



Original Contribution

CORRELATION BETWEEN PLASMA MALONDIALDEHYDE AND CERULOPLASMIN ACTIVITY IN PATIENTS WITH MALIGNANT HAEMATOLOGICAL DISEASES

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ABSTRACT

The plasma level of ceruloplasmin (Cp) as an antioxidant protein in patients suffering from different malignant haematological diseases was studied. Significantly higher level of Cp was detected in malignant haemopathies ($p < 0.001$) in comparison to the controls. Lipid peroxidation (MDA) as a marker of oxidative stress in the plasma of patients with malignant haemopathies was also increased in comparison with healthy volunteers ($p < 0.05$). Moreover, a positive correlation was observed between the levels of plasma Cp and MDA in patients with malignant haemopathies ($R = 0.407$, $p = 0.74$) but not in the controls. After polychemotherapy, the oxidative stress significantly progressed and appeared to be compromised by augmented activity of ceruloplasmin in these patients.

Key words: Ceruloplasmin, lipid peroxidation, malignant haemopathies

INTRODUCTION

Small amounts of reactive oxygen species (ROS), such as $-O_2$, H_2O_2 , OH^{\cdot} , are constantly generated in aerobic organisms as a consequence of aerobic respiration and substrate oxidation (Hurst *et al.*, 1997; de Zwart *et al.*, 1999) ROS are involved in cell growth, differentiation, progression and death (Ghosh *et al.*, 1998). An imbalanced production of ROS plays a role in the pathogenesis of a number of human diseases such as ischemia/reperfusion injury, atherosclerosis, cancer, neurodegenerative diseases and allergy (Wiseman *et al.*, 1996). It is known that ROS are found to be involved in both initiation and promotion of multistage carcinogenesis (Cerutti, 1985) and tumour cells are more susceptible to oxidative stress than the surrounding normal cells. Patients with cancer exhibit increase in lipid peroxidation products and impaired antioxidant status (Abdel-Aziz and El-Naggar, 1997). Several anticancer drugs such as adriamycin, mitomycin C, bleomycin, etc,

are known to bring about their tumoricidal actions by a free radical-dependent mechanism (Mimnaugh.E. *et al.*, 1985). The enzymatic and non-enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), ascorbic acid (vitamin C), α -tocopherol (vitamin E) glutathione (GSH), β -carotene, and vitamin A (Scott MD *et al.*, 1989). In addition, there exists in human plasma the acute-phase glycoprotein - ceruloplasmin (Cp) a 132kDa copper binding glycoprotein. Cp has been considered a type of plasma antioxidant due to its ability to react with and scavenge toxic oxygen species such as superoxide and hydrogen peroxide (Ozgunes *et al.*, 1995; Harris *et al.*, 1997).

We have already reported that erythrocyte SOD activity was decreased and CAT activity was significantly increased for patients with myelo- and lymphoproliferative diseases in comparison with healthy volunteers (Kutchukova *et al.*, 2001).

In furtherance of this nature of work, we now aimed in the present study to investigate the plasma levels of Cp in patients with different malignant haematological diseases before and after different regimens of polychemotherapy. The

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correlation between the level of Cp and lipid peroxidation products (MDA), a marker of oxidative stress, was also investigated in those patients.

MATERIALS AND METHODS

Patients

This study was carried out on 51 patients suffering from different malignant haematological diseases (**Table 1**). Malignant group included myeloproliferative

diseases like acute myeloblastic leukaemia (AML), chronic myeloblastic leukaemia (HML), polycythaemia vera (PV) and lymphoproliferative diseases like non-Hodgkin's lymphoma (NHL), Hodgkin's diseases (HD), malignant lymphoma (ML), acute lymphoblastic leukaemia (ALL), chronic lymphoblastic leukaemia (HLL), multiple myeloma (plasmacytoma). A group of 64 healthy volunteers, aged between 14 and 55 years, was chosen as Control.

Table 1: Details of the patients

<i>Diagnosis</i>	<i>N</i> <i>/m:f/</i>	<i>Age</i> <i>(years)</i>	<i>Therapy</i>
Controls	64 (36:28)	14-55	
Malignant:	51 (22:29)	20-82	
a) Myeloproliferative Disorders and Multiple myeloma (Malignant plasmacytoma)	27		vincristin, alceran, cyclophosphamid, cyclomin, hydroxyurea, heparin (for polycythaemia vera)
b) Lymphoproliferative Disorders.	23		ABVD (for HD); CVP; CHOP and BACOP (for NHL)

CHOP - cyclophosphamide, doxorubicin, vincristine, prednisolone

BACOP - bleomycin, adriamycin, cyclophosphamide, vincristine, prednisolone

ABVD - adriamycin, bleomycin, vinblastine, dacarbazine

CVP - cyclophosphamide, vincristine, prednisolone

Chemotherapy

Patients were treated with different regimens of mono- and polychemotherapy that comprised the following: CHOP (cyclophosphamide, doxorubicin, vincristine prednisolone), CVP (cyclophosphamide, vincristine, prednisolone), ABVD (adriamycin, bleomycin, vinblastine, dacarbazine), BACOP (bleomycin, adriamycin, cyclophosphamide, vincristine, prednisolone), Hydroxyurea, etc.

Lipid peroxidation

Lipid peroxidation was evaluated by the Thiobarbituric Acid Reactive Substances method (TBARS). This method evaluated malondialdehyde (MDA) reactive products, the last products of lipid breakdown caused by oxidative stress. The optical density was determined at 532 nm using Ultraspec III UY/ Visible Spectrophotometer (Plaser *et al.*, 1966).

Ceruloplasmin

The method for the determination of the

oxidase activity of Cp in plasma is based on the ability of Cp to oxidise substrates such as o-dianisidine yielding a yellow product. The optical density was determined at 540 nm using Ultraspec III UV/Visible Spectrophotometer (Harris *et al.*, 1997).

RESULTS

Results of determination of plasma ceruloplasmin in patients with Malignant Haematological Diseases are given on **Figure 1**. The concentration of ceruloplasmin in patients with malignant haematological diseases was significantly increased compared to controls (mean 27.48 mg/l vs 8.58 mg/l, $p < 0.0001$). The values of ceruloplasmin in patients with malignant diseases after therapy remained very high (mean 29.62 mg/l).

Results of determination of plasma lipid peroxidation products (MDA) in patients with Malignant Haematological Diseases are given on **Figure 2**. Lipid peroxidation in the plasma of patients with malignant diseases was slightly increased as compared to the control group, (mean 2.26 μ M vs 1.88 μ M, $p > 0.05$). However, in patients treated with PHT lipid peroxidation products were found to be significantly higher in comparison to the

patients in the group with malignant diseases before therapy (mean $2.48 \mu\text{M/l}$, $p < 0.001$).

Moreover, a positive correlation was also observed between MDA level and ceruloplasmin activity in patients with

malignant diseases ($R=0.407$, $p=0.74$, Correlation analysis) but not in the controls (**Figure 3**).

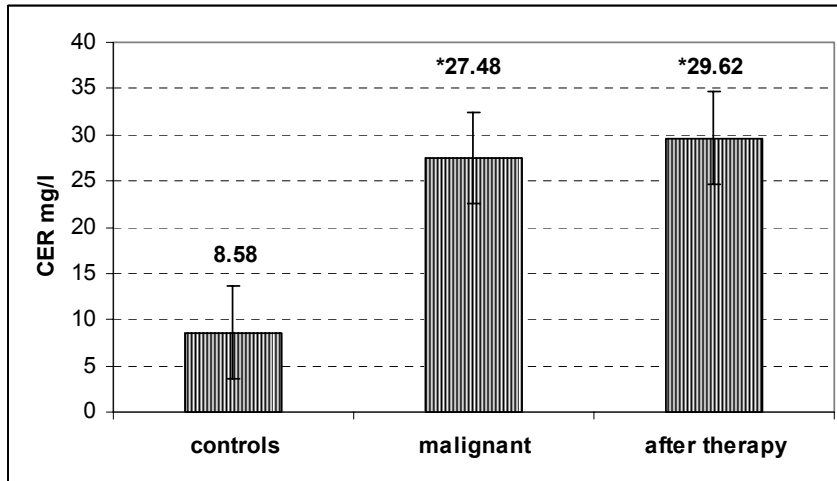


Figure 1. Results of determination of plasma ceruloplasmin in patients with Malignant Haematological Diseases: * $p=0.001$ compared to controls;

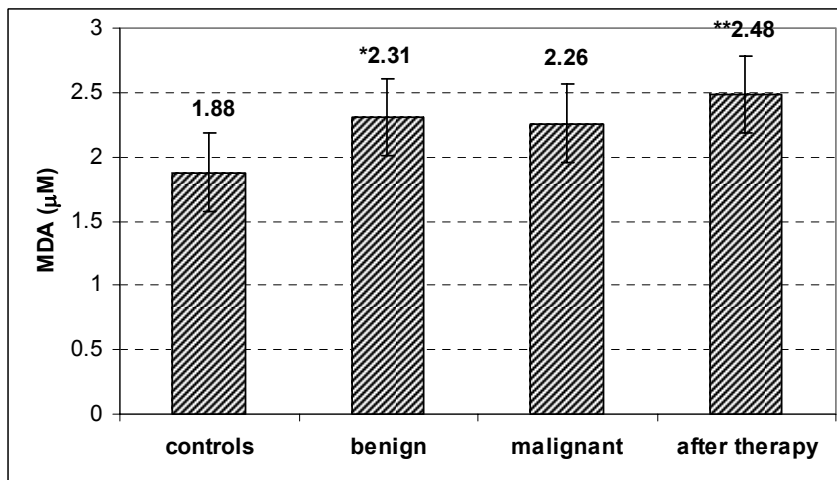


Figure 2: Results of determination of plasma lipid peroxidation products (MDA) in patients with Malignant Haematological Diseases: * $p=0.05$ compared to controls; ** $p < 0.001$ compared to the group with Malignant Haematological Diseases before therapy.

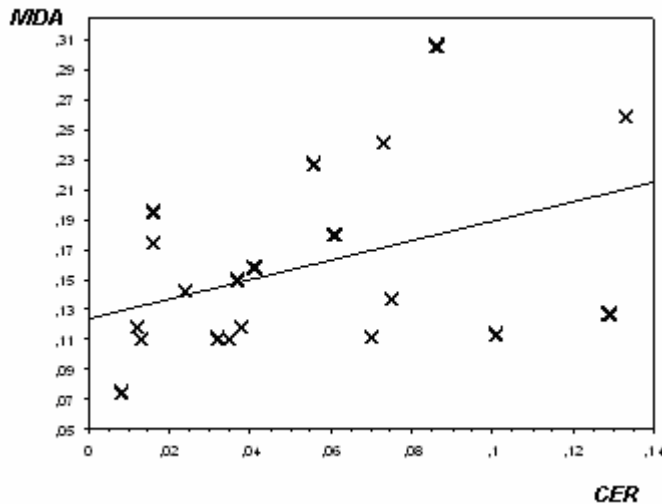


Figure 3. Correlation between MDA and Ceruloplasmin activity for patients with malignant haemopathies ($R=0.407$, $p=0.74$, Correlation analysis).

DISCUSSION

Over 95% of plasma copper is bound to Cp and this fact is an evidence of role of Cp in copper transport. The copper atom of Cp is a prerequisite for copper utilisation in the biosynthesis of cytochrome C oxidase and that Cp can transfer copper to metal-free superoxide dismutase. In addition, Cp, as a growth factor, can be considered a regulatory function of the protein; it is mediated by the enzymatic ability of Cp to convert Fe (II) to Fe (III). A further activity of Cp is at the border between a regulatory and an enzymatic function. Increased levels of Cp are found in patients who have chronic infections, degenerative diseases, leukaemia, Hodgkin's diseases, and other malignant tumours (Harris *et al.*, 1997).

Our results suggest an oxidative stress presented in non-treated patients with malignant haematological diseases demonstrated by the increased levels of MDA as a consequence of abnormality in antioxidative metabolism due to the cancer process. The oxidative stress might lead to compensatory increased level of the ceruloplasmin in these patients. The positive correlation observed between MDA level and ceruloplasmin activity in patients with malignant diseases confirmed that the oxidative stress appears to be compromised by augmented activity of ceruloplasmin in these patients. After polychemotherapy, the oxidative stress significantly progresses in consequence of the effect of the different regimens of polychemotherapy: CHOP, CVP, ABVD and BACOP. Our further aim of the research is to follow up the oxidative status of the patients during the different regimens of mono- and polychemotherapy.

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