

OXIDATIVE STRESS AND IMMUNOGLOBULIN LEVELS IN PATIENTS WITH HODGKIN'S LYMPHOMA

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ABSTRACT

Objective: To assess, oxidative stress (by measuring malondialdehyde "MDA" which reflects lipid peroxidation, and total antioxidant status "TAS"), and immunoglobulin levels (IgA, IgG, IgM) in patients with Hodgkin's lymphoma before and one month after starting cytotoxic drugs, in comparison with controls.

Design: Case-control study

Setting: This study was conducted in the Hospital of Nuclear Medicine and Department of Pharmacology, College of Medicine, University of Mosul.

Patients and Methods: Twenty-three patients with Hodgkin's lymphoma were included in this study together with 24 apparently healthy subjects taken as a control. Initially for both patients and control, serum MDA, TAS and immunoglobulin levels (IgA, IgG, IgM) were measured and reported one month from starting specific cytotoxic drugs for the patient groups and after one month gap period for the control using especial commercial kits from Randox company (UK), for measuring TAS and immunoglobulin levels, while measurement of MDA was done by laboratory method.

Results: Before starting cytotoxic regimen, there was a highly significant rise in serum MDA concentration in patients with 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 a significant reduction in TAS and serum immunoglobulin levels. 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 TAS and immunoglobulin levels with the exception of IgM which showed insignificant reduction.

Conclusion: This study revealed a rise in the oxidative stress; to which patients with Hodgkin's lymphoma were liable to, by the effect of specific cytotoxic regimen. This is reflected by the rise in the serum level of MDA and a reduction in the TAS after cytotoxic regimen. It also indicated a reduction in immunity of such patients during cytotoxic regimen as reflected by a reduction in immunoglobulin levels.

INTRODUCTION

It is well recognized that reactive oxygen species (ROS) are produced in a well regulated manner to help to maintain homeostasis on the cellular level, and normal healthy tissues^[1]. Also ROS are involved either directly or indirectly in aging process^[2] and in various clinical disorders including cancer^[3]. Oxygen free radicals have been suggested to exert their cytotoxic effects on membrane phospholipids^[4], which stimulate the process of lipid peroxidation, which is a chain of reactions providing a continuous supply of free radicals^[3] and the end product of these reactions are malondialdehyde (MDA), ethane and pentane^[4]. Antioxidants allow aerobic organism to withstand daily episodes of oxidative stress by counteracting the adverse effect of free radicals, which are produced by metabolic activities within the body. In addition antioxidants deal or inhibit oxidation of molecules such as carbohydrates, fat, protein and DNA^[5]. Aggressive chemotherapy is the cornerstone of cancer therapy. Some reports showed that endogenous antioxidants are reduced in patients

with cancer, while other showed that administration of antineoplastic agents during cancer chemotherapy results in much greater degree of oxidative stress than is induced by cancer itself^[6]. Reports about immunoglobulins in patients with lymphoma before and after cytotoxic regimen are few and controversial. The aim of this study is to assess oxidant/antioxidant status and immunoglobulin levels (IgA, IgG, IgM) in patients with Hodgkin's lymphoma, before and after specific cytotoxic regimen and in comparison with controls.

PATIENTS AND METHODS

This study was conducted in the Hospital of Nuclear Medicine and the Department of Pharmacology, College of Medicine, University of Mosul, from Jan 2005 to Sept. 2006. Patients included in this study, should meet certain criteria for selection.

Criteria for selection of cases:

Diagnosis of cases of Hodgkin's lymphoma (proved by histopathology) was done by a

specialist in this field for which specific cytotoxic regimen were given (adriamycin, bleomycin, vinblastine and dacarbazine" ABVD"), no cardiovascular, respiratory, renal or hepatic diseases or DM, non smoker, cooperative and compliant patients. Out of 29 cases interviewed, only 23 fulfilled the criteria of selection and included in this study. They were 4 females and 19 males with a mean age \pm SD of 47.39 \pm 8.86 year (ranged between 28 and 59 year). 24 apparently healthy non-smoker subjects were also included, as a control group. They were 19 males and 5 females with a mean age \pm SD of 19.54 \pm 8.73 year (ranged between 28 and 60 year). Initially for the control and, before starting cytotoxic regimen for the patients, a 7 ml venous blood sample were taken for the assay of serum MDA, total antioxidant status (TAS) and immunoglobulin levels (IgA, IgG, IgM). After one month gap-period for the control and one month from starting specific cytotoxic regimen for the patients, 7 ml venous blood sample were taken for assay of the same parameters.

Planned cytotoxic regimen:

ABVD 6 cycles, days 1 & 15 of each cycle, every 28 days, as follows

Adriamycin 25 mg/m² IV days 1 & 15

Bleomycin 10 mg/ m² IV days 1 & 15

Vinblastin 6 mg/ m² IV days 1 & 15

Dacarbazine 375mg/ m² IV days 1 & 15

MATERIALS

1. Measurement of MDA in the serum:

The serum concentration of MDA was estimated by the method of Buege and Aust (1978)

2. Measurement of TAS:

Total antioxidant status (TAS) was measured by peroxidase/H₂O₂/ABTs colorimetric assay using kits from Randox company (UK).

3. Measurement of serum immunoglobulins (IgA, IgG, IgM):

Immunoglobulin levels were measured using immunoturbidmetric method with kits from Randox Company (UK).

Statistical analysis:

Standard statistical methods were used to determine the mean and standard deviation (SD).

Paired and unpaired t-tests were used to compare the results among the same group and with controls. Difference between observations were considered significant at P<0.05

RESULTS

There were insignificant differences between the levels of the parameters under assay (MDA, TAS, IgA, IgG, IgM) initially and after one month gap-period in the control group (Table-1).

Table 1. Variation of measured parameters in control group after one month.

Parameters	Mean \pm SD		P-value
	Initial	After one month	
IgA (mg/dl)	221.88 \pm 39.89	216.67 \pm 43.68	NS
IgG (mg/dl)	866.67 \pm 149.28	855.42 \pm 137.93	NS
IgM (mg/dl)	173.13 \pm 28.51	173.96 \pm 27.66	NS
MDA (mg/l)	1.26 \pm 0.32	1.25 \pm 0.30	NS
TAS (μ mol/l)	1.52 \pm 0.15	1.52 \pm 0.15	NS

By comparing control in the initial state, with the Hodgkin's lymphoma patients before starting cytotoxic regimen, there was a highly significant increase in MDA serum levels in

patients with lymphoma in comparison to controls (P<0.001), while the other parameters under assay showed insignificant difference (Table-2).

Table 2. Comparison of Hodgkin's lymphoma patients before treatment with controls.

Parameters	Mean \pm SD		P-value
	Patients before treatment	Controls	
IgA (mg/dl)	216.74 \pm 36.45	221.88 \pm 39.89	NS
IgG (mg/dl)	831.74 \pm 72.40	866.67 \pm 149.28	NS
IgM (mg/dl)	158.91 \pm 24.59	173.13 \pm 28.51	NS
MDA (mg/l)	2.22 \pm 0.25	1.26 \pm 0.32	< 0.001
TAS (μ mol/l)	1.62 \pm 0.53	1.52 \pm 0.15	NS

By comparing controls after one month gap period with the Hodgkin's lymphoma patients after one month from starting cytotoxic regimen, there was a significant reduction in the

levels of TAS ($P < 0.005$), immunoglobulins IgA ($P < 0.001$), IgG ($P = 0.001$), and IgM ($P = 0.001$) with a significant increase in MDA serum level in comparison to controls (Table-3).

Table 3. Comparison of Hodgkin's lymphoma patients on chemotherapy with controls after one month period.

Parameters	Mean \pm SD		P-value
	Patients after one month of chemotherapy	Controls after one month	
IgA (mg/dl)	179.57 \pm 20.11	216.67 \pm 43.68	<0.001
IgG (mg/dl)	726.52 \pm 83.41	855.42 \pm 137.93	0.001
IgM (mg/dl)	156.30 \pm 24.32	173.96 \pm 27.66	0.001
MDA (mg/l)	2.71 \pm 0.18	1.25 \pm 0.30	<0.001
TAS (μ mol/l)	1.21 \pm 0.41	1.52 \pm 0.15	<0.05

By comparing patients with Hodgkin's lymphoma before and one month after starting cytotoxic regimen, there was a significant reduction ($P < 0.001$) in immunoglobulin levels

(with the exception of IgM which showed insignificant reduction), with a significant reduction in TAS, while MDA serum levels showed a raised levels (Table-4).

Table 4. Effect of chemotherapy on measured parameters in Hodgkin's lymphoma patients.

Parameter	Mean \pm SD		P-value
	Before chemotherapy	After Chemotherapy	
IgA (mg/dl)	216.74 \pm 36.45	179.57 \pm 20.11	<0.001
IgG (mg/dl)	831.74 \pm 72.40	726.52 \pm 83.41	<0.001
IgM (mg/dl)	158.91 \pm 24.59	156.30 \pm 24.32	NS
MDA (mg/l)	2.22 \pm 0.25	2.71 \pm 0.18	<0.001
TAS (μ mol/l)	1.62 \pm 0.53	1.21 \pm 0.41	<0.001

DISCUSSION

Hodgkin's lymphoma represents approximately 30% of all malignant lymphomas, and despite the recent treatment success rate, the pathogenesis is still largely unknown. 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^[3,7]. 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^[8]. 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^[9]. Our study revealed a highly significant rise in MDA and reduction in TAS in serum of patients with Hodgkin's lymphoma before and one month after starting chemotherapy as compared to control. Abou-Seif et al (2000), reported that the MDA serum level and osmotic fragility of RBC in patients with 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^[10]. There have been a few previous reports on antioxidant enzyme abnormalities in patients with Hodgkin's disease. Bewick et al (1987), reported significantly lower activities of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) without changes of catalase activities in patients with Hodgkin's disease^[11]. On the other hand, Gonzales et al (1984), found significantly higher activity of erythrocyte SOD without changes of GPX activity in patients

with Hodgkin's disease^[12]. Because the cellular antioxidants status depends on the stage of malignancy and the histological pattern of tumor, the different histological types and clinical stages of patients with Hodgkin's disease, can account for different reported results of antioxidant enzymes^[13]. The sum of endogenous and food derived antioxidants represents the total antioxidant capacity of extracellular fluids, which integrates the cumulative effects of all antioxidants present in the plasma and body fluid and may give more relevant biological information as compared to that obtained by the measurement of individual antioxidant parameters^[14]. That is why we concentrate in this study on measuring total antioxidant status (TAS) rather than individual antioxidant parameters. Also in agreement with our results, Guven et al (2000), who found a significantly higher concentration of MDA in plasma and erythrocytes of patients with Hodgkin's lymphoma compared to control group and concluded that the antioxidant system is impaired in Hodgkin's disease^[13]. Zhu et al (2004) reported that chemotherapy depletes antioxidant capacity of cancer patients^[15]. On the other hand Gadjeva et al (2005), reported that plasma MDA levels were found to be significant 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 polychemotherapy, the oxidative stress and the imbalance of antioxidant enzyme systems significantly progress in patients with lymphoproliferative hematological disease^[16]. Also in agreement with the finding of this study, a study conducted by Kaya et al (2005), who reported a rise in serum MDA and a significant reduction in antioxidant parameters SOD and GPX, in patients with Hodgkin's lymphoma 7 days after receiving adriamycin, bleomycin, vincristine and dexamethasone (ABVD) treatment protocol^[17]. With regard immunoglobulin levels in patients with Hodgkin's lymphoma after one month from starting cytotoxic regimen, this study revealed a highly significant reduction in immunoglobulin levels with the exception of IgM which showed insignificant difference, but with significant differences in comparison with controls. Douer

et al (1981), by comparing immunoglobulin levels in splenectomized Hodgkin's disease patients in remission and splenectomized healthy subjects following trauma as controls reported that controls showed higher IgA and lower IgM than the normal and IgM level of patients group were decreased below normal^[18]. Solanki et al (1990), reported that the mean levels of IgG and IgA were found to be significantly decreased in malignant lymphoma and IgM levels were found to be increased in 3 cases with non-Hodgkin's lymphoma^[19]. Oborilora et al (2004) by measuring immunoglobulins (IgM, IgA, IgG) reported a significant signs of immunotoxicity manifested mainly by a significant reduction in IgG levels in patients with follicular lymphoma receiving cytotoxic regimen^[20]. In a study conducted by Zignol et al (2004), by assessing humoral immunity to poliomyelitis, tetanus, hepatitis B, measles, rubella and mumps in children after chemotherapy, he concluded that chemotherapy induced different rates of loss of protective antibody titers^[21]. In the same field, Luczynski et al (2004) reported that in the course of maintenance treatment in children with ALL, constant suppression of immune system of both humoral and cellular response can be observed^[22].

In conclusion, patients with Hodgkin's lymphoma were under great oxidative stress during cytotoxic regimen as manifested by a rise in MDA serum level and a reduction in TAS, also impairment of immunity can be anticipated during such period in those patients.

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