

Investigate of correlation between CA125 levels in serum with infected (SBP) and non-infected ascitic fluid existence, in cirrhosis patients

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ABSTRACT

Introduction and Objectives: Cancer antigen 125 is a glycoprotein macromolecule which was first identified on ovarian carcinoma cells. It has been reported that serum CA 125 levels are highly elevated in chronic liver diseases, generally and in liver cirrhosis, specifically. So, it seems that there would be a relation between the levels of serum CA 125 and ascitic fluid in patients with liver cirrhosis. Accordingly, the purpose of this study was to find that reliable correlation exists between them.

Materials and Methods: The participants of the study included of liver cirrhotic patients with ascites. A total of 213 patients were studied in three groups: the control group consisted of healthy subjects not suffering from cirrhosis. And the two patient groups included patients without SBP ascites (group B) and patients with SBP ascites (group C). The blood samples were taken from both the patient groups and the control group in fasting conditions. In addition, ascitic fluid sample was obtained only from the patient groups. Then, on ascitic fluid samples of the patient groups the bacterial culture test and blood counts were performed. On blood samples after making serum, CA 125 was measured using ELISA method.

Results: The three groups were unified by age and gender. The mean of CA 125 in case group one and case group two was significantly different ($p = 0.0001$, for each group) from that of the control group.

Discussion: According to the results of the present study, it is concluded that CA 125, as a completely special marker, can be used for identifying the presence or absence of ascites in cirrhotic patients.

KEYWORDS: liver cirrhosis, ascitic fluid, cancer antigen 125 (CA 125)

INTRODUCTION

Tumor marker or cancer antigen 125 is a glycoprotein macromolecule with molecular weight of 220 KD, which was initially detected on cells 433 of ovarian carcinoma (OVCA) (1-2). For this reason, it is applied to diagnose ovarian cancer since the antigen represents an increase in more than 80% cases with ovarian cancer (3-4).

Although specificity of the antigen is low, and it is elevated in most malignancies such as malignant and benign tumors of endometrial and endocervical regions of uterus, pancreas and breast, it has been reported that serum CA 125 level shows a high rise in liver chronic disease, in general and in liver cirrhosis, in particular (1,5). Different studies have revealed that it is more likely to be a strong relationship between the formation of ascitic fluid and CA 125 level in liver cirrhosis accompanied with ascitic fluid formation. In other words, ascitic fluid formation plays a key role in the rise of CA 125 level. Regarding this close relation, helpful suggestions are made (6, 7) in a limited number of studies carried out in this field that CA 125 can be even more sensitive to the minimum level of ascitic fluid in liver cirrhotic patients and be of more usefulness than ultrasound sonography (1).

Although the origin of CA 125 in cirrhosis accompanied with ascitic fluid is not completely understood, this antigen is probably formed as a result of the response to the mechanical stress resulted from ascitic fluid presence and accumulation by the lining of the peritoneum, pleura, and then being released into blood through this epithelium.

Various studies have obtained controversial results. In a research conducted by Zuckerman and et.al, for example, CA 125 has found application as an ascitic fluid level marker in patients with chronic liver disease. This is while, in Xiao and Liu study, it was concluded that higher level of CA 125 is associated with liver cirrhosis disease, not with ascitic fluid level.

Therefore, the main aim of the present study was to find a reliable relationship between serum CA125 level and ascitic fluid in patients with liver cirrhosis. Moreover, the attempts were made to prove the following research hypothesis:

- 1- Is there a really strong relationship between ascitic fluid level and increased level of serum CA 125 antigen?

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- 2- Is there any logical relationship between serum CA 125 antigen level and presence or absence of SBP infected ascitic fluid in cirrhotic patients?

If proved, these hypothesizes would be of widely use in measuring serum CA 125 in patients whose ascitic fluid chemical and bacteriological examine are difficult, or the presence or absence of ascitic fluid in their body is unclear, and also it would work in patients who need strict control of diet, needless to ascitic fluid thoracentesis (fine-needle aspiration).

METHODS AND MATERIALS

The cases of the study were the patients with liver cirrhosis. The diagnosis of cirrhosis was based on clinical, laboratory, endoscopy and sonography grounds in these patients. Moreover, the presence of ascitic fluid was diagnosed through clinical examinations and sonography. And spontaneous bacterial peritonitis was determined to exist since the ascitic fluid contained more than 250000/ml white blood cells and culture was positive.

There were three groups of participants in the study, the control group and the two treatment groups. 71 patients with no sign of cirrhosis served as the control cases (Group A) referred to gastrointestinal clinics due to digestive disorders and then referred to laboratory to undergo Anti-IgG HP serological test to identify them with *Helicobacter pylori* infection. After receiving negative test result, explaining the study to them and asking their permissions, their serum samples were used to achieve the aims of the present study. The treatment groups were divided into two groups each consisted of 71 cases without infected ascitic fluid with SBP (Group B) and with infected ascitic fluid with SBP (Group C). A blood and an ascitic samples were taken from the treatment patients, and only a blood sample was also taken from the control ones. Blood specimens were obtained under fasting condition. Microbial culture test and blood cells counting were carried out on ascitic fluid specimens in treatment group patients. And measurement of CA 125 and nitric oxide level was conducted on blood specimens after making serum.

Criteria for excluding the cases from the Study

- **Renal failure:** All the taken blood samples underwent urea and creatinine tests to determine patients suffering from **renal failure**. The participants showed blood urea greater than 40 mg/100 ml and also creatinine more than 1.2 mg/100 ml were excluded from the study.
- **Diabetes:** The patients with diabetic symptoms, whose fasting blood sugar level was historically confirmed to be consistently elevated above normal level (120 mg/ dl serum) and glycosylated hemoglobin higher than 6.7%, were left out of the research.
- **Heart failure:** The cases, diagnosed by the internal medical specialist and through experimental results, with serum LDH level higher than 500 IU/L, serum CK-MB more than 25IU/L and serum CTN-I more than 1.3 Nano grams/ ml were confirmed that they suffer from heart failure, so were excluded from the study.
- **Prostate cancer:** This criterion was detected through clinical examinations and laboratory results. Being diagnosed with Serum PSA more than 4 mg/liter and free serum PSA more than 1 nanogram/ ml, the male participants were left out of the research.
- **High blood pressure:** The cases were not included in the study if they were identified to have high blood pressure after investigating their disease history and measuring blood pressure greater than 160.90.
- **Alcohol consumption:** Since alcohol consumption causes cerotic condition in liver, so patients with alcohol consumption history were excluded.
- **Having other kinds of cancers:** Patients who were consecutively suffering from other malignancies including ovarian and uterine cancers or liver encephalopathy identified by internal medical specialist were excluded from the investigation based on disease symptoms, since these diseases might affect CA 125 level.
- **Ethics Statement:** Because this study was to better identify and easier the presence of ascites fluid and also the rate it will be in cirrhotic patients, this study is part of the process of diagnosis and treatment in patients with Liver Cirrhosis. However, the study explained to patients to see that this study was to improve the complications of cirrhosis and better control in these patients. Also the patient's written consent. Ethics Committee of Research Deputy of Tabriz University of Medical Sciences also has a License number of 2563.54 dated 10.5.2011 has approved this study.
- **Patient Consent:** Of each patient separately in a five-page form an informed consent was obtained and patients with full knowledge and informed consent willing to participate in this study. Patients were given confidence the results of this study will be achieved confidentiality will be protected.

Serum CA 125 level was determined using ELISA kits of CA 125 made in Ray Biotech, Inc. with product number of ELH-CA125-001

RESULTS

Comparing age mean in the groups:

A total of 213 participants were examined in this investigation; one-third of whom were non-cirrhosis patients selected as the control group, other third of patients suffering from liver cirrhosis were selected as the first treatment group and their ascitic fluid was without SBP infection. Finally, the remaining third of patients included ones suffering from liver cirrhosis whose ascitic fluid was with SBP infection (PMN>250000 or positive culturing). They were selected as the second treatment group. The control group consisted of 41 male (57.7%) and 30 female (42.25%) cases; the first treatment group involved 38 male (53.52%) and 33 female (47.46%); and the second treatment group involved 39 male (54.92%) and 32 female (45.07%). In control patients, the minimum and maximum ages were 25 and 74 years, respectively, including mean age of 46.15±11.21. The minimum and maximum participants' ages in case group 1 were also 31 and 69 years, with mean age of 44.16±15.34. And finally in the participants of the treatment group 2, 29 and 71 years were identified to be the minimum and maximum ages with mean age of 45.33±13.24. Using one-way ANOVA, it turned out that there wasn't observed any statistically significant differences between control group's mean age and case group's (Table 1). In other words, the treatment group and control group had been matched well regarding age.

age	N	Mean±SD (age)	f	p-value	CI _{95%}
control group (non-cirrhosis cases)	71	46.15±11.21	.256	.625	-8.42 to 6.11
First treatment group (cirrhotic patients with infectious Ascite fluid)	71	44.16±15.34			
control group (non-cirrhosis cases)	71	46.15±11.21	.211	.754	-9.11 to 4.31
The second treatment group (cirrhotic patients without infectious Ascite fluid)	71	45.33±13.24			

Table 1: comparing mean age in the control group and **treatment** groups

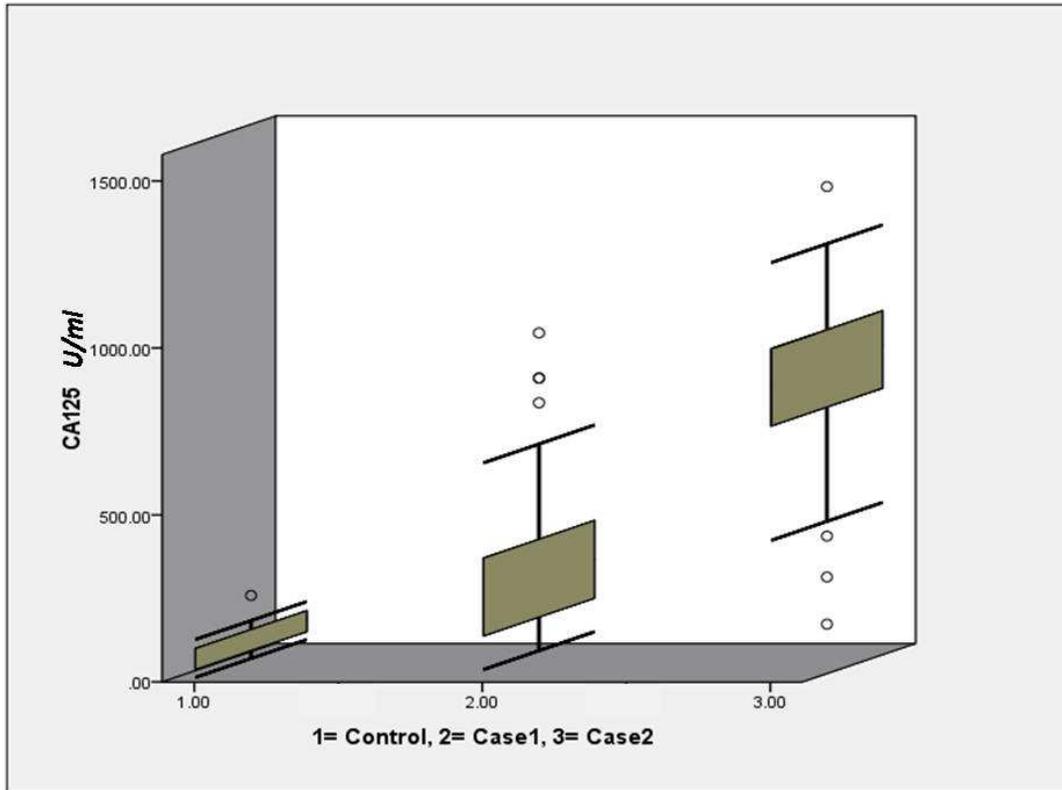
In this study, in order to analyze serum CA 125 level, the average of serum CA 125 was first identified for each group separately, then they were compared using one-way ANOVA and SPSS version 16. In these tests, $p < 0.05$ was considered to be significant.

Comparing CA-125 blood serum mean in three studied groups:

Considering Table 2 and graph 1, serum CA 125 level in cirrhotic patients without infectious ascitic fluid (the 1st treatment group) was significantly different from that of cirrhotic ones with infectious ascites (the 2nd treatment group), and also that of the control patients (i.e. non-cirrhosis individuals). A considerable increase was observed in serum CA 125 level in both treatment groups. Results obtained from this comparison have been shown as Mean ± Standard Deviation, so that means of serum CA 125 level in control, treatment group 1 and treatment group 2 were 73.10±34.46, 350.01±99.47 and 760.45± 254.60 (ml), respectively. P-value was .0001 in comparison of CA 125 mean between the control group and treatment group 1 (cirrhosis patients without infectious ascitic fluid), and confidence intervals varied from 64.94 to 81.26 and from 326.46 to 373.54, respectively. P-value was equal to .0001 in comparison of CA 125 mean between control group and case group 2 (cirrhosis patients with infectious ascitic fluid), and confidence intervals were in the range of 46.94 to 81.26 and from 700.18 to 820.71, respectively. Finally p-value equaled to .0004 compared with CA125 mean in the 1st and 2nd treatment groups.

serum CA125 level Groups	n	Mean±SD (age)	f	p-value	CI _{95%}
control group (non-cirrhotic cases)	71	73.10 ± 34.46	335.59	.0001	64.94 to 81.26
The first treatment group (cirrhotic patients with infectious ascite fluid)	71	99.47 ± 35.47			326.46 to 373.54
control group (non-cirrhotic cases)	71	73.10 ± 34.46	662.84	.0001	6494.11 to 81.26
The second treatment group (cirrhotic patients without infectious Ascite fluid)	71	254.60 ± 760.45			700.18 to 820.71
The first treatment group (cirrhotic patients with infectious Ascite fluid)	71	99.47 ± 350.01	8.34	.004	326.46 ± 373.54
The second treatment group (cirrhotic patients without infectious Ascite fluid)	71	254.60 ± 760.45			700.18 ± 820.71

Table 2: comparing serum CA125 mean in three study groups



Graph 1: comparing CA125 tumor antigen mean in three study groups

DISCUSSION

Cancer antigen 125 has a mucin-like glycoprotein structure and a molecular weight more than 200kd. CA 125 is one of the main markers of endometriosis and non-mucin tumors of ovary. Recent studies carried out

have concluded that this antigen is generated by all tissues with coelmic epithelium origins. The term coelmic may be referred to epithelial cells of visceral and abdominal tissues wall formed at embryonic stage (10). Newly done researches have indicated that CA 125 is elevated under many malignant and benign conditions including pleura inflammation, endometriosis and etc. Cirrhosis disease is considered as one of these conditions (11, 12).

Incidence of liver cirrhosis is probable to occur at all ages among human, and mostly it causes long-term side effects (13). Clinical course for patients suffering from cirrhosis is complex, usually accompanied with complex side effects (14). One of the cases in which CA 125 antigen indicated highest increase is counted to be liver cirrhosis (15). Our findings revealed that the amount of this antigen had a significant rise in both cirrhotic patients with infectious ascitic fluid and cirrhotic patients without infectious ascitic fluid. On the other hand, the rate of increase in cirrhotic patients with infectious ascitic fluid was more than that in patients without infectious ascitic fluid. As mentioned in the introduction, in the study carried out by Zukerman and et.al in 1999 (2), they considered CA 125 as a sign of identifying ascitic fluid level in liver chronic disease, which is in conformity with the findings of present research. This is while, the study conducted by Xiao and Li in 2003 (1) concluded that higher amount of CA 125 is attributed to liver cirrhosis disease, but not to ascitic fluid level. The results achieved from our study rejected findings of Xiao and Li (1), and completely proved that an increase in ascitic fluid of cirrhotic patients would result in an increase in CA 125 antigen accordingly. The problem with these scholars' works may be because of the lack of proper proportion between the treatment patients and control ones which were not equal in the number. In addition, Qureshi and et.al (16) in 2014 concluded that there was a normal correlation between CA 125 and ascitic fluid amount which nearly confirms our findings. This lower correlation might be due to the reasons that firstly, their samples were fewer than the present study's; secondly, they had not identified the presence or the absence of bacterial infection in ascitic fluid taken from patients, while in this study they were completely separated into two distinguished groups. Another point is that Qureshi and et al. (16) had not screened patients for other diseases such as renal failure, diabetes, cardiovascular diseases, prostate disorders, blood pressure, alcohol consumption, and other cancers, so not excluded them from the study, for these diseases may effect both CA 125 and nitric oxide amount. Besides, Devarbhavi and et.al (17) in 2002, in term of cirrhotic patients with and without ascitic fluid infection, observed that CA 125 antigen is increased considerably in cirrhotic patients especially in those with infected ascitic fluid, considering that the reason for such an increase was induced by the special response of peritoneum against ascitic fluid accumulated in peritoneal cavity, confirming this study findings. They also mentioned that being aware of the reason increasing CA 125 antigen in cirrhotic patients may prevent unnecessary surgical interventions. Therefore, on the basis of highly elevated amount of CA 125, we would even make use of it to diagnose ascitic fluid with infection in cirrhotic patients, in addition to diagnosing the existence or the absence of ascitic fluid.

The mechanism of CA 125 antigen rise has not been completely understood in cirrhotic patients yet. Though, in Zeimet and et.al in 1997 (18) research which was done in culture medium and used peritoneal mesothelial, they observed that these cells are secreting CA 125 five times more than in ovarian cancer cells. The hypothesis which is more probable in this field is that an increase in the antigen in blood of patients with cirrhosis may be due to the increase in lymphatic absorption of ascitic fluid (19). Another factor leading to a large increase in this antigen in cirrhotic patients' blood is associated with CA 125 lower clearance by liver, and in patients who have ascitic fluid the penetration of peritoneal membrane may be of help to rise blood antigen (20).

Conclusion:

According to results obtained from present study, it was concluded that CA125 can be used as an excellent and fully appropriate marker to detect the presence or absence of ascitic fluid in cirrhotic patients.

REFERENCES

1. Xiao WB and Liu YL. *Elevation of serum and ascites cancer antigen 125 levels in patients with liver cirrhosis*. J Gastro and Hepato; 18: 1315-1316, 2003.
2. Zukerman E, Lanir A, Sabo E, Rozenvald TZ, Matter I, Yeshurun D and Eldar S. *Cancer Antigen 125: A sensitive marker of ascites in patients with liver cirrhosis*. The Am J Gastro, 94(6): 1613-1618, 1999.
3. Bast RC, Feeney M, Lazarus M et al. *Reactivity of a monoclonal antibody with human ovarian carcinoma*. J Clin Invest; 68: 1331-1337, 1981.
4. Bast RC, Klug TL, St. John E et al. *A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer*. N Engl J Med; 309: 883-887, 1983.
5. Devarbhavi H, Kaese D, Williams AW et al. *Cancer antigen 125 in patients with chronic liver disease*. Mayo Clin Proc; 77: 538-541, 2002.

6. Deschenes M, Michel RP, Albert E et al. *Elevation of CA125 level is due to abdominal distension in liver transplantation candidates*. Transplantation; 72: 1519-1522, 2001.
7. Sari R, Yildirim B, Sevinc A et al. *Sensitivity of CA 125 in patients with liver cirrhosis in the presence of ascites*. Am J Gastroenterol; 96: 253-254, 2001.
8. Kabawant SE, Bast RC, Bhan AK et al. *Tissue distribution of a coelomic epithelium-related antigen recognized by the monoclonal antibody OC125*. Int J Gynecol Pathol; 2: 275-285, 1984.
9. So Young Bae, Jun Haeng Lee, Jun Young Park, Da-min Kim, Byung-oon Min, Poong-Lyul Rhee, Jae J. Kim. *Clinical Significance of Serum CA-125 in Korean Females with Ascites*. Yonsei Med J., 54(5):1241-1247, 2013.
10. Halila H, Stenman UH, Seppälä M. *Ovarian cancer antigen CA 125 levels in pelvic inflammatory disease and pregnancy*. Cancer.; 57(7):1327–1329, 1986.
11. Pittaway DE, Douglas JW. *Serum CA-125 in women with endometriosis and chronic pelvic pain*. Fertil Steril.; 51(1):68-70, 1989.
12. So Young Bae, Jun Haeng Lee, Jun Young Park, Da-min Kim, Byung-Hoon Min, Poong-Lyul Rhee, Jae J. Kim. *Clinical Significance of Serum CA-125 in Korean Females with Ascites*. Yonsei Med J., 54(5): 1241–1247, 2013.
13. Deshmukh A, More UK, Tilak MA, Sontakke AN, Deshmukh UD. *Role of Nitric Oxide in Liver Cirrhosis*. Indian Journal of Basic & Applied Medical Research, 6 (2): 546-550, 2013.
14. Chu C J, Lee F Y, Wang S S, Chang F Y, Tsai Y T. *Hyper dynamic Circulation of Cirrhotic Rats: Role of Nitric Oxide*. Scand J Gastroenterol, 32(8):841-6, 1997.
15. Piekarska A, Piekarski J, Zbońska J. *Elevated levels of cancer antigen CA-125 in patients with chronic liver diseases, with and without ascites*. E&C Hepatology, 3(1): 30-33, 2005.
16. Qureshi MO, Dar FS, Khokhar N. *Cancer Antigen-125 as a Marker of Ascites in Patients with Liver Cirrhosis*. Journal of the College of Physicians and Surgeons Pakistan. 24 (4): 232-235, 2014.
17. Devarbhavi H, Kaese D, Williams AW, Rakela J, Klee GG, Kamath PS. *Cancer antigen 125 in patients with chronic liver disease*. Mayo Clin Proc., 77(6):538-41, 2002.
18. Zeimet AG, Offner FA, Marth C, et al. *Modulation of CA 125 release by inflammatory cytokines in human peritoneal mesothelial and ovarian cancer cells*. Anticancer-Res., 17: 3129-31, 1997.
19. DiBaise JK, Donovan JP. *Markedly elevated CA 125 in hepatic cirrhosis*. J Clin Gastroenterol, 28: 159-61, 1999.
20. Collazos J, Genolla J, Ruibal A. *CA 125 serum level in patients with non-neoplastic liver diseases. A clinical and laboratory study*. Scand J Clin Lab Invest. 52: 201-6, 1992.