



Evaluation of Free Radicals and Some Other Biochemical Parameters in Leukemia and B-Thalassemia in Erbil City

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Abstract

Background: Free radicals have been shown to be involved in the etiology of degenerative diseases, and medical conditions such as heart disease, cancer and ageing. Various biochemical markers are indicators of pathological state in both thalassaemia and leukemia. Increased oxidative stress and decreased antioxidant factors play a key role in the pathogenesis of both diseases. High ROS production has been associated with significant decrease in antioxidant defense mechanisms leading to protein, lipid and DNA damage.

Aim: To conduct comparative analysis of MDA and as a marker of oxidative stress in patients with β -thalassaemia and leukemia.

Materials and methods: This research work was conducted on a total number of 95 subjects in which 20 were control participants and 75 patients (35 males and 40 females) mean age (59.2 ± 3.5 years) The levels of serum lipids, MDA, total Sialic acid, catalase and total protein, were measured in all participants.

Results: In both groups of patients β -thalassaemia and leukemia Serum MDA and total Sialic acid levels were significantly higher when compared to the controls ($p < 0.001$). The results showed significant decrease ($p < 0.001$) in Catalase activity of thalassaemic and leukemic patients in comparing with healthy control group. No significant differences were observed in the comparison of total protein levels between patients' group and healthy control group.

Conclusion: Oxidative stress is involved in pathogenesis of thalassaemia and leukemia, as evident from elevated malondialdehyde levels and decreased catalase activity. Elevated levels of sialic acid and total proteins suggest the role of glycoproteins in carcinogenesis processes.

Keywords: Free radicals; Leukemia; β -Thalassaemia

Introduction

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases. Free radicals are implicated in the pathogenesis of multi-stage process of carcinogenesis. They are proposed to cause DNA base alterations, strand breaks, damage to tumor suppressor genes and enhanced expression of proto-oncogenes [1-4]. Lipid hydroperoxide, malondialdehyde, and decreased blood levels of antioxidants catalase, superoxide have been reported in cancer patients. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition [5] Thalassaemia major can result in severe complications and even death due to absence of hemoglobin A synthesis and the patients are more dependent on

transfusion of blood [6]. Iron metabolism disorders are common in the human including both iron deficiency anemia and excessive iron storage. Iron is essential for oxidation-reduction catalysis and bioenergetics, but unless appropriately shielded, iron plays a key role in the formation of toxic oxygen radicals that can attack all biological molecules. Hence, specialized molecules for the acquisition, transport (transferrin), and storage (ferritin) of iron in a soluble nontoxic form have evolved [7] Reactive oxygen species (ROS) degrade polyunsaturated lipids, forming malondialdehyde that mainly exists in the enol form [8]. This compound is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts which are referred to as advanced lipoxidation end products [9]. The effect of OS to the cells may either be acute or chronic. Chronic OS results from little

oxidative damage which accumulates during the life cycle of the cell and subsequently disrupts essential cellular functions and triggers many cancers [10-12], including solid tumors such as prostate carcinoma melanoma [13], and several hematopoietic malignancies such as acute lymphoblastic leukemia (ALL), [14] myelodysplastic syndrome (MDS), [14] and myeloid leukemia; chronic myeloid leukemia (CML) and acute myeloid leukemia (AML) [15,16]. The objective of our study was to measure the intensity of oxidative damage at the cellular and molecular levels through measurements of lipid peroxidation and protein carbonyls. The study also aimed to investigate the oxidative profile in CML patients through the verification of main enzymatic antioxidant defenses (catalase [CAT] and superoxide dismutase [SOD]), nonenzymatic antioxidants (vitamins A and E), and essential micronutrients (selenium, magnesium, and zinc) in the blood of CML patients.

Materials and Methods

Subjects

The study subjects consisted of 32 clinically diagnosed CML patients and 32 clinically diagnosed β -thalassemia major at Nanakaly hospital in Erbil patient. The controls consisted of 32 age- and sex matched healthy volunteers. The control subjects were selected from healthy subjects, and their clinical blood profiles were within the normal range, and the general health status was normal.

Blood sample

Blood from thalassemic, Leukemic patients and controls was collected, the samples were centrifuged at 3000 rpm for 10 min to separate serum. The separated serum was collected for further analysis in polythene tube with cork and stored at -200C .

Biochemical Analyses

a) Determination of Malondialdehyde (MAD) level

Serum Malondialdehyde level was determined by a spectrophotometric method using the Randox assay kit the principle of method based on reaction with thiobarbituric acid (TBA) at $90 - 100^{\circ}\text{C}$ (18). MDA and TBA react together in the TBA test reaction, to produce a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2 - 3 at 90°C for 15 minutes.

b) Determination the activity of catalase

catalase activity was assayed by by a spectrophotometric method using the Randox assay kit the principle of method based on the utilization of H_2O_2 by the enzyme. The color developed was read at 620 nm.

c) Determination of Sialic acid level

Measurement of Sialic acid based on reaction of sialic acid with acid ninhydrin reagent. The absorbance of colored product was measured at 470nm.

d) Determination of Total protein level

Total, protein Total protein was measured using a colorimetric kit based on the biuret method.

e) Biostatistical analysis

Statistical analyses were performed with the SPSS 10.0 pocket programme for Windows (SPSS Inc., Chicago, IL, USA). The data were expressed as mean values \pm standard deviation ($X \pm SD$). The level of statistical significance was defined as $P < 0.05$ (Table 1).

Table 1: Comparison of Serum MDA levels between control and patients group.

Parameters	leukemia	Thalassemia	P-Value
MDA (nmol/ml)	4.476 \pm 3.08	4.940 \pm 1.19	p< 0.001

Results

As presented in Figure 1 The mean level of MDA was significantly higher in CML cases (4.476 \pm 3.08 nmol/ml) as compared to control cases ((1.59 \pm 0.28 nmol/ml). The elevated levels of MDA in patients are a marker of increased oxidative stress, Also The level of MDA as depicted in Figure(2), was increased highly significant(p<0.001) in β - thalassemic patients (4.940 \pm 1.19 nmol/ml) in comparasion to healthy control (1.59 \pm 0.28 nmol/ml), Table 2 shows the status of Catalase activity, total Sialic Acid (TSA) and Total Proteins In controls, Thalassemic and leukemic subjects. The activity of catalase, was significantly decreased in both Thalassemic (24 \pm 22.5 (KU/L) and leukemic patients (26.25 \pm 24 KU/L) (as compared to control subjects (205.36 \pm 41.83 KU/L). The serum TSA level was significantly increased both Thalassemic (64.25 \pm 4.33 $\mu\text{g}/\text{mL}$) and leukemic patients (79.60 \pm 6.93 $\mu\text{g}/\text{mL}$) as compared to control subjects (51.60 \pm 3.51 $\mu\text{g}/\text{mL}$). Significant difference was obtained for serum Total Proteins levels between patients and control.

Table 2: The results of main biochemical parameters in healthy control and patients group.

Parameters	Healthy Control	leukemia	Thalassemia	P-value
Catalase (KU/L)	205.36 \pm 41.83	26.25 \pm 24	.24 \pm 22.5	<0.001
TSA ($\mu\text{g}/\text{mL}$)	51.60 \pm 3.51	79.60 \pm 6.93	64.25 \pm 4.33	<0.001
Total Proteins	8.17 \pm 0.29	12.01 \pm 0.95	14.01 \pm 1.05	<0.05

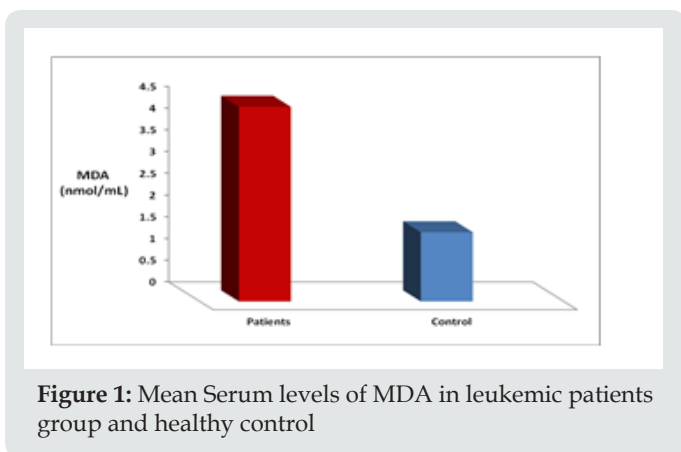


Figure 1: Mean Serum levels of MDA in leukemic patients group and healthy control

Discussion

Present study revealed elevated malondialdehyde concentration in Thalassemic patients indicating increased lipid peroxidation. The oxidative stress causes lipid peroxidation to generate wide range of products including MDA. Previous investigations demonstrated that malondialdehyde plays important roles in the processes of carcinogenesis through their interaction with DNA and formation of DNA-MDA adduct. This DNA-MDA adducts induce mutations in various genes such as tumor suppressor genes and oncogenes as well as cell cycle alterations in different cancers [17]. Elevated levels of lipid peroxidation along side decreased concentrations of antioxidant capacity have been severally reported in patients with head and neck cancers [18,19]. Elevated levels of lipid peroxidation and MDA are largely linked to the damage of red blood cell membranes enriched by polyunsaturated fatty acids. The concentrations of free radicals increase in different cancers leading to enhanced lipid peroxidation. On the other hands, the levels of antioxidant capacity decreased following enhancement of ROS production in compensatory manner [20]. MDA is a good biomarker of oxidative damage. The extent of lipid peroxidation denotes the amount of free oxygen radicals generated, which have not been eliminated by the defense mechanism. There are increasing evidences proposing that oxidative stress is a major damaging factor. This is due to the effect of continuous blood transfusions in these patients which leads to peroxidative tissue injury by the secondary iron overload. Absence of beta globin chains lead to accumulation of unpaired alpha globin chains. Excess presence of the alpha globin chains is a primary reason for the cellular oxidative damage and also iron overload. As a result of both high plasma iron and high intracellular non hemoglobin iron in beta thalassemia, there is an enhanced generation of ROS [21,22]. Moreover, repeated blood transfusion causes iron overload which increases free radical formation and peroxidative damage of tissues. In such condition, depletion of endogenous antioxidants may be expected. Peroxidative damage of lipids was indicated by the increase in serum MDA levels following oxidation of polyunsaturated fatty acids [23].

In the Present study Figure 2, we found that the concentration of malondialdehyde was increased in leukemic patients when

compared to healthy controls. Free radicals play an important role in the pathophysiology of myeloid leukemia, and that high levels of free radicals may cause oxidative stress in haematopoietic cells if the antioxidant defense system is not potential. Oxidative stress may occur in patients with leukemia due to the higher number of mature and immature myeloid series cells as well as other unknown factors. Malondialdehyde (MDA) which is a stable end product of free radical induced-lipid peroxidation was used as a surrogate marker for oxidative damage to tissues. Anti-oxidants, which control the oxidative stress state, represent a major line of defense regulating overall true state of health. The disturbance of the pro-oxidant/antioxidant balance, resulting from elevated free-radical production, antioxidant enzyme inactivation, and excessive antioxidant consumption, is the causative factor in oxidative damage. These free radicals set up a chain reaction that changes cell permeability, denatures proteins, alters enzyme activity, decreases neurotransmitter transmission, causes breakage of DNA, and degrades structural proteins. Thus, free radicals are directly responsible for causing genetic mutations and carcinogenesis.

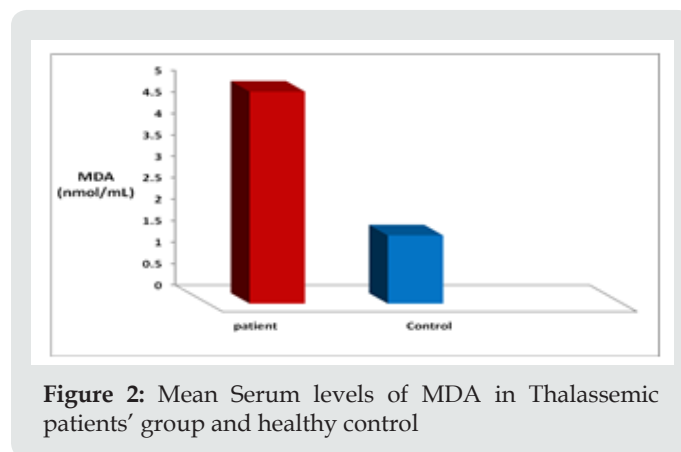


Figure 2: Mean Serum levels of MDA in Thalassemic patients' group and healthy control

In our study, we found that the activity of Catalase was decreased in leukemic and Thalassemic patients when compared to healthy controls. Catalase activity in patients with β -thalassemia major is decreased as compared to normal healthy control. Decrease in the activity of CAT could be due to increase in the lipid peroxidation product, malondialdehyde which can form cross links, there by in activating several membranes bound enzymes. The body has multiple defense mechanisms against free radical-mediated tissue damage. Among them, antioxidants are the primary lines of defense. Under normal circumstances, there is a critical balance between pro-oxidizing and antioxidantizing forces. Antioxidants interact with free radicals in several Cancer cells under sustained ROS stress tend to heavily utilize adaptation mechanisms and may exhaust cellular ROS-buffering capacity. A relationship between leukemia and oxidative stress has been observed. Leukemic cells produce higher amounts of ROS than nonleukemic cells because the former are experiencing sustained oxidative blockade [24].

The current study shows that the total Sialic Acid (TSA) levels were significantly increased in patients group. Serum TSA

has also been used as a tumor marker for a number of different cancers including colorectal, prostate, and breast cancers [25,26]. Cell surfaces and membrane components play a prominent role in neoplastic behavior. Neoplasms often have an increased concentration of TSA on the tumor cell surface, and sialo glycoproteins are shed or secreted by some of these cells, which increases the concentration in blood. Moreover, cancer cells have been associated with an increased activity of sialyltransferase, leading to an increased amount of TSA on the cell surface, thus increasing the plasma or salivary concentration [27,28]. TSA concentrations have been reported to be related not only to diagnosis, but also to staging, prognosis, and detection of early recurrence. It has been suggested that evaluations of the serum glycoconjugate levels may be useful in early detection and staging of oral precancerous conditions and oral cancer [29].

Conclusion

It may be concluded from the finding of the study that accumulation of free radicals contributes to antioxidant depletion and dysfunction in both Thalassemic and leukemic patients. high levels of serum malondialdehyde with poor antioxidant level indicates that oxidative stress may be a major cause for the pathogenesis of beta thalassemia and leukemia.

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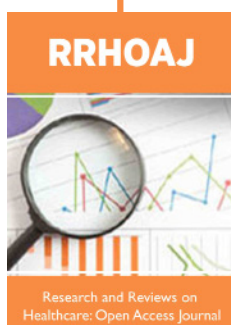
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