Diagnostic value of serum copper, zinc and plasma fibrinogen in cirrhotic patients with and without hepatocellular carcinoma

Serum bakır, çinko ve plazma fibrinojen düzeylerinin hepatosellüler karsinomu olan ve olmayan sirotik hastalarda diagnostik önemi

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SUMMARY: *The differential diagnosis of liver tumors and cirrhosis remains a difficult problem, particularly in the early stage of a tumor and when the tumor is associated with cirrhosis. Increased copper and decreased zinc levels in the blood have been reported to occur in various malignant tumors including hepatocellular carcinoma (HCC). The aim of this study was to investigate the diagnostic value of serum copper (SCu), zinc (SZn) and plasma fibrinogen (PF) levels in cirrhotic patients with and without HCC. Seventy seven patients were studied. Of these, 44 had liver cirrhosis, 20 had HCC associated with liver cirrhosis, and 13 had hepatic metastases. Mean SCu level was higher in patients with HCC (149.88 ± 42.22) and than in patients with liver cirrhosis (98.27±36.92) (p<0.001) and hepatic metastases (119.38±38.27) (p=0.0219). No significant differences were observed in the levels of SZn in patients with or without hepatic carcinoma. Mean PF levels were higher in patients with both HCC (351.20± 130.02) and hepatic metastases (362.14 ± 98.29) than that in patients with liver cirrhosis (236.18 ± 107.80) (p<0.001). In all patients, SCu level were positively correlated with PF (r = 0.415, p < 0.05). However, SZn level was not correlated with any of these parameters. In the differential diagnosis of HCC, sensitivity and specificity of SCu was 50% and 84% respectively.at a cut-off value of 140 µg/ dL, Also, sensitivity and specificity of PF were 35% and 91% at a cut-off value of 400 mg/dL, respectively. The combination of SCu and PF did not improve the diagnostic value. In conclusion, SCu and PF levels had a poor sensitivity but a high specificity in discriminating of HCC from liver cirrhosis.*

Key words: Cirrhosis, copper, fibrinogen, hepatic metastase, hepatocellular carcinoma, zinc

ÖZET: *Karaciğer tümörleriyle sirozun ayırıcı tanısı, özellikle tümörün erken safhasında ve tümör sirozla birlikte olduğu zaman zor olmaktadır. Kanda artmış bakır ve azalmış çinko seviyeleri, hepatosellüler karsinomayı (HKK) da içeren çeşitli tümörlerde rapor edilmiştir. Bu çalışmanın amacı HKK'sı olan ve olmayan sirotik hastalarda, serum bakır (SCu), çinko (Szn) ve plazma fibrinojeninin (PF) diagnostik değerini araştırmaktır. 77 hasta çalışmaya alındı. 44 hastada siroz, 20 hastada sirozla ilişkili HKK ve 13 hastada karaciğer metastazı vardı. Ortalama SCu seviyesi HKK'sı olan hastalarda (149.88 ± 42.22), sirozu (98.27 ± 36.92) (p<0.001) ve karaciğer metastazı olan hastalardan (119.38±38.27) (p=0.0219) daha yüksekti. Karaciğer kanseri olan ve olmayan hastaların SZn seviyeleri arasında anlamlı bir fark bulunmadı. Ortalama PF seviyesi hem HKK'sı (351.20±130.02), hem de karaciğer metastazı (362.14±98.29) olan hastalarda, sirozu olan hastalardan (236.18±107.80) daha yüksekti (p<0.001). SCu seviyesi, PF seviyesi ile bütün hastalarda pozitif bir korelasyon gösteriyordu (r = 0.415, p < 0.05). Fakat SZn seviyesi bu parametrelerin hiçbiri ile korele değildi. HKK'un ayırıcı tanısında, SCu'nın sensitivitesi ve spesifisitesi 140 µg/dL'lik cut-off değerinde sırasıyla 50% ve 84% bulundu. 400 mg/dL'lik cut-off değerinde PF'inin sensitivitesi ve spesifisitesi de sırasıyla % 35 ve % 91 olarak bulundu. SCu ve PF kombinasyonu diagnostik değeri arttırmadı. Sonuç olarak, HKK'nın karaciğer sirozundan ayrılmasında SCu ve PF değerlerinin düşük bir sensitivitesi mevcut iken yüksek bir spesifisitesi bulunmaktadır.*

Anahtar sözcükler: Siroz, bakır, çinko, fibrinojen,karaciğer metastazı, hepatosellüler karsinoma

An increased serum copper (SCu) level has been observed in lymphomas (1) and in some solid tumors (2), particularly of the breast (3), gastrointestinal tract (4), and lung (5). Many clinical studies suggest that the SCu level is correlated with the clinical course of tumors (1, 5). Cupremia has also been proposed as a good prognostic index in neoplastic patients, since it seems to be correlated with the extension of the neoplastic disease (2).

The significance of SCu levels in liver disease is

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Table 1. Mean values of all groups (Mean ± SD)

	Cirrhosis	HCC	Hepatic metastases
Age (yrs)	52.92 ± 7.89	56.57± 13.61	55.33 ± 9.96
Male/Female	32/ 12	14/ 6	9/ 4
SCu (µg/ dL)	98.27 ± 36.92*	149.88 ± 42.22*	119.38 ± 38.27*
SZn (µg/ dL)	82.16 ± 32.04	84.59 ± 29.25	78.85 ± 11.16
PF (mg/ dL)	236.18 ± 107.80*	351.20 ± 130.02*	362.14 ± 98.28*

* $p < 0.001$ with analysis of variance

still unclear. It is known that the copper level in the cirrhotic liver, especially of alcoholic origin, is more or less increased (6). An excess of toxic-form copper, free copper, may cause DNA damage in the presence of free radicals and oxygen radicals. Such DNA damage by the radicals has been considered to be responsible for hepatic necrosis and hepatocellular carcinoma (HCC) in Long Evans Cinnamon (LEC) rats (7). The value of determining SCu level in the differential diagnosis between hepatic tumor and cirrhosis has been reported (8), and it is also proposed that serum copper levels may be used as a marker for the detection of HCC, especially for AFP-negative HCC (9, 10). Morever, SCu estimation may be valuable in the assessment of therapeutic effect and prognosis in patients with HCC.

Reports of the significance of zinc values in the biological fluids of cancer patients have been variable. Low serum zinc levels have been observed in the serum of patients with cancer of the bronchus and colon, but not in other forms of cancer (11). Decreasing zinc levels have been noted from chronic hepatitis through cirrhosis to primary liver cancer and decompensated patients have lower levels than those with well-compensated disease (12).

It has also been reported that those mean plasma fibrinogen (PF) levels higher in patients with HCC than that in patients with liver cirrhosis (10).

Since the performance of these tests is available to many laboratories, is not time-consuming, and is less costly than many other procedures, we were interested in determining whether SCu, Zinc and PF levels have any predictive value in patients with liver cirrhosis, with and without HCC.

MATERIALS AND METHODS

Patient selection: Our study included 77 patients. Of these 44 had liver cirrhosis, 20 had HCC associated with liver cirrhosis, and 13 had hepatic metastases. In all cases the diagnosis was confirmed histologically. All patients with hepatic carcinoma (HCC and hepatic metastases) had no specific treatment.

Serum biochemical determinations: On admission, SCu and SZn levels were determined by the calorimetric method. PF level was determined by the standard method. Normal values of SCu, SZn, and PF in our laboratory varied from 70 to 140 µg/dL, 70 to 120 µg/dL, and 200 to 400 mg/dL, respectively.

Statistical analysis: All results were expressed as means (Mean ± SD). Group mean values were compared by "analysis of variance" and "student's t" tests. In addition, statistical analysis was performed using the correlation matrix test. The results were considered significant if $p<0.05$.

RESULTS

Results were reported in tables. By Child-Pugh score, 20 patients with liver cirrhosis (45.45%) were in Child-A, 15 (34.10%) in Child-B and 9 (20.45%) Child-C. SCu, SZn and PF levels were not significantly different among patient groups with liver cirrhosis to Child-Pugh score.

Age and sex were comparable in all groups. Analysis of variance showed that only mean SCu and PF levels were significantly different in all groups ($p<0.001$). Mean SCu levels were higher in patients with HCC than in those with liver cirrhosis ($p<0.001$, student's t test) and patients with hepatic metastases ($p=0.0219$, student's t test).

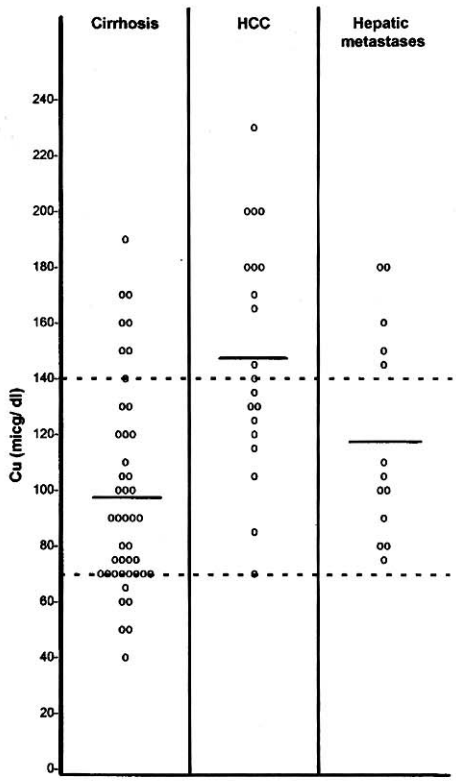

Figure 1. SCu levels of patients

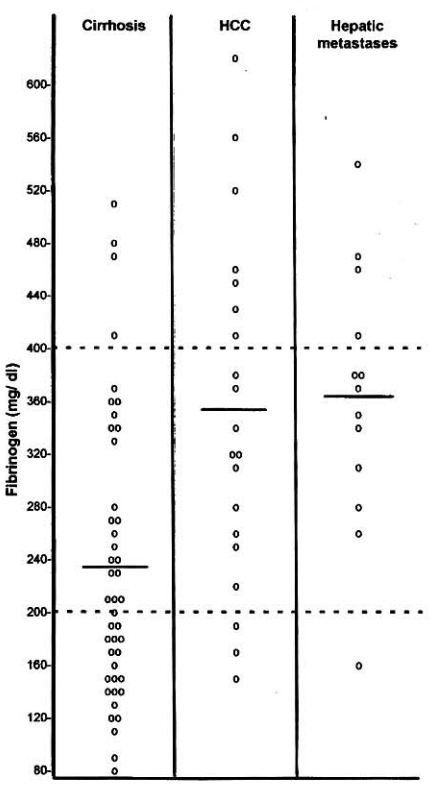

Figure 2. PF levels of patients

Also mean SCu levels were higher in patients with hepatic metastases than that in those with liver cirrhosis (p=0.0389, student's t test). No significant differences were observed in the levels of SZn in patients with and without hepatic carcinoma. Mean PF levels were higher in patients with HCC and hepatic metastases than that in those with liver cirrhosis (p<0.001, student's t test). However, there was no significant change in the mean value of PF in patients with HCC when compared with that of patients with hepatic metastases (Table1). There was no association between diameters of tumors and these parameters.

In all patients the SCu level was positively correlated with PF (r=0.415, p<0.05). However, SZn level was not correlated with any of these parameters.

To assess the value of SCu and PF in the differential diagnosis of HCC and cirrhosis, we determined the sensitivity and specificity of SCu and PF. SCu and PF levels of patients were shown in Figure 1 and 2, respectively. Sensitivity and specificity of SCu were 50% and 84% respectively at a cut-off value of 140 µg/dL. Also, sensitivity and specificity of PF were 35% and 91% at a cut-off value of 400 mg/dL, respectively. Cut-off values were determined as the upper ranges of our laboratory values. The combination of SCu and PF did not improve the diagnostic value.

DISCUSSION

Alterations in trace element concentrations may be observed in patients with chronic liver disease. The levels of SCu and urinary Cu in liver cirrhosis are higher than normal (9). Furthermore, there is an increase in hepatic copper content in liver cirrhosis (13), the most probable cause of which is a defect in the metabolism of copper in this disease. Reduced zinc levels in both serum (14) and

liver tissue (15) have been well documented in cirrhotic patients and multiple mechanisms, including poor intake and decreased absorption, appear to be responsible for it (16).

Increased copper and decreased zinc levels in the blood have been reported to occur in various malignant tumors including HCC (4, 10), but whether these abnormalities are causal or sequential is unclear. Copper overload is known to exert an inhibitory effect on chemical hepatocarcinogenesis in animals (17), and the low incidence of HCC in patients with Wilson's disease could be explained by the presence of an excess amount of copper in hepatocytes protecting them from the oncogenic consequences of liver cirrhosis (18). Development of HCC is also rare in primary biliary cirrhosis, where copper accumulation occurs during its course (19). The low incidence of HCC in this disease can be ascribed to the fact that liver cirrhosis usually occurs only at a late stage of the disease, but the accumulated copper possibly inhibits hepato-carcinogenesis (20).

The differential diagnosis of liver tumors and cirrhosis remains a difficult problem, particularly in the early stage of the tumor and when it is associated with cirrhosis. It has been affirmed that only 63% of hepatic tumors were diagnosed when the patient was alive (21).

In many studies, SCu and PF levels may be used as markers for early detection of HCC (9, 10). Miatto *et al* reported that, SCu had a good sensitivity (80%) and a high specificity (92%) at a cut-off value of 160 µg/dL; when the cut-off level was raised to 170 µg/dL, the specificity increased to 100%, with a sensitivity of 77% (10). In that study, the PF level had a lower diagnostic value than did SCu. However, the combination of SCu and PF improved the diagnostic value slightly. In a previous study, we reported that SCu and PF levels were higher in patients with hepatic carcinoma than that in patients with liver cirrhosis and that these levels should be used as a screening test in all cirrhosis to detect neoplastic degeneration (22). In that study however only 60% of patients with HCC was associated with liver cirrhosis.

In this study, all patients with HCC were associated with liver cirrhosis. We found significantly higher levels of SCu and PF in patients with both HCC and hepatic metastases than that in those with liver cirrhosis. SCu and PF levels had a poor sensitivity but a high specificity in discriminating HCC from liver cirrhosis. At a cut-off value of 140 µg/dL, the maximum of our range, sensitivity and specificity of SCu was 50% and 84%, respectively. Also, at a cut-off value of 400 µg/dL, sensitivity and specificity of PF was 35% and 91%, respectively. In all patients, SCu level was positively correlated with PF ($r = 0.415$, $p < 0.05$). The combination of SCu and PF did not improve the diagnostic value.

Although SCu level was higher in patients with HCC than that in patients with hepatic metastases, it seems that differential diagnosis between HCC associated with cirrhosis and hepatic metastases is impossible by measurment of SCu and PF levels. However, these diseases may be discriminated easily with other diagnostic procedures. This data is similar to a study in which SCu levels increased significantly in patients with hepatic metastases (23).

To conclude, determination of SCu and PF levels do not appear to have great diagnostic value and they should not be used as a screening test in cirrhotic patients to detect early neoplastic degeneration. However, SCu and PF levels may be useful in the differential diagnosis between hepatic tumor and cirrhosis, because of their high specificity.

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