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Research Article

Oxidant/Antioxidant Status and their Relations to Chemotherapy in Non-Hodgkin's Lymphoma

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ABSTRACT

Objectives: Oxidative stress is one of several factors which contribute to the development of non-Hodgkin's lymphoma. The aim of the study was an assessment of the activity of oxidant/antioxidant status in patients with non-Hodgkin's lymphoma, before and one month after starting the specific cytotoxic regimen. Patients and methods: This study was conducted on 146 adult patients who were diagnosed as non- Hodgkin's lymphoma, besides 60 adult healthy persons served as controls. Their age ranged from 22 to 65 years, the protocol of chemotherapy was CHOP. All subjects gave written informed consent. Initially for both patients and control, serum MDA and SOD were measured and reported one month from starting specific cytotoxic drugs for the patient groups. Results: Before starting cytotoxic regimen, there was a highly significant rise in serum MDA concentration in patients with non-Hodgkin's lymphoma in comparison with control. After one month from starting cytotoxic regimen, there was a highly significant rise in serum MDA concentration and serum levels of SOD showed insignificant difference between NHL lymphoma patients before chemotherapy and controls, while the levels were significantly decreased in patients after one month of starting treatment compared to them before treatment and compared to controls. By comparing the period before and after starting cytotoxic regimen in patients with lymphoma, there was a significant rise in MDA and a significant reduction in SOD. Conclusion, patients with non-Hodgkin's lymphoma were under great oxidative stress during cytotoxic regimen as manifested by a rise in MDA and a reduction in SOD on comparison to those of controls, these results suggest that chemotherapy destroys the oxidant/antioxidant equilibrium in the body serum level.

Key words: NHL. Superoxide dismutase, Malondialdehyde.

INTRODUCTION

Non-Hodgkin's lymphomas (NHL) are a heterogeneous group of lymphoproliferative malignancies with differing patterns of behavior and responses to treatment, types of NHL vary significantly in their severity, from slow growing to very aggressive. Non-Hodgkin lymphomas can occur at any age and are often marked by lymph nodes that are larger than normal, fever, and weight loss. In Egypt, non- Hodgkin's lymphoma the fifth most common cancer in both sexes**.** Aggressive non-Hodgkin's lymphoma represents around 60% of all lymphoma as in the Western world and even more in Egypt[1]. Oxidative stress is defined as a type of physiological stress on the body caused by the damage done by free radicals inadequately neutralized by antioxidants. It has long been known that oxidative stress is an essential mechanism by which chemotherapy works to treat cancer. Oxidative stress caused by the increased production of reactive oxygen species and/or decreased efficacy of the antioxidant system is implicated in the pathogenesis of various disease entities, such as arteriosclerosis, malignant tumors, and autoimmune diseases[2]. Reactive oxygen species (ROS) are normal degradation products of aerobic cellular metabolism and hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), and hydroxyl radicals (OH^*). In pathologic conditions such as ischemia-reperfusion and certain metabolic derangements, excessive production results in oxidative stress and free radical damage to lipids, proteins, and DNA that has been associated with the initiation, promotion, and progression of carcinogenesis and other chronic diseases[3]. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals. This process, leading to the production of lipid peroxides and their by-products, and ultimately the loss of membrane function and integrity. Lipid peroxidation is widely accepted to be involved in the pathogenesis of several human diseases including cancer[4]. Measurement of Malondialdehyde (MDA) levels in serum provides a suitable in vivo index of lipid peroxidation and represents a non-invasive biomarker of oxidative stress often clinically employed to investigate radical-mediated physiological and pathological conditions[5]. The aim of the present study is to assess the oxidant/antioxidant serum levels of MDA and SOD before and one month after specific cytotoxic regimen and in comparison with

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controls in order to evaluate their prognostic significance. Also, to investigate the relationship between levels of these markers and other different prognostic factors (LDH, ESR) in patients with non- Hodgkin's lymphoma.

PATIENTS AND METHODS

This study was conducted on 146 adult patients who were diagnosed as non- Hodgkin's lymphoma in the Medical Oncology Department at National Cancer Institute, Cairo University. The staging procedure was done by detailed history, careful physical examination, chest radiograph with CT chest if the radiograph is positive, abdominal and pelvic CT and bone marrow examination with core biopsy from the posterior iliac crest. There were no age restrictions (22 to 65 years). Patients were excluded if they had any previous treatment with chemotherapy or radiotherapy. All patients gave written informed consent. Besides 60 adult healthy persons served as controls. The protocol of chemotherapy was CHOP (cyclophosphamide + doxorubicin + vincristine + prednisone). CHOP was given in eight consecutive 14-day courses unless progressive disease developed. Clinical data and follow up information were obtained by reviewing the patients' medical records[6].

Sampling

Blood samples were taken from each subject (controls and patients before chemotherapy). Further blood samples were obtained from the NHL patients one month after starting chemotherapy. Ten ml fasting venous blood sample were taken for assay. Five ml blood put in tubes containing EDTA for analysis of the blood count. The other five ml blood left to clot at room temperature to separate sera after centrifuging for measuring other parameters. Sera were stored at –70°C till the time of analysis.

All patients were subjected to the following laboratory assessments:

Hemogram: including hemoglobin concentration, total leucocytic count (TLC), platelet count using Coulter counter and examination of Lishman or Wright-stained peripheral blood smears.

Determination of serum alanine transaminase (ALT) and serum aspartate transaminase (AST) levels by using the method recommended by Committee on enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology[7]. The test was performed using already commercially available kit from Boehringer-Mannhiem Company, Germany.

Determination of serum urea level was done by colorimetric method according to Tietz[8]. The Kit from Croma Test Company Spain.

Determination of serum creatinine by enzymatic method utilizes a multi-step approach ending with a photometric end-point reaction. The enzyme creatinine amidohydrolase is used to convert creatinine to creatine. Creatine is broken down to sarcosine and urea by creatine amidinohydrolase. Further enzyme linked steps with sarcosine oxidase and peroxidase yield a colored chromogen read at 545nm according to Young[9].

Erythrocytic sedimentation rate (ESR) was measured by standard laboratory technique (normal values <20 mm/h) according to Westergren[10].

Measurement of Lactate dehydrogenase (LDH) enzyme activity in the serum was estimated by the kinetic procedure according to the recommendations of the Committee on the Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology[11], using pyruvate as substrate at 37ºC. The reaction was monitored at 340 nm. The test was performed by using the commercially available kit from Bio-Merieux Company, France.

Measurement of MDA concentration in the serum was estimated by the method of Buege and Aust[12]. Assay of oxidative damage in the serum of the patients as well as healthy control samples was assessed by measurement of products of lipid peroxidation in serum by the thiobarbituric acid (TBA) method. MDA, which is a stable end product of fatty acid peroxidation, reacts with TBA at acidic conditions to form a complex that has maximum absorbance at 532 nm.

Measurement of SOD activity in the serum was assayed according to the method of Sun[13]. This method employs xanthine and xanthine oxidase to generate superoxide anion which reacts with nitro blue tetrazolium (NBT) and then measured by the degree of inhibition of the reaction one unit of enzyme provides 50% inhibition of NBT reduction. Results were expressed as U/ml.

Statistical analysis

Statistical analysis Statistical analysis was performed using the SPSS for Windows package (version 11.0, SPSS, Chicago, IL, USA). Data were presented as range, mean ±SD and number (%). Comparisons between the groups (healthy controls, patients with NHL (before treatment) and patients with NHL (after treatment) was performed using T test or one way ANOVA as a parametric tests. Correlations between serum biomarkers were analyzed with the Pearson's correlation method. P- Value of < 0.05 was considered statistically significant.

RESULTS

One hundred and forty six patients were included in the study, 85 were males and 61 were females. The median age was 42 years (range 22- 65 years). Seventeen patients had positive family history. Six patients died through the study. The demographic and biochemical characteristics of the control and NHL groups were illustrated in table (1).

Results of determination of serum MDA, SOD, LDH, WBCs and ESR are given in table (2). WBCs count showed insignificant difference between controls and patients before and after chemotherapy while there was significant decrease in WBCs count NHL lymphoma patients after chemotherapy compared to them before treatment (5.64 vs. 6.94, $p < 0.0001$). ESR was significantly higher in patients before and after treatment than in controls (mean 32.28 and 26.02 vs. 10.40, $p<0.0001$ and $p<0.001$ respectively), it decreased in patients after treatment but this decrease was not statistically significant ($p>0.05$) tables (2, 3, 4). The concentration of serum MDA was significantly increased

Table 1: demographic and biochemical characteristic of Control and NHL groups at diagnosis (Mean ± SD)

Parameters	Controls	NHL Patients
Number	60	146
Sex (male/female)	38/22	85/61
Age	42± 2.1	41 ± 1.5
Family history		
Positive (%)	---	17 (11.6%)
Negative (%)	----	129 (88.4%)
Red blood cells (x 10^6 cells/mL)	4.67 ± 0.33	3.86 ± 0.4
Hemoglobin (g/dL)	13.0 ± 1.1	10.3 ± 0.6
Leucocytic count ($\times 10^9$/L)	6.290 ± 1.797	6.94 ± 3.41
AST (IU/L)	29.1 ± 1.71	25 ± 2.6
ALT (IU/L)	28.27 ± 1.01	31.72 ±1.45
Urea (mg/dL)	16.2±2.6	17.5 ± 2.6
Creatinine (mg/dL)	0.85±0.05	0.92 ± 0.01

Table 2: Variation of measured parameters in controls and NHL patients before treatment (Mean ± SD)

Parameters	Controls	NHL Patients before chemotherapy	p-value
No.	60	146	
MDA (nmol/ml)	0.282± 0.114	0.807 ± 0.257	<0.0001
SOD (U/ml)	16.204± 8.681	13.708± 8.823	NS
LDH (IU/L)	333.00± 102.02	869.31± 1034.67	<0.008
WBCs ($\times 10^9$/L)	6.290± 1.797	6.94± 3.41	NS
ESR(mm/h)	10.40± 4.36	32.28± 16.86	<0.0001

P<0.05 considered significant

in NHL lymphoma patients before and after chemotherapy compared to controls (mean 0.8079 and 0.5803 vs. 0.2828, p<0.0001 and p<0.0001 respectively) and in NHL lymphoma patients before treatment compared to them after treatment (p<0.0001). Results of determination of serum levels of SOD showed insignificant difference between NHL lymphoma patients before chemotherapy and controls (mean 13.708 vs16.204, p>0.05), while the levels were significantly decreased in patients after treatment compared to them before treatment and compared to controls (mean 10.078 vs. 13.708 and 10.078 vs16.204, p <0.0001, p <0.002 respectively) tables (2,3,4). Serum LDH enzyme activity was significantly increased in NHL lymphoma patients before chemotherapy compared to them after treatment and compared to controls (mean 869.31 vs. 437.40 and 333.00, p <0.0001, p <0.008 respectively). By comparing NHL lymphoma patients after chemotherapy and controls, there was no statistically significant difference in the serum LDH enzyme activity (p>0.05). Normalization of serum LDH occurred after treatment in only patients achieving complete response tables (2, 3, and 4). Also, the results showed a positive correlation were observed between MDA level and SOD and LDH activity (R= 0.168 and R=0.340, p=0.01 respectively) figure 1.

DISCUSSION

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals. This process, leading to the production of lipid peroxides and their byproducts, and ultimately the loss of membrane function and integrity. Lipid peroxidation is widely accepted to be involved in the pathogenesis of several human diseases including cancer[14]. Measurement of Malondialdehyde (MDA) levels in serum provides a suitable in vivo index of lipid peroxidation and represents a non-invasive biomarker of oxidative stress often clinically employed to investigate radical-mediated physiological and pathological conditions[15]. It is well known that oxidative stress may be associated not only with initiation, but also with promotion and progression in the multi-stage carcinogenesis model. In fact, the abnormal production of cellular oxidants or the imbalance of the antioxidant control systems have been linked to mutation (induced by oxidant-induced DNA damage), as well as modification of gene expression[16]. SOD is the key enzyme required for the removal of O_2 by converting it to hydrogen peroxide (H_2O_2), which can be eliminated by CAT and peroxidases. Catalase helps in neutralizing the toxic effect of H_2O_2 converting it to water and non-reactive oxygen species, thus it prevents the generation of hydroxyl radicals and protects cells from oxidative damage[14]. They also act as anti-carcinogens and inhibitors at initiation and promotion/transformation stage in carcinogenesis. Mutation caused by potassium superoxide in mammalian cells is blocked by SOD[17]. Our study revealed a highly significant rise in MDA and reduction in SOD in serum of patients with non-Hodgkin's lymphoma before and one month after starting chemotherapy as compared to controls. Our results suggested an oxidative stress presented in non-treated patients with NHL demonstrated by the increased levels of MDA and decreased levels of SOD as a consequence of abnormality in antioxidative metabolism due to the cancer process. One month after starting chemotherapy, the oxidative stress was significantly higher than controls as a result of the effect of the chemotherapy. Imad[18] found a highly significant rise in MDA and reduction in TAS (total antioxidant status) in serum of patients with malignant lymphoma before and one month after starting chemotherapy as compared to controls and concluded that patients with malignant lymphoma were under great oxidative stress during cytotoxic regimen as manifested by a rise in MDA serum level and a reduction in TSA. Abou-Seif et al[19], reported that the MDA serum level and osmotic fragility of RBC in patients with malignant lymphoma were higher before and after treatment in comparison with control group whereas plasma L-ascorbic acid concentration were lower than control group and concluded that the hematological complications and autoimmune hemolytic anemia might be attributed to the oxidative stress produced by malignant lymphoma. There have been a few previous reports on antioxidant enzyme abnormalities in patients with non- Hodgkin's disease.

Table 3: Variation of measured parameters in controls and NHL patients after one month of starting treatment (Mean ± SD)

Parameters	Controls (Mean± SD)	Patients after chemotherapy	p –value
No.	60	140	
MDA (nmol/ml)	0.282 ± 0.114	0.580 ± 0.0190	<0.0001
LDH (IU/L)	333.00 ± 102.02	437.40 ± 295.66	NS
SOD (U/ml)	16.204 ± 8.681	10.078 ± 5.444	<0.002
WBCs ($\times 10^9$/L)	6.290 ± 1.797	5.64 ± 1.72	NS
ESR (mm/h)	10.40 ± 4.36	26.02 ± 21.20	<0.001

P<0.05 considered significant

Table 4: Significance of measured parameters in NHL patients before and after one month of starting treatment (Mean ± SD)

Parameters	Patients before chemotherapy	Patients after chemotherapy	p –value
No.	146	140	
MDA (nmol/ml)	0.807± 0.257	0.580± 0.019	<0.0001
SOD (U/ml)	13.708± 8.823	10.078± 5.444	<0.0001
LDH (IU/L)	869.31± 1034.67	437.40± 295.66	<0.0001
WBCs ($\times 10^9$/L)	6.94± 3.41	5.64± 1.72	<0.0001
ESR (mm/h)	32.28± 16.86	26.02± 21.20	<0.01

P<0.05 considered significant

Bewick et al[20], reported significantly lower activities of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) without changes of catalase activities in patients with malignant lymphoma. On the other hand, Gonzales et al[21], found significantly higher activity of erythrocyte SOD without changes of GPX activity in patients with malignant lymphoma, because the cellular

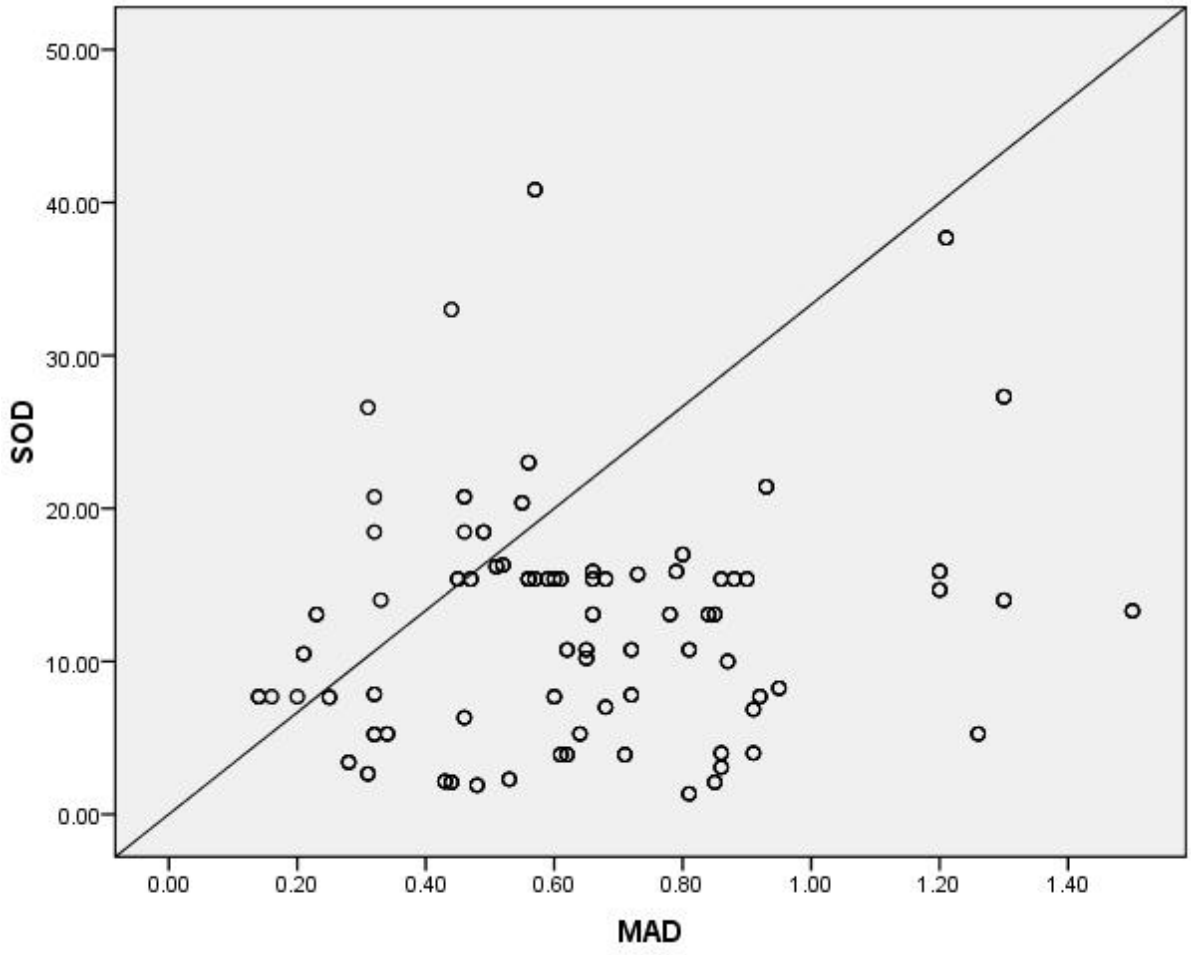

Figure 1: Correlations between SOD (U/ml) and MDA (nmol/ml) parameters in NHL patients

antioxidants status depends on the stage of malignancy and the histological pattern of tumor, the different histological types and clinical stages of patients with malignant lymphoma, can account for different reported results of antioxidant enzymes. Guven et al[22], also was in agreement with our results, who found a significantly higher concentration of MDA in plasma and erythrocytes of patients with malignant lymphoma compared to control group and concluded that the antioxidant system is impaired in malignant lymphoma. Zhu et al[23] reported that chemotherapy depletes antioxidant capacity of cancer patients. On the other hand Gadjeva et al[24], reported that plasma MDA levels were found to be significantly higher in patients treated with cyclophosphamide, vincristine, prednisolone (CVP), or adriamycin, bleomycin, vinblastine and dacarbazine (ABVD), in comparison to control group and concluded that after poly-chemotherapy, the oxidative stress and the imbalance of antioxidant enzyme systems significantly progress in patients with lympho-proliferative hematological disease. Also in agreement with the finding of this study, a study conducted by Kaya et al[5], who reported a rise in serum MDA and a significant reduction in antioxidant parameters SOD and GPX, in patients with lymphoma seven days after receiving adriamycin, bleomycin, vincristine and dexamethasone (ABVD) treatment protocol. Our results were in agreement with Hossam et al[25] who concluded that MDA level was elevated in NHL and HL patients, before and after treatment on comparison to that of controls. But SOD, GSH levels were decreased before and after treatment at all. Several studies have evaluated enzymatic antioxidant activities in patients with hematological malignancies and their findings are not consistent. Caroline et al[26] reported increased superoxide dismutase (SOD) and GPX in patients with hematological malignancies but Sonali and Medhusnata[17] and Guven et al[22], reported the reverse. Recently, Ravi and his colleagues[27] found that the level of marker of lipid peroxidation (MDA) and DNA damage were found to be raised significantly in patients with lymphoma and multiple myeloma in comparison to healthy controls. Their results indicate that oxidative stress and DNA damage activity increase progressively with the progression of disease. Moreover, the results of this study showed a positive correlation between MDA level and SOD activity in patients with non-Hodgkin's lymphoma. These results were in agreement with Gadjeva et al[24] who found a positive correlation between MDA level and ceruloplasmin activity (antioxidant) in patients with malignant hematological diseases including malignant lymphomas. In conclusion, patients with non-Hodgkin's lymphoma were under great oxidative stress during cytotoxic regimen as manifested by a rise in MDA and a reduction in SOD on comparison to those of controls, these results suggest that chemotherapy destroys the oxidant/antioxidant equilibrium in the body serum level. As regards we observed the patients only after one month in the course of chemotherapy protocol. We did not quantify the contribution arising from the patients' response. Many drugs used in cancer chemotherapy form free radicals as a result of their metabolic activities. On the other hand, tumor regression itself induces free radicals[5]. We suggest that the change in oxidant/ antioxidant parameters could be due to both the direct effect of chemotherapy and the indirect effect of tumor regression. Further experimental and clinical studies of the role of ROS in tumor biology may lead to new strategies for cancer therapy**.**

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