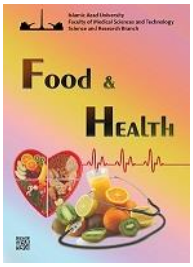

Food & Health Journal
Journal homepage: fh.srbiau.ac.ir

Serum levels of malondialdehyde, glutathione peroxidase and superoxide dismutase in patients with non-Hodgkin's lymphoma in Zahedan

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ARTICLE INFO

Original Article

Article history:
Received 23 January 2020
Revised 10 March 2020
Accepted 19 May 2020
Available online 15 June 2020

Keywords:
Non-Hodgkin lymphoma
Malondialdehyde
Glutathione peroxidase
Superoxide dismutase

ABSTRACT

Imbalance in the production of oxidative molecules and antioxidant activity plays an important role in carcinogenesis. This study was performed to evaluate the serum level of malondialdehyde (MDA) as oxidative damage marker, superoxide dismutase (SOD) as well as glutathione peroxidase (GPx) as an antioxidant defense system in non-Hodgkin's lymphoma (NHL). Twenty-five NHL patients and twenty-five healthy individuals were included in the study. The data showed that lower activity of enzymatic antioxidants (GPx, SOD) and higher MDA levels in NHL patients than in the control group. The results suggest that increased serum MDA and decreased SOD and GPx activity may be due to oxidative stress, which may play an important role in NHL formation. the role of oxidative stress in the pathogenesis of the NHL has not been extensively studied. Therefore, the present study aimed to measure the level of MDA as well as GPx and SOD activity in blood tissue collected from NHL patients compared with the control group.

1. Introduction

There has been a significant outbreak of various cancers in recent decades. In Iran, cancer is the third leading cause of death and lymphoma is the ninth most common cancer (1). Non-Hodgkin's lymphoma (NHL) is a heterogeneous disease of lymphoproliferative malignancy (2, 3). Risk factors for NHL malignant lymphomas are not well understood, but some risk factors that may cause NHL to include age, sex, race, genetic factors and family history of lymphoma, environmental risk factors such as chemical exposure, radiation therapy, and Chemotherapy and some occupations. Such as the rubber industry, veterinarians, uranium miners, wood industry, metalworking, farmers, chemists (4-6). Oxidative stress indicators are important factors in the assessment of disease status as well as the effects of antioxidants on health promotion. The risk of lymphoma development is associated with decreased antioxidant activity (7, 8). Superoxide dismutase (SOD) is one of the antioxidant enzymes that can scavenge superoxide radicals. SOD is a very important antioxidant defense against oxidative stress that catalyzes the conversion of superoxide anion to H_2O_2, which is eventually converted to oxygen and water by catalase or glutathione peroxidase (9). Elevated SOD levels can be an important therapeutic strategy in the pathology of oxidative stress. glutathione peroxidase (GPx) is a selenocysteine-dependent enzyme that converts hydrogen peroxide to water (10). SOD and GPx can directly balance the oxidant attack and protect cells from DNA damage. GPx, a whole-body oxidoreductase enzyme, was first identified in 1957 as protecting red blood cells from H2O2 (11). GPx plays an important role in redox homeostasis, neutralizing excessive oxidation, and measuring and transmitting oxidative signals, and extends to survival, growth regulation, differentiation, apoptosis, and gene activation (12). MDA is an indicator of oxidative damage assessment. MDA is caused by the peroxidation of unsaturated fatty acids in membranes (13). Under physiological conditions, MDA easily binds to

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functional groups of proteins, lipoproteins, DNA, and RNA (14) to form additive compounds and induce mutagenesis (15). Increasing the level of adducts is a symbol of a pathological condition (16, 17). The predictive role of oxidative stress in lymphoma has already been investigated with conflicting results (18-20). Tom et al. (7) showed that the worst results were those with reduced expression of antioxidant enzymes such as catalase, glutathione peroxidase, and MnSOD. Andreadis et al. (21) showed overexpression of these genes was associated with a worse prognosis. They found that overexpression of antioxidants was associated with poor predictor scores. However, evaluating the studies proposes that enzymatic antioxidant systems may be therapeutically useful in lymphomas. Oxidative stress indicators are important factors in assessing the condition of the disease as well as the effects of antioxidants on health promotion. Case-control findings of lymphoma proposed that oxidative stress has an important role in the NHL development. However, the role of oxidative stress in the pathogenesis of the NHL has not been extensively studied. Therefore, the present study aimed to measure the level of MDA as well as GPx and SOD activity in blood tissue collected from NHL patients compared with the control group.

2. Materials and methods

This study was Case-Control (25 people) study done in Zahedan city (Sistan and Baluchestan province).

2.1. Study population

2.1.1.Patients group

Blood samples were taken from 25 patients with diagnosed and pathological lymphoma. They had no other known cancers or systemic diseases and are currently being treated. The criteria for entering the study were people with lymphoma (non-Hodgkin's) whose disease was confirmed by a pathologist Patients did not have secondary malignancies and congenital chromosomal abnormalities such as Fanconi's anemia and Bloom's Syndrome, and in terms of hematology criteria and bone marrow samples, chemotherapy protocols were appropriate to them. Besides, the demographic information of patients including age, sex, height, weight, and place of residence was performed and body mass index (BMI) was calculated.

2.1.2.Control group

Blood samples were collected from 25 healthy people in Zahedan city who had no known cancers or systemic disease.

2.2. Samples collection

The cases matched healthy controls according to age, sex, and ethnicity. 7 ml of blood samples were collected in non-coagulated tubes and stored for 25 minutes at 25˚C. Then, at 3000 rpm, the blood was centrifuged in a refrigerated centrifuge for 10 minutes, carefully collected the serum and stored at -70˚C until oxidative stress enzymes are measured. Oxidative stress enzymes are measured according to standard protocols.

2.3. Measurement of oxidative stress enzymes

The method of measuring the antioxidants studied is summarized in Table 1.

Table 1. Description of oxidant and antioxidant marker measurement variables.

Parameters	Role of variables	Type of variables	Assessment method	Scale of variables
Lymphoma	Dependent	Qualitative-nominal	Based on pathological findings	Have/ Do not have
SOD	Dependent	Quantitative - constantly	By spectrophotometric laboratory kit	U/gr Hb
MDA	Dependent	Quantitative - constantly	By Cumene hydroperoxide method	Mmol/L
GPx	Dependent	Quantitative - constantly	By Thiobarbituric acid method	Mmol/L

2.3.1.Malondialdehyde (MDA) assay

Lipid peroxidation analysis was focused on evaluating MDA levels, using the thiobarbituric method. MDA levels is usually determined on the basis of its reaction to thiobarbituric acid (TBA), where one MDA molecule with two TBA molecules reacts at high temperatures and under acidic conditions to give colorimetrically measured pink chromophores (MDA-TBA adduct) at 535 nm (22).

2.3.2.Superoxide dismutase (SOD) assay

A commercial assay kit (Randox Laboratories Ltd, Crumlin, UK) was used to measure the SOD. If SOD is present in the sample, conversion of superoxide radicals to oxygen inhibits the production of formazane. This dye is the product of p-iodonitrotetrazolium, xanthine and xanthane oxidase interaction to form superoxide radicals. Then SOD activity was conducted at 505 nm using spectroscopy (23).

2.3.3.GPx enzyme assay

In a coupled reaction, GPx activity was assayed by detecting changes in NADPH level. The substrates used were hydrogen peroxide and cumene hydroperoxide (Sigma, St. Louis, Mo.); they were reduced by GPx with glutathione as the reduction agent. With NADPH as the substratum the GSSG was reduced by GR. The NADPH amount in a LKB Ultraspec 4050 was calculated by the A340. Before the assay performance, all the reagents had been pre-warmed to room temperature. In the

reaction mixture, the final concentrations of these reagents were 0.5 mM peroxide (cumene hydroperoxide or hydrogen peroxide), 1 mM GSH, 0.1 U of GR, 5 mM K2HPO4, 0.2 mM EDTA, 0.2 mM NaN3, and 0.1 mM NADPH. For the reaction mixture bovine serum albumin was used as a negative control. The behavior of the enzyme was estimated from the linear portion of the absorbance curve. One unit of GPX activity was specified as the amount of enzyme needed to cause 1 nmol oxidation of NADPH per min under the conditions of assay mentioned above. At least two separate samples were tested for each point, and each sample was tested at least twice each time in duplicate. Coefficient of extinction was 6,200 M-1 cm-1 (24).

2.4. Statistical analyses

Statistical analysis was carried out using version 21 of SPSS software (SPSS Inc., Chicago, IL, USA). Means ± standard deviation was determined and after evaluating the one-way variance (ANOVA) the student t-test was used to determine the significance. Statistically significant was $p<0.05$.

3. Result

3.1. SOD activity

As shown in Table 2, there is a significant difference in the activity of superoxide dismutase enzyme in the lymphoma group with the control group, so that the activity of superoxide dismutase antioxidant enzyme was significantly lower in patients than the control group ($p<0.01$).

Table 2. Superoxide dismutase (SOD) activity in patients and control group.

Variable	Group	Mean ± SD	T	Df	P value
SOD (n/ul)	Case	140.05± 58.545	-12.556	198	≤ 0.01
	Control	256.84 ± 72.273*			

*The level of SOD was significantly lower in non-Hodgkin lymphoma than control subjects ($p<0.01$).

3.2. Serum levels of MDA

Statistical analysis has shown that mean of malondialdehyde serum levels in the lymphoma group and the control group was 7.2 and 3.68 respectively.

Table 3. The mean ± standard deviation of serum levels of malondialdehyde (MDA) enzyme in in patients and control group.

Variable	Group	Mean ± SD	T	Df	P value
MDA (n/ml)	Case	7.2 ± 1.511	22.057	198	≤ 0.01
	Control	3.68 ± 0.506*			

*The level of MDA was significantly higher in non-Hodgkin lymphoma than control subjects ($p<0.01$).

There was a statistically significant difference between case and control groups so that MDA analysis indicated a significant increase in MDA activity in the lymphoma group compared with the healthy group ($p<0.01$, Table 3).

3.3. Serum GPx levels

Based on the Table 4, the T-test findings revealed that there was a substantial difference between the activity of glutathione peroxidase enzyme in both groups of patients with lymphoma and control, resulting in a significantly lower GPx activity enzyme in the lymphoma group than the healthy group ($p<0.01$).

Table 4. The mean ± standard deviation of glutathione peroxidase (GPx) activity in in patients and control group.

Variable	Group	Mean ± SD	T	Df	P value
GPx (u/g Hb)	Case	36.77 ± 9	22.057	198	≤ 0.01
	Control	44.77 ± 9.25*			

*The level of GPx was significantly lower in non-Hodgkin lymphoma than control subjects ($p<0.01$).

4. Discussion

Some human diseases are caused by an imbalance between the production of oxidative molecules and the ability of biological antioxidant systems to detoxify these oxidative stress mediators. Oxidative stress plays an important role in altering metabolic pathways, vascular networks, and penetration of macrophages in the tumor cells. These changes can not only affect tumor progression but also the compatibility of cancer cells with oxidative stress and potentially lead to increased resistance to treatment, angiogenesis, and increased risk of metastasis. Therefore, oxidative stress may be of particular importance in the prognosis of cancer. Although SOD and GPx are widely regarded as an antioxidant molecule and have been shown to inhibit oxidative stress in various diseases and cancers, little is known about its in patients with NHL. In this study, the amount of oxidant (MDA) and antioxidant (SOD and GPx) enzymes in non-Hodgkin's lymphoma blood samples were investigated and compared with healthy controls. The data showed higher serum MDA levels and lower SOD and GPx levels in the NHL patient group than the healthy control group. These findings suggested that the NHL may be caused by reduced antioxidant defenses. According to some research, lower antioxidant activity may occur as a consequence of the overproduction of oxidant molecules (25, 26). The serum levels of the antioxidant enzyme in patients are controversial. In agreement with this study, Tsamesidis et al. (27) reported a significant increase in ROS and MDA production and a decrease in antioxidant defense mechanisms in Hodgkin lymphoma patients. It has been reported SOD activity significantly decreased in malignant lymphomas (28). Moreover, some studies have reported that the activity of SOD, GPx, and GSH decreased with DNA damage and cancer (29, 30). High levels of MDA in the present study suggest that non-Hodgkin's lymphoma may cause free radical scavenging and increased oxidative stress, especially when the activity of antioxidant enzymes such as SOD and GPx is reduced. GPx and SOD are antioxidant enzymes that can detoxify hydrogen peroxide, superoxide, and lipid peroxides. As long as the system was intact, recovery in the oxidative attack was

achieved, but when GPx and SOD enzymes were inhibited, as seen in the NHL group, the removal of free radicals was delayed and the MDA level was increased. In contrast, it has been reported SOD activity significantly increased in myelocytic, monocytic, and lymphocytic leukemia cells and patients with malignant lymphoma (31). Additionally, a significant increase in GPx and SOD activity has been suggested in patients with oral cancer (32). These results may be explained by the fact that in the early stages of cancer, lipid peroxidation and oxidative stress increase, so the body tries to fight it by increasing the level and activity of antioxidants (33). In summarize, the increased levels of oxidative stress in the NHL may be due to increased local production of free radicals. Disruption of the antioxidant system and a reduction of antioxidant enzymes such as SOD and GPx increase lipid peroxidation products, including MDA, which can lead to DNA damage and carcinogenesis.

5. Conclusion

The data showed that there was a considerably higher level of MDA in the NHL group than in the healthy group. In addition, the patient group had lower levels of the antioxidant enzymes SOD and GPx than the healthy group. The present study helps to better understand the oxidative statuses of non-Hodgkin's lymphoma.

Acknowledgments

The authors would like to thank all participants (case and control) in this study for their patience and support.

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