

## ORIGINAL ARTICLES

### Oxidase Activity of Ceruloplasmin and Some Acute Phase Reactant and Trace Element Concentrations in Serum of Patients with Chronic Lymphocytic Leukemia

M Gundogdu<sup>1</sup>, H Kaya<sup>2</sup>, I Gulcin<sup>3</sup>, F Erdem<sup>1</sup>, K Cayir<sup>1</sup>, M Keles<sup>1</sup>, A Yilmaz<sup>4</sup>

1 Department of Internal Medicine, Ataturk University, Medical School, Erzurum, Turkey

2 Department of Internal Medicine, Mustafa Kemal University, Medical School, Hatay, Turkey

3 Department of Chemistry, Science and Art Faculty, Erzurum, Turkey

4 Department of Biochemistry, Nenehatun Obstetric and Gynecology Hospital, Erzurum, Turkey

#### Correspondence to:

Dr Mehmet GUNDOGDU, MD, Ataturk University, Medical School, Department of Internal Medicine, 25240 Erzurum, Turkey

Phone: +90 442 2361212-1723

Fax: +90 442 2361301 or 3172294

E-mail: gundogdu@atauni.edu.tr or yilmazadnan69@hotmail.com

#### ABSTRACT

In the present study, we aimed to investigate the parameters in serum of patients with chronic lymphocytic leukaemia (CLL) and correlate with the cancer stage. The serum concentrations of ceruloplasmin, a-1-acid glycoprotein, albumin, transferrin, copper, zinc, manganese, and ceruloplasmin oxidase activity were measured, and compared with those from a healthy control group. The serum from 34 patients with CLL were extracted before chemotherapy. Serum transferrin, albumin and Zinc concentrations were lower in patients with CLL while serum a-1-acid glycoprotein, ceruloplasmin, copper concentrations, and ceruloplasmin oxidase activity were higher in CLL patients when compared with the control group. Although serum manganese concentration was lower in CLL groups than in the control group, the difference was not statistically significant. Serum transferrin concentration was lower in the early stage group compared with the advanced stage. Serum ceruloplasmin level positively correlated with serum ceruloplasmin oxidase activity in patients from the early stage group. Serum ceruloplasmin level positively correlated with serum ceruloplasmin oxidase activity in patients with advanced stage. In conclusion, increased serum ceruloplasmin oxidase activity, ceruloplasmin, a-1-acid glycoprotein, copper levels and decreased transferrin and albumin, unchanged manganese levels are associated with CLL and appear to be a consequence of the disease itself.

#### Introduction

Chronic lymphocytic leukaemia (CLL) is a haematologic neoplasm characterised by the proliferation and accumulation of relatively mature appearing lymphocytes in the blood, bone marrow, lymph nodes, spleen, liver and other organs. The disease is seen rarely in those below the age of 30; most patients with CLL are over 60 years of age. CLL increases in incidence exponentially with age; by 80 the incidence rate is 20 cases per 100,000 persons per year. Asian countries such as Japan and China have an incidence of CLL only 10 per cent that in the United States and other Western countries.<sup>1,2</sup> The major causes are not known nor is there detailed understanding about how the elusive origin(s) may relate to clinical expression, basic biological mechanisms, or pathogenesis.<sup>2,3</sup>

The acute-phase proteins are a family of proteins synthesised by the liver whose levels change in response to infection, injury and neoplasia. In response to injury, the levels of some acute-phase proteins such as serum amyloid A, ferritin, and ceruloplasmin (Cp), increase. In contrast, levels of others, such as Albumin, prealbumin and transferrin (Trf), fall.<sup>5</sup>

Alpha-1-acid glycoprotein (AAG) is a 44 kDa plasma protein and its concentration in plasma increases approximately two to four fold following tissue injury. It has been speculated that AAG plays an important role in inflammation, and may be the best laboratory parameter for the pre-therapeutic prognostic evaluation of lung cancer patients.<sup>6,7</sup>

Albumin contains 17 disulphide bridges and has a single remaining cysteine residue and it is this residue that is responsible for the capacity of albumin to react with and neutralise peroxy radicals. This property is important in view of the role that albumin plays in transporting free fatty acids in the blood. Albumin also has a single high-affinity copper binding site that transfers newly absorbed copper to the liver.<sup>8,9</sup>

A major contributor to the antioxidant defence system of human plasma is reported to be ceruloplasmin. Ceruloplasmin acts as an antioxidant by several mechanisms<sup>10,11,12,13,14</sup> inhibiting iron-dependent lipid peroxidation (LP) and HO formation from hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) via its ferroxidase activity,<sup>12</sup> reacting with and scavenging H<sub>2</sub>O<sub>2</sub> and superoxide anion, and inhibiting copper-induced LP by binding copper ions.<sup>10,12</sup> Ceruloplasmin is a plasma glycoprotein that is

primarily synthesised by the liver and secreted into the blood. It binds about 60% of plasma copper and is considered a copper transport protein. Ceruloplasmin permits the incorporation of iron into Trf without the formation of toxic iron (Fe) products.<sup>14, 15,16</sup>

Trace elements exist in very low concentrations in the body and consist of 0.01% of total body weight. It is well known that certain major and minor elements play an essential part in a number of biological processes.<sup>16</sup>

To our knowledge, there is no available data on serum AAG, Albumin, Trf, ceruloplasmin, copper, zinc, manganese concentrations and ceruloplasmin oxidase activity in patients with CLL. Therefore, in the present study, we aimed to investigate these parameters in serum of patients with CLL and correlate with the cancer stage.

## Materials and Methods

### Patients

Thirty-four patients (29 males) with CLL comprised the patient group, and 26 healthy subjects were taken as control group (23 males), with the age range being 39 - 78 years (mean  $\pm$  SD;  $60.1 \pm 10.3$ ) for the patients and 37 - 66 years (mean  $\pm$  SD;  $57.6 \pm 11.9$ ) for the controls. CLL patients were staged according to the Rai et al,<sup>17</sup> classification: 5 were in stage I; 12 in stage II; 7 in stage III, and 10 in stage IV. CLL patients were divided into two groups as early stage (stage I and II) and advanced group (stage III and IV). The patients were taking no antileukaemic therapy and were newly diagnosed cases.

### Blood Sampling

CLL patients and healthy control subjects were recruited into the study after obtaining their informed consent. Venous blood (8 ml) was taken from controls and patients. The blood samples were centrifuged and the serum samples obtained were stored at  $-80^{\circ}\text{C}$  until analysis.

### Biochemical Measurements

Serum ceruloplasmin, albumin, AAG and transferrin levels were determined by nephelometric method (Beckman Array 360 Protein System, Minnesota, Brea, USA). Serum transferrin, AAG, ceruloplasmin and albumin were expressed as mg/dL, and g/dL respectively.

Serum ceruloplasmin oxidase activity was measured according to the method of Schosinski et al.<sup>18</sup> The method is based on the ability of ceruloplasmin to oxidise substrate such as o-dianizidine (3,3,-dimethoxybenzidine)

yielding a yellow product. Briefly, 0.75 ml of 0.1 M acetate buffer, pH 5, in 2 tubes was mixed with 0.05 ml of serum sample and kept for 5 min at  $30^{\circ}\text{C}$ . To both tubes 0.2 ml of 0.25 % o-dianizidine dihydrochloride was added and one mixture was incubated at  $30^{\circ}\text{C}$  for 5 min, and the other for 15 min. The reaction was stopped by adding 2 ml of 9 M sulfuric acid. The optical density was determined at 540 nm using a spectrophotometer (CECIL CE 3041, Cambridge, UK). Ceruloplasmin oxidase activity was expressed as U/dL.

The ceruloplasmin oxidase activity was calculated as: Cp oxidase activity (U/l) =  $(A_{540}^{15\text{ min}} - A_{540}^{5\text{ min}}) \times 625$

For trace element assays, all of the materials (glass and plastic) used were thoroughly cleaned with hot solution of nitric acid (20%, v/v) for 48 hours and rinsed five times with deionized water. Serum samples were diluted with deionized, double distilled water for element measurements, when required. Zn, Cu, and Mn measurements were carried out with an atomic absorption spectrometer (Unicam 929, UK). Serum Cu, Zn and Mn were expressed as mg/dL and ng/dL, respectively.

### Statistical Analysis

The findings were expressed as the mean  $\pm$  SD. Statistical and correlation analyses were undertaken using the Mann-Whitney U-test and Spearman's rank correlation coefficient test, respectively. A p value  $< 0.05$  was accepted as statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 11.0, Chicago, IL, USA).

## Results

The results from patients with CLL and control group are summarised in Table I. As seen from Table I, although serum Mn concentration was lower in CLL cancer groups

**Table I Mean  $\pm$  SD of Trf, AAG, Cp, Albumin, Cu, Zn, Mn Levels and Cp Oxidase Activity in Serum of Patients with Chronic Lymphocytic Leukemia and Control Group**

	Control group	Early stage (I+II)	Advanced stage (III+IV)	Total patient group
Trf (mg/dL)	276.2 $\pm$ 36.0	181.4 $\pm$ 33.1 <sup>a</sup>	227.6 $\pm$ 44.8 <sup>a,d</sup>	204.5 $\pm$ 45.3 <sup>a</sup>
AAG (mg/dL)	89.3 $\pm$ 16.0	120.1 $\pm$ 26.9 <sup>a,c</sup>	141.2 $\pm$ 31.5 <sup>a</sup>	130.7 $\pm$ 30.8 <sup>a</sup>
Cp (mg/dL)	31.5 $\pm$ 5.8	62.7 $\pm$ 19.6 <sup>a,c</sup>	78.9 $\pm$ 13.9 <sup>a,c</sup>	70.8 $\pm$ 18.6 <sup>a</sup>
Cp oxidase activity (U/dL)	16.7 $\pm$ 4.1	22.2 $\pm$ 7.3 <sup>b</sup>	25.3 $\pm$ 5.5 <sup>a,c</sup>	23.8 $\pm$ 6.5 <sup>a</sup>
Albumin (g/dL)	4.3 $\pm$ 1.0	3.7 $\pm$ 0.46 <sup>a,c</sup>	3.6 $\pm$ 0.43 <sup>a,c</sup>	3.6 $\pm$ 0.44 <sup>a</sup>
Cu ( $\mu\text{g/dL}$ )	71.8 $\pm$ 14.0	91.2 $\pm$ 9.8 <sup>a,c</sup>	98.8 $\pm$ 7.7 <sup>a,c</sup>	95.0 $\pm$ 9.5 <sup>a</sup>
Zn ( $\mu\text{g/dL}$ )	83.1 $\pm$ 5.2	66.1 $\pm$ 9.4 <sup>a</sup>	61.9 $\pm$ 5.8 <sup>a</sup>	64.0 $\pm$ 8.0 <sup>a</sup>
Mn (ng/dL)	116.0 $\pm$ 17.4	115.1 $\pm$ 11.5	112.2 $\pm$ 7.6	113.7 $\pm$ 9.7

a:  $p < 0.005$ , b:  $p < 0.01$ , c:  $p < 0.001$ , when compared to control group.

than in the control group; this difference was not statistically significant ( $p > 0.05$ ). Serum Trf, albumin and Zn concentrations were lower in patients with CLL while serum AAG, Cp, Cu concentrations, and Cp oxidase activity were higher when these patients compared with control group. Serum Trf concentration was lower in early stage group compared with advanced stage group. However, among all stages, no significant differences were found in the basis of above mentioned parameters. As seen from table, as the stage of the disease increased, higher concentrations of serum AAG, Cp, Cu and Cp oxidase activity, and lower concentrations of serum albumin and Zn were determined.

Serum Cp level positively correlated with serum Cp oxidase activity ( $r = 0.81$ ,  $p < 0.001$ ) in patients with early stage group.

Serum Cp level positively correlated with serum Cp oxidase activity ( $r = 0.74$ ,  $p < 0.001$ ) in patients with advanced stage. However, no other correlation could be found among the parameters in patients with CLL and healthy controls.

## Discussion

Several factors such as serum acute phase reactants (APR) have been reported to be useful markers for staging the disease and predicting the prognosis in patients with cancer.<sup>5,6,7</sup> In this study, we aimed to investigate the importance of APR and some trace elements in patients with CLL.

The activity of antioxidant enzymes, mainly of cellular location, plays a crucial role in oxidant carcinogenesis. A contribution of Cp to antioxidant defense can be of great importance, in regard to its plasma location. Increased production of some of acute phase proteins, also Cp, that often accompanies neoplastic process, supposedly is caused by interleukin-1 (IL-1) and tumour necrosis factor (TNF) released by macrophages. In various carcinomas the oxidase activity and the concentration of Cp were reported to be elevated.<sup>12</sup>

Of the extracellular antioxidants, Cp permits the incorporation of Fe into Trf without the formation of toxic Fe products. Trf inhibits iron-ion dependent OH formation from  $H_2O_2$ . Iron-catalyzed reactions are limited by the presence of Trf in human plasma. Under physiologic conditions, Cp is also important in the control of membrane lipid oxidation, probably by direct oxidation

of cations, thus preventing their catalysis of lipid peroxidation.<sup>15,16</sup> We found that increased serum Cp oxidase activity and Cp level in patients with CLL. This increase of Cp oxidase activity and Cp level in serum may be due to a compensatory mechanism. By keeping iron in  $Fe^{3+}$  state, Cp presents it from undergoing the redox cycles necessary to initiate toxic effects.<sup>16</sup>

In one study, Agroyannis et al,<sup>19</sup> decreased serum Trf and increased Cp levels were reported in patients with gastrointestinal cancer. Zowczak et al,<sup>12</sup> documented significantly increased Cp oxidase activity in various cancer patients. Taysi et al,<sup>16</sup> and Varela et al,<sup>20</sup> reported significantly increased Cp in serum of laryngeal cancer patients.

Our results for Cp oxidase activity, Cp, and Trf, levels in serum of patients with CLL are in agreement with those obtained by Zowczak et al,<sup>12</sup> Varela et al,<sup>20</sup> and Taysi et al's<sup>16</sup> studies.

AAG is one of positive acute phase proteins and its concentration in plasma increases approximately two to four fold following tissue injury. It has been speculated that AAG plays an important role in inflammation and cancer, but its exact biological function is still unclear.<sup>21</sup> Our results showed significant decrease in the mean albumin level and significant increase in the mean AAG level in the serum of patient groups comparison the control group. This latter result is in agreement with the results of other researchers.<sup>7</sup>

Recent studies draw attention to trace elements. Zn, and Mn are important elements in the preservation of immune resistance and both Zn and Cu are required for numerous biochemical functions and for optimal activity of the immune system. Zn is involved in the function of approximately 80 enzymes in body.<sup>16</sup>

Cu and Fe can catalyze the formation of the highly reactive hydroxyl radicals from  $H_2O_2$  via the Haber-Weiss reaction and decompose of lipid peroxides to peroxy and alkoxy radicals, which favor the propagation of lipid oxidation. The competition of Zn for Fe binding sites is particularly relevant taking in account that Zn deficiency facilitates intracellular Fe accumulation.<sup>22</sup>

In this study, serum Cu level was found to be elevated while serum Zn level was found to be decreased. These data confirm the earlier studies.<sup>16,23</sup> Contrarily,

unchanged serum Zn and Cu were determined gastrointestinal system and cervical cancer cases, respectively.<sup>24, 25</sup> As both metals, together with Mn, are the prosthetic group of Superoxide dismutases (SODs), any alterations in their levels may affect activities of the enzyme. The relationship between SOD activity and estimated trace elements remains to be enlightened. Nevertheless, this may cause oxidative stress or may further increase the existed stress.<sup>16</sup>

There are contradictory results on the alterations in serum or tissue trace elements and concentrations of serum antioxidant protein in various cancers. The mechanisms by which these alterations occur in certain cancers need to be elucidated. It is also obscure that whether these alterations are a cause or a consequence of the malignancy.

As a conclusion, in our opinion, alterations in the level of trace elements and antioxidant proteins are important for many metabolic processes, in CLL may not be a reason for, but in fact a consequence of the disease itself. These parameters and other acute phase proteins might have important metabolic roles in cancer progression.

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