Original Article

Determination of Reactive Oxygen Species and Some Antioxidants in Iraqi Patients with Myelodysplastic Syndrome

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Abstract

Background: A diverse set of clonal hematopoietic illnesses known as myelodysplastic syndrome (MDS) have decreased survival rates, risk factors for leukemia transformation, and a variety of clinical manifestations. This makes the clinical relevance of an accurate diagnosis and risk classification of MDS patients. **Objectives:** Evaluation of serum levels of reactive oxygen species (ROS) and some antioxidants in patients with MDS. **Materials and Methods:** A total of 20 healthy individuals as the control group (12 men and 8 women), and 45 MDS patients (25 males and 20 females). MDS samples were collected from several Iraqi locations. The serum oxidative stress level was assessed by measuring ROS using an automated colorimetric method. Superoxide dismutase (SOD) and glutathione peroxidase (GPx) in serum were determined using an enzyme-linked immunosorbent assay approach. Moreover, glutathione reductase (GR) was measured by using Ellmam's reagents methods. **Results:** The ages of the participants ranged from 20 to 65 years, with mean ages of 38 ± 4.43 and 40 ± 5.93 years for patients and healthy people, respectively. The prevalence was higher in males than females among patients (62.5% and 37.5%, respectively). ROS levels were found to be significantly higher in the patient group (33.64 µmol/L) than that of control (16.31 µmol/L) ($P = 0.041$). Antioxidants, such as SOD and GPx, levels were significantly higher in MDS patients (12.9 and 78.65 U/L, respectively) compared with the control group (2.04 and 10.09 U/L, respectively; $P < 0.05$). Serum level of GR deceased in MDS patients compared with control groups (43.26 and 71.46 pg/mL, respectively; $P = 0.014$). **Conclusion:** ROS, GPx, and SOD increased in patients compared with control in contrast to GR that decreased in patients so all these indicators were significantly associated with MDS.

Keywords: GPx, GR, myelodysplastic syndrome, reactive oxygen species, SOD

Introduction

Myelodysplastic syndromes (MDSs) are clonal stem cell malignancies characterized by cytopenias, defect hematopoiesis, dysplasia in one or more myeloid cell lineages, and increased risk of development of acute myeloid leukemia.[1] Oxidative stress or reactive oxygen species (ROS) as a term, the oxidative stress explains the amplifying of free radicals by the human body, these reactions are due to damage to biomolecules, and the consumption of oxygen occurs in 1%–2% that occur by mitochondria, due to the formation of ROS normally, this chain of reactions will cause dysfunction to the mitochondria, because of the increase in the reactive molecules that found in the mitochondria.[2] Hematopoietic cells that appear to grow and migrate in part several oncogenic tyrosine kinases produce ROS as a result. There are conflicting views on whether therapeutic techniques should aim to diminish or enhance oxidative stress in leukemia cells.[3] Hepcidin suppression in MDS patients with iron overload may potentially be mediated by ROS,[4] as a result of their ability to prevent CCAAT/enhancer-binding protein alpha (C/EBPα) and signal transducer and activator of transcription 3 (STAT-3)

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from binding to the hepcidin promoter and suppressing the hepcidin gene. As a result, it was anticipated that after deferasirox treatment improved oxidative stress measurements, blood hepcidin levels would increase.[5] In a previous study, subsets of bone marrow cells were examined for ROS levels and for the expression of 28 genes that code for important antioxidant enzymes. Moreover, a specific signature of antioxidants has been identified, called "antioxidants" for different subgroups of MDS or secondary acute myeloid leukemia that made regression of expression levels of antioxidants that could cause disease progression.[6] Superoxide dismutase (SOD) is defined as a catalysis enzyme that alternately has catalysis function to the disputation of super-oxide-radical (O_2^-) due to hydrogen peroxide (H_2O_2) and regular molecular oxygen (O_2). As a by-product of oxygen metabolism, superoxide is produced and, if unchecked, can lead to a variety of cell damage. Another harmful substance that is, broken down by other enzymes like catalase is hydrogen peroxide. Consequently, SOD serves as a crucial antioxidant defense in almost all live cells that are exposed to oxygen.[7]

Glutathione sulfhydryl-containing peptide glutathione is the predominant nonprotein thiol in eukaryotic cells. The glutathione-containing electrons in the sulfhydryl group can interact with the cell's weak biochemical electron structures, including lipids, to prevent damage to proteins, deoxyribose nucleic acid (DNA), and fats. The products of this interaction, known as glutathione complexes, build up inside the cells and have the potential to develop into hazardous chemicals. These molecules need to be carried outside of the cell, and adenosine triphosphate-dependent plasma membrane vectors are used to do this. Cell surface enzymes then break down the extracellular molecules, which are then eliminated in the kidneys as mercapturic acid.[8] Glutathione peroxidase (GPx) generally, these compounds belongs to the enzyme family, and has peroxidase activity, these kinds' primary job is to shield cells from oxidative harm.[9] In biology, the specialized role of GPx is to convert free hydrogen peroxide to water and lipid hydroperoxidase to alcohol.[10] The chemical structure of glutathione reductase (GR) consists of three constituents of amino acids named γ-glutamyl-cysteinyl-glycine, GR is considered a ubiquitous substrate-specific antioxidant enzyme, has many functions inside the cell, due to the significant role glutathione metabolism plays in the systemic regulation of sulfur metabolism in all living cells. According to the expression of GR, an increase or decrease in glutathione levels may result in oxidative stress on intracellular surfaces.[11]

To know the role of ROS and antioxidants in the occurrence or complications of MDS, we evaluated the level of ROS, GR, GPx, and SOD of patients with MDS, which may be an introduction to other studies targeting these indicators in developing a treatment or diagnostic method for the MDS in Iraq.

Materials and Methods

The current study was conducted in a DNA laboratory at the University of Babylon's College of Sciences' Biology Department, Babylon, Iraq. The current study's sample collecting and practical activities took place from June 2019 to November 2020 MDS patients at the Merjan Teaching Hospital, Samples were gathered from the Al-Hussein Medical City in Karbala Province, the National Center for Research and Treatment of Hematology at Al-Mustaniriyah University in Baghdad Province, and the Al-Furat Al-Awset for Tumors and Blood Disease. Both men and women were among the patients. Blood and bone marrow aspirate samples were taken from each patient via vein puncture, placed in ethylene diamine tetraacetic acid tubes and gel tubes for serum separation by centrifugation, and then stored at −20°C until they were utilized to evaluate oxidative stress. The study comprised of 50 patients with MDS. Patients were 25 male and 20 female MDS patients group with ages ranging from 20 to 65 years old. In contrast, the study involved 20 individuals who appeared to be in good health, including 12 men and 8 women with average ages of 20–65 years.

Determination of oxidative stress

The serum oxidative stress level was evaluated by measuring ROS using the automated colorimetric method for measuring total oxidant status (Wismerll Co. Ltd., Tokyo, Japan).[12]

Analyses of antioxidant parameters

SOD and GPx in serum were determined using an enzyme-linked immunosorbent assay (ELISA) approach using enzymatic ELISA kits manufactured by Cohesion Bioscience (Suzhou, China).[13]

Glutathione reductase

According to Rotruck *et al.*,[14] reduced glutathione was measured by using Ellmam's reagents methods that were performed manually.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (IBM Corp., Armonk, NY, USA) program version 20. Presented as means and standard deviation. Independent sample *t* test was used to compare means between two groups. When the *P* value was lesser than 0.05 considered statistically significant, whereas the *P* value was greater than (0.05) considered statistically nonsignificant.[15]

Ethical approval

The study was conducted following the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before samples were taken. The study protocol, the

subject information, and the consent form were reviewed and approved by a local ethics committee according to document number 124 on April 2, 2019.

Results

The current study included 45 MDS cases and 20 healthy people as a control group. The ages of the participants ranged from 20 to 65 years, with a mean age of 38 ± 4.43 and 40 ± 5.93 years for patients and healthy people, respectively, as shown in Table 1. The percentage of males was higher than females in patients (62.5% and 37.5%, respectively), and healthy people (60% and 40%, respectively), without significant differences appearing ($P > 0.05$).

ROS levels were found to be significantly higher in the MDS patient group (33.64 μmol/L) than that of the control (16.31 μmol/L) in the current investigation ($P = 0.041$) as shown in Table 2. SOD and GPX levels were significantly higher in MDS patients (12.9 and 78.65 U/L, respectively), than that of the control group (2.04 and 10.09 U/L, respectively; $P < 0.05$) in the antioxidant results as shown in Tables 3 and 4. Serum level of GR in MDS patient and control groups (43.26 and 71.46 pg/mL, respectively), showed in Table 5, a concurrently significant decline ($P = 0.014$).

Table 1: Demographic properties of cases and control groups

Age properties	Patient	Control	*P* value
Age range (years)	20–65	20–65	
Mean ± standard deviation	38 ± 4.43	40 ± 5.93	0.558
Standard error	0.66	1.33	
Gender			
Males (%)	25 (62.5)	12 (60)	0.471
Females (%)	20 (37.5)	8 (40)	0.393
Total number	45	20	

S: significant association ($P < 0.05$)

Table 2: Compared mean of ROS between control and MDS patient groups

ROS concentration (μmol/L)	Patient	Control	*P* value
Mean	33.64	16.31	0.041 [S]
Standard deviation	±5.42	±2.81	
Standard error	0.81	0.042	
Total number	45	20	

S: significant association ($P < 0.05$)

Table 3: Mean of SOD between control and MDS patient groups

SOD concentration (U/L)	Patient	Control	*P* value
Mean	12.9	2.04	0.048 [S]
Standard deviation	±2.51	±0.09	
Standard error	0.37	0.02	
Total number	45	20	

S: significant association ($P < 0.05$)

Discussion

According to the current study, MDS patients had significantly higher serum total ROS levels than the control group. High ferritin or over-accumulation of iron is a well-known factor contributing to increased oxidative stress through the Fenton reaction and by other mechanisms. At the same time, anemia itself is related to oxidative stress, even in iron-deficient patients.[16,17] Ghoti *et al.*[5] showed that hypoxia-induced by severe anemia is directly related to reduced hepcidin production, a key negative regulator of iron absorption and release from macrophages. They maintained that anemia-induced ROS, and ROS-induced *C/EBPα* and *STAT-3* genes and DNA binding are implicated in this phenomenon. Moreover, Ivars *et al.*[18] reported increased hepcidin levels during iron chelation treatment with deferasirox. Another possible cause of high oxidative stress in MDS patients might be high cytokinemia, due mainly to tumor necrosis factor alpha (TNF-α), which is known as a producer of oxygen radicals.

In a present study, the serum level of ROS associated with an increased level of SOD and GPX may be elucidated by the protective role of these antioxidants to reduce distractions caused by ROS. Previous studies showed that the most important adaptation of organisms to aerobic life was the development of an effective antioxidant system to prevent intracellular damage caused by ROS.[18,19]

Hematopoietic cells are particularly susceptible to oxidative damage brought on by the buildup of free

Table 4: Compared mean of GPx between control and MDS patient groups

GPx concentration (U/L)	Patient	Control	*P* value
Mean	78.65	10.09	0.008 [S]
Standard deviation	±9.75	±1.99	
Standard error	1.454	0.445	
Total number	45	20	

S: significant association ($P < 0.05$)

Table 5: Compared mean of GR between control and MDS patient groups

GR concentration (pg/mL)	Patient	Control	*P* value
Mean	43.26	71.46	0.014 [S]
Standard deviation	±7.81	±10.7	
Standard error	1.164	2.39	
Total number	45	20	

S: significant association ($P < 0.05$)

radicals, hence the possible effects of ROS on these cells are of particular relevance.[20] The current study's findings corroborated those of Evans *et al.*,[21] Dalle-Donne *et al.*,[22] and Ghoti *et al.*,[23] which demonstrated that in comparison with normal cells, BM cells from MDS patients had decreased GR levels and greater intracellular GPX concentrations. Importantly, those with MDS who also have high ROS, low GR, and high superoxide/peroxide ratios have lower overall survival rates.[24] Direct biomolecule destruction and/or deregulation of ROS-dependent signaling pathways are two outcomes of increased intracellular ROS generation that have long been known to exist.[21,25] Blood cell precursor cells such as blasts or erythroid precursors have been shown to have higher levels of oxidation in response to DNA oxidative damage in CD34+ cells.[17]

Additionally, in patients with MDS,[26] and other clinical diseases, oxidative stress is associated with DNA hypermethylation.[27] The majority of ROS is absorbed by proteins,[28] and oxidation can cause protein aggregation, polymerization, unfolding, or conformational changes that result in structural or functional loss. Although there are many different oxidative alterations that proteins can go through, the majority of them involve the addition of carbonyl groups to the proteins.[29] However, the main mechanisms for protein carbonylation are the direct metal-catalyzed oxidation of particular amino acid residues (lysine, arginine, proline, and threonine), and the secondary interactions of nucleophilic amino acid side-chains with ROS-induced lipid peroxidation products such as 4-hydroxynonenal.[30] These modifications are likely to have a considerable effect on cell signaling.[31] Increased cytokinesis, especially TNF-α known producer of oxygen radicals, may be a factor in MDS patients' increased oxidative stress.[32]

Furthermore, previous studies showed that oxidative stress is associated with the development and progression of some hematological malignancies such as MDS,[12,18-20] but the role of specific ROS generated and the involvement of the main antioxidant pathways remain unknown.[18,19]

Conclusion

Present results showed that the ages of the participants ranged from 20 to 65 years, with an age mean equal to 38 ± 4.43 and 40 ± 5.93 years for patients and healthy people, respectively, so most MDS patients are males. In lab analysis, ROS, SOD, and GPX levels were significantly higher in MDS patients than in the control group ($P < 0.05$), whereas serum levels of GR were significantly reduced in MDS patients compared with control groups ($P = 0.014$).

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Conflicts of interest

There are no conflicts of interest.

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