 0.05$) between TNFα versus albumin and prothrombin concentration. No significant correlations were found between TNFα versus ALT and AST. In the control group, TNFα showed positive significant correlations with ALT, no significant correlations were found between TNFα versus all other parameters (table 5). Hanug et al., [36] found that TNFα was correlated better with indices of hepatic dysfunction than with parameters of hepatic inflammation. However, Mammmaey et al., [41] found that TNFα was positively correlated with serum ALT and Nelson et al., [42] found that TNFα is correlated with markers of hepatocellular injury, including ALT. Yuan et al., [37] found that there was significant correlation between TNFα levels and bilirubin in chronic hepatitis patients. These differences may be attributed to different number of cases and underlying pathology. In addition, it has been reported that not only host factors, but also viral factors may affect the biochemical and histological changes of the liver [46].

No significant correlation ($p < 0.05$) was found between TNFα versus AFP except in HCC ($p < 0.05$). (table 4,5). Yuan et al., [37] and Serrenkova et al., [47] reported that no significant correlation ($P > 0.05$) was found between TNFα versus AFP. There were no significant differences in TNFα levels between early and advanced stages of HCC (table 6).

The sensitivity of TNFα in HCC cases was 100%, the specificity was 40% and the accuracy was 58.8% (cut off value = 10 pg/ml). The positive predictive value was 43.1% and the negative predictive value was 100%.

Although AFP is more specific than TNFα in HCC diagnosis. TNFα is more sensitive. TNFα is useful not only for AFP -positive HCC diagnosis, but also for AFP-negative HCC. The data indicate the complementary diagnostic value between AFP and TNFα. The simultaneous assay of both can increase the diagnostic accuracy in HCC because serum

TNFα is helpful in the early diagnosis of small HCC and in the diagnosis of AFP-negative HCC. Periodic follow up of TNFα concentration would be useful in patients with chronic liver diseases.

Factors as nutritional abnormalities such as cachexia and alteration in carbohydrate, protein, lipid and trace mineral metabolism are well known in chronic liver diseases and these alterations persist and progress in most patients until death and have been associated with increase in TNFα levels (40).

Also, the interpretation of serum TNFα should consider the co-existence of other conditions which may lead to its upregulation such as bacterial infection (48), parasitic infestation (49) and fever (50).

In conclusion, TNFα is non-specific marker, and could be used in association with AFP in diagnosis of HCC cases. TNFα has a higher sensitivity than AFP, but lower specificity as it was elevated in benign and inflammatory diseases. It is recommended that periodic follow up of TNFα concentrations would be useful in patients with chronic liver diseases for early detection of HCC.

REFERENCES

1- **Badvie S (2000) :** Hepatocellular carcinoma. Postgrad. Med. J., 76: 4-11.

2- **Jenkins RL and Lally AL (2002) :** Hepatocellular carcinoma In: Bland KI and Sarr MG (eds). The practice of general surgery. WB Saunders company Philadelphia London New York. P 633.

3- **Takats PG, Jones SR, Penn R and Cullen MH (1996) :** Alpha feto – protein heterogeneity: what is its value in managing patents with germ cell tumors? Clinical Oncology; 8: 323- 6.

4-**Butch AW, Massoll NA, and Alex AP (2002) :** Tumor markers. In: Bishop ML, Dubin-Engelkirk JL and Fody EP (eds). Clinical chemistry: principles, procedures and correlations. 5th ed. Lippincott Wollams and Winlkins. P:526.

5-Di Bisceglie AM, Carithers RL and Gores GJ (1998) : Hepatocellular carcinoma. Hepatology; 28 (4): 1161-1165.

6-Deuffic S, Poynard T, Buffat L and Valleron AJ (1998) : Trends in primary liver cancer. Lancet; 350:214-217.

7- Okuda K (2000) : Hepatocellular carcinoma. J Hepatol; 32 (suppl): 225-37.

8- Van Deventer SJH (1997) : Tumor necrosis factor alpha and Crohn's disease. Gut; 40:433-8.

9- Manogue KR, Van Deventer SJH and Cerami A (19991) : Tumor necrosis factor alpha or cachectin. In: Thomson AW, (ed). Cytokine handbook. London: Academic Press. P: 241-56.

10- Le Moine O, Marchant A, De groote D, Azar C, Goldman M and Deviere J (1995) : Role of defective monocyte release in tumor necrosis factor alpha over-production in alcoholic cirrhosis. Hepatology; 221: 1436-9.

11- Fausto N (2000) : Liver regeneration. J Hepatol; 32 (suppl. 1): 19-31.

12- Rosado JA, Rosenzweig I, Harding S and Sage So (2001) : Tumor necrosis factor alpha inhibits sore-mediated Ca2+ entry in the human hepatocellular carcinoma cell line Hep G2. Am J Cell Physiol; 280 (6):C 1636-44.

13-Beutler B (1994) : An evolutionary approach to the TNFα receptor/ligand family. Ann NY Acad Sci; 730: 118-33.

14- Larrea E, Garcia N, Qian C, Civeira MP, and Prieto J (1996) : Tumor necrosis factor alpha gene expression and the response to interferon in chronic hepatitis C: Hepatology; 23 (2): 210-7.

15- Byrne A and Reen DJ (2002) : Lipopolysaccharide induces rapid production of interleukin -10 by monocytes in the

presence of apoptotic neutrophils. J Immunol; 168 (4): 1968- 77.

16- **Vikhanskaya F, Falugi C, valente P (2002) :** Human papillomavirus type 16 E6-enhanced susceptibility to apoptosis induced by tumor necrosis factor in A2780 human ovarian cancer cell line. Int J Cancer ; 20; 97 (6): 732-9 .

17- **Yared G, Hussain KB, Nathani MG, Moshier JA, Dosescu J, Mutchnick MG, and Naylor PH (1998) :** Cytokine-mediated apoptosis and inhibition of virus production and anchorage independent growth of viral transfected hepatoblastoma cells. Cytokine; 10 (8): 586-95.

18- **Locksley RM, Killeen N, and Lenardo MJ (2001) :** The tumor necrosis factor alpha and tumor necrosis factor R super families: Integrating mammalian biology. Cell; 104: 487-501.

19-**Mizuhara H, O, Neille E, Seiki N, Ogawa T, Kusonoki C, Otsuka K and Satoh S (1994) :** T cell activation – associated hepatic injury: mediation by tumor necrosis factor alpha and protection by interleukin-6. J Exp Med; 179: 1529- 37.

20-**Szabo G, Catalano D, Bellerose G, and Mandrekar P (2001) :** Interferon alpha and alcohol augment nuclear regulator factor kB activation in HepG2 cells, and interferon alpha increases pro-inflammmatory cytokine production. Alcohol Clin Exp Res; 25(8): 1188- 97.

21- **Such J, Hillebrand DJ, Guarner C, Berk L, Zapater P, westengard J, Peralta C, Soriano G, Pappas J, and Runyon BA (2001) :** Tumor necrosis factor alpha, interleukin-6 and nitric oxide in sterile ascetic fluid and serum from patients with cirrhosis who subsequently develop ascetic fluid infection. Dig Dis Sci; 46 (11):2360-6.

22-**De Vita JR, Hellman S and Ro-**

senberg S (1997) : Cancers of the gastrointestinal tract. Cancer; 32 (5): 1093.

23- **Griner PF, Mayewski JP, Mushlin AI (1981) :** Selection and interpretation of diagnostic tests and procedures principles and applications. Ann Intern Med; 94:557.

24- **Brechot C (2001) :** Molecular basis of hepatitis B and hepatitis C related chronic liver diseases. In: Arias IM, Boyer JL, Chrisari FV, Fausto N, Schachter D, and Shafritz DA (eds). The liver: Biology and pathobiology. 4th edition. Lippincott Williams and Wilkins. Philadelphia. P 802.

25- **Paterlini P, Poussin K, and Kew M (1995) :** Selective accumulation of the X transcript of hepatitis B virus in patients negative for HBsAg with hepatocellular carcinoma. Hepatology; 21: 313-21.

26- **Park CC, Bissell MJ and Barcellos-Hoff MH (2000) :** The influence of microenvironment on the malignant phenotype. Mol Med Today; 6:324-9.

27- **Bioulac-Sage P, Le Bail B and Winnock M (2000) :** Occurrence of hepatocellular carcinoma in nonfibrotic livers. Hepatology; 32 (6): 1411-2.

28-**Marrero JA, Gosh M, Nour K, Su JL, Fontana RJ, Cojecvaram HS and Lock AS (2001) :** Des gamma carboxy prothrombin is a more sensitive and reliable marker for hepatocellular carcinoma than alpha fetoprotein in American patients. Hepatology; 34 (4): 14- 24.

29- **Dan Y, Shiana S, Teratani T, Obi S, Sato S, Hammamura K, Koike Y, Shiratori Y, and Omata M (2000) :** Tumor markers represent clinical profiles of hepatocellular carcinoma. Gastroenterology; 118(4): 114-9.

30- **Lamerz R (1997) :** Alpha-fetoprotein isoforms and their clinical significance.

Anticancer Res; 17(4B): 2927-30.

31- **Ankoma-Sey V, McGee A, Chalal S, Clarke J, Clark C, Chung A and Sellin J (2000) :** Do levels of alpha-fetoprotein predict inflammatory activity or fibrosis in chronic hepatitis C infected patient? Gastroenterology; 188 (4): 1190 – 7.

32-Yao D, Huang Z, Chen S, Huang J, Lu J, Xiao M, and Meng X **(1998) :** Diagnosis of hepatocellular carcinoma by quantitative detection of hepatoma-specific bands of serum GGT. Am J Clin Pathol; 110: 743-9.

33- **Kelsten ML, Chan DW, Bruzek DJ and Rock RC (1988) :** Monitoring hepatocellular carcinoma by using a monoclonal immunoenzymometric assay for alpha-fetoprotein. Clin Chem; 34:76-81.

34- **EL-Shaer OS, Zidan M, Atta H and Tolba F (1998) :** Significance of alpha fucosidase and N-acetyl neurminic acid as markers for hepatocellular carcinoma. J Egypt Med Ass; 2: 84-8.

35- **Johnson PJ (2001) :** The role of serum alpha fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liver Dis; 5(1): 145 -59

36- **Haung YS, Hwang SJ, Chan CY, Wu JC, Chao Y, Chang FY and Lee SD (1999) :** Serum levels of cytokines in hepatitis C- related liver diseases: a longitudinal study. Zhonghua Yi Xue Za Zhi (Taipei; 62(6): 327-33.

37- **Yuan AL, Luo YH, and Liu SD (1994) :** Tumor necrosis factor alpha levels in patients with chronic liver diseases and its relationship to pathogenesis. Zhonghua Nei Ke Za Zhi, 33 (10): 672-4.

38- **Kallinowski B, Haseroth K, Marinos G, Hanck C, Stremmel W, Theilmann L, Singer MV and Rossol S (1998) :** Induction of tumor

necrosis factor R type p55 in patients with chronic hepatitis C virus infection. Clin Exp Immunol; 111 (2): 269-77.

39-Webber EM, Bruix J, Pierce RH and fausto N (1998) : Tumor necrosis factor primes hepatocytes for DNA replication in the rat. Hepatology; 28: 1226-34.

40- Tilg H, Wilmer A, Vogel W, Herold M, Nolchen B, Judmaier G and Huber C (1992) : Serum levels of cytokines in chronic liver diseases. Gastroenterology; 103: 264-74.

41- Mammaev SN, Lukina EA, Shul, pekova IUO, Levina AA and Ivashkin VT (2001) : Cytokine regulation of liver inflammation and fibrosis during chronic hepatic diseases. Klin Lab Diagn: (12): 37-40.

42- Nelson DR, Lim HL, Marousis CG, Fang JW, Davis GL, Shen L, Urdea MS, Kolberg JA, and Lau JY (1997) : Activation of tumor necrosis factor-alpha system in chronic hepatitis C virus infection. Dig Dis Sci; 42(12): 2487-94.

43- Yee L, Tang J, Herrera J, Kaslow RA and van Leeuwen DJ (2000) : Tumor necrosis factor alpha gene polymorphisms in patients with cirrhosis from chronic hepatitis C virus infection. Genes Immuno; 1 (6): 386-90.

44-Toyoda M, Kakizaki S, Horiguchi N, Sato K, Takayama H, Takagi H, Nagamine T and Mori M (2000) : Role of serum soluble Fas/souble Fas ligand and tumor necrosis factor alpha on response to interferon alpha therapy in chronic hepatitis C. Liver; 20 (4):305-11.

45- Zhang DF, Ren H, Jia XP and Zhou YS (1993) : Serum tumor necrosis factor in the pathogenesis of clinical hepatic failure of hepatitis C virus and/or hepatitis B virus infection. Clin Med J (Engl); 106 (5): 335-8.

46- **Kuboki M, Shinzawa H, Shao L, Ishibashi M, Yoshii E, Suzuki K, Saito K, Saito T, Togashi H, Takahashi T, Yasumura S, and Fukao A (1999) :** A cohort study of hepatitis C virus infection in an hepatitis C virus epidemic area of Japan: age and sex-related seroprevalence of anti-hepatitis C virus antibody, frequency of viraemia, biochemical abnormality and histological changes. Liver; 19:88-96.

47- **Semenkova LN, Dudich EL, Dudich IV, Shingarova LN and Korobka VG (1997) :** Alpha fetoprotein as a tumor necrosis factor resistance, resistance factor the human hepatocellular cell line HepG2. Tumor Biol; 18(1):30-40.

48- **Gao JJ, Xue Q, and Morrison DC (2001) :** Bacterial DNA and lipopolysaccharide induce synergistic production of tumor necrosis factor alpha through a post-transcriptional mechanism. Hepatogastroenetrology; 166-6855-60.

49- **Klein J and Horejsi V (2000) :** Immuonology. 3rd ed. Blackwell Science. P 291. 303-14 and 541-73.

50- **Oppenheim JJ, Russetti FW, and Faltynek C (1994) :** Cytokines. In: Stiles DP, Terr AI, and Parslow TG (eds). Basic and clinical immunology. 8th ed. Appleton and Lange p:109.

القيمة التشخيصية للعامل المهلك للأورام- ألفا مقارنة بالفا-فيتو بروتين فى مصل الدم فى مرضى سرطان خلايا الكبد الأولى

د. حسام غانم * ، د. صلاح أغا *
د. مصطفى أبو زيد** ، د. إيمان فوزى*

من أقسام الباثولوجيا الإكلينيكية - والجراحة العامة - كلية طب المنصورة

يعد سرطان الكبد الأولى من أكثر السرطانات شيوعاً فى العالم وغالباً مايكتشف فى مراحله الأخيرة وهناك عوامل كثيرة تسبب هذا المرض مثل الالتهاب الكبدى الوبائى سى وبى بالإضافة إلى تليف الكبد وبعض الكيماويات.

لايزال التشخيص المعملى لسرطان الكبد الأولى غير مرضى، يستخدم ألفا- فيتو بروتين فى تشخيص هذا المرض ولكن بعض الحالات تعطى نتائج زائفة .

العامل المهلك للأورام ألفا له أنشطة كثيرة على كافة أجهزة الجسم مثل مقاومة الأورام والمناعة والالتهاب وكذلك مقاومة بعض الفيروسات والبكتريا والطفيليات. ويصدر أساساً من خلايا الماكروفاج.

تم هذا البحث على ثمانين شخصاً منهم ٢٠ حالة من الالتهاب الكبدى، ٢٠ حالة تليف الكبد و ٢٥ حالة من سرطان الكبد بالإضافة إلى ١٥ حالة ضابطة، وخضعوا جميعاً للتحاليل التالية :

١- وظائف الكبد (بيليروبين، انزيمات الكبد ا ل ت، ا س ت ، البومين).

٢- تركيز البروثرومبين.

٣- دلالات الفيروسات بى و سى .

٤- ألفا-فيتو بروتين .

٥- العامل المهلك للأورام ألفا فى مصل الدم.

ووجد أن العامل المهلك للأورام ألفا يزيد فى جميع الحالات المرضية بالمقارنة بالحالات الضابطة وأعلى منسوب له كان فى سرطان الكبد.

لايوجد اختلاف ذو دلالة إحصائية بالنسبة للعامل المهلك للأورام ألفا بين مرضى الالتهاب الكبدى ومرضى تليف الكبد.

العامل المهلك للأورام ألفا أكثر حساسية (١٠٠٪) عن ألفا-فيتو بروتين (٨٠٪) وأقل دقة (٤٠٪) للعامل المهلك للأورام ألفا و (٦٤٪) بالنسبة ألفا-فيتو بروتين.

وإستنتجنا أن العامل المهلك للأورام ألفا يمكن استخدامه فى التشخيص المبكر لسرطان الكبد عند متابعة حالات الالتهاب الكبدى وتليف الكبد. ويمكن استخدام العامل المهلك للأورام ألفا بالإضافة إلى ألفا-فيتو بروتين فى تشخيص سرطان خلايا الكبد الأولى.