RESEARCH ARTICLE

Investigation of the Antioxidant Status in Multiple Myeloma Patients: Effects of Therapy

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Abstract

Background: Multiple myeloma is a malignant silent incurable plasma cell disorder. The present study aimed to assessed the activation of the oxidative stress pathway in afected patients Materials and Methods: Advanced oxidation protein products (AOPPs), malondialdehyde (MDA), adenosine deaminase (ADA), total antioxidant capacity (TAC) levels, glutathione, ascorbic acid (vitamin C), α-tocopherol (vitamin E) in addition to related enzymes glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R) and superoxide dismutase (SOD) were analyzed in sixty patients with multiple myeloma before and after one month treatment with induction therapy. Results: The results of the study showed a significant elevation in AOPPs, MDA, ADA levels in patients with multiple myeloma before and after treatment in comparison to healthy control samples In contrast TAC glutathione, vitamin C and E, and the antioxidant enzymes levels were decreased significantly. On comparing samples of MM patients after treatment, there was significant increase of TAC glutathione, vitamin C and E, and the antioxidant enzymes in parallel with decreasing AOPPs, MDA and ADA levels in comparison with samples of patients before treatment. Conclusions: The results indicate oxidative stress and DNA damage activity increase in MM and are alleviated in response to therapy.

Keywords: Multiple myeloma - oxidation byproducts - antioxidants - antioxidant enzymes - total antioxidant capacity

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Introduction

Multiple myeloma (MM), also recognized as plasma cell myeloma or Kahler's disease a type of white blood cell normally responsible for producing antibodies (Raab et al., 2009), Multiple myeloma is a malignant still incurable plasma cell disorder (Campanella et al., 2012), it is a B-cell malignancy characterized by the accumulation of clonal population of plasma cells (Xuan-li et al., 2013). In MM, collections of abnormal plasma cells accrue in the bone marrow, where they impede with the production of normal blood cells. Most cases of myeloma also feature the production of a Para protein an abnormal antibody which can cause kidney complications. Bone lesions and hypercalcemia are furthermore encountered (Raab et al., 2009). It is a example primary malignancy of the bone associated with malignant plasma cells that secrete monoclonal immunoglobulins into the serum, the urine or else both (Hideshima and Anderson, 2002). The B cells, T cells and macrophages undergo immunologic alterations related to marrow invasion. Such variations confer an increased exposure to infections, particularly those caused by pneumococcus, tetanus and diphtheria organisms (Munshi, 1997; Kyle and Rajkumar, 2009).

The MM cell clone produces an excess of monoclonal (M proteins) and free light chain proteins. Serum protein electrophoresis is used to recognize patients with MM and other serum protein disorders. Serum protein electrophoresis will identify an M protein as a narrow peak or "spike" in the γ, β or α2 regions of the densitometer tracing. If MM is strongly suspected and electrophoresis is normal, serum immune fixation may be more sensitive in identifying a small M protein (Ravaud et al., 1996; O'Connell et al., 2005).

Initial treatment of MM depends on the patient's age and co morbidities. In recent years, high-dose chemotherapy with autologous hematopoietic stem-cell transplantation has become the preferred treatment for patients under the age of 65. Prior to stem-cell transplantation, these patients receive an initial course of induction chemotherapy (Kyle and Rajkumar, 2008). Autologous stem cell transplantation (ASCT), the transplantation of a patient's own stem cells after chemotherapy, is the most common type of stem cell transplantation for MM. It is not curative, but does prolong overall survival and complete remission. Allogeneic stem cell transplantation, the transplantation of a healthy person's stem cells into the affected patient, has the potential for a cure, but is only available to a small percentage of patients Furthermore, there is a 5-10% treatment-associated mortality rate (Kyle and Rajkumar,

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2004).

Free radicals are molecules prepared highly reactive as a result of the attendance in them of atoms with one or more unshared electrons. In general, free radicals form following the break of a covalently bound molecule where each part takes one of the shared electrons, with the loss of an electron. on the other hand, in biological systems, free radicals form mostly by electron transfer. Free radicals induce breaks in DNA and chromosomes (Dierickx et al., 1999; Kaya et al., 2005). Reactive oxygen species and other free radicals mediate phenotypic and genotypic changes leading from mutation to neoplasia in all cancers including MM.

A number of defense mechanisms a recurrent in the body to stop the formation of reactive oxygen species (ROS) and the damage resulting from their presence. Plasma and tissue antioxidants are classified as primary, secondary and tertiary antioxidant defenses. Primary defense is the best-defined prevention mechanism against free radicals. It prevents radical formation bybinding iron atoms to high-affinity positions in the protein molecules in the extracellular matrix (Sharma et al., 2009). Advanced oxidation protein products (AOPPs) are a marker of oxidative damage, AOPPs are a family of oxidized, di-tyrosine containing protein compounds that are produced by monocyte activation, myeloperoxidase release, and interaction through re active oxygen species (Camilla et al., 2011). AOPPs result from the action of chlorinated compounds on proteins, leading to the formation of dityrosine residues and consequently to the protein cross-linking, aggregation and precipitation (Krzystek-Korpacka et al., 2008). Glutathione is involved main cell function containing vitamin C metabolism, chelating of copper ions and bio transformation of foreign substances and intermediate oxygen metabolites GSH is synthesized mostly in liver (Mehde et al., 2013). Vitamin E is a fat soluble and acts as a free radical scavenger to prevent lipid peroxidation of polyunsaturated fatty acids and block nitrosamine formation (Estakhri et al., 2013).

In furtherance of this nature of work, the present study aimed to investigate the levels of several antioxidants in patients with MM before and after taking initial treatment.

Materials and Methods

The sampling procedure was done in 30 male patients (47.88±8.36 years) with MM before start of any therapy and after one month treatment with induction therapy comparing with 30 male healthy (48.16±6.95) years. None of these patients received antioxidant medicines or foods. Patients were chosen from the patients referred to the Al-Yarmuk Teaching Hospital and Medical City in Iraq. All patients were subjected to a detailed history taking, thorough clinical examination,Routine hematological and biochemical assessments were carried out and laboratory investigations including Five to seven ml were collected from each subject by vein puncture, Venous blood samples have been collected into two vacationer tubes, one containing EDTA for measurement of blood hemoglobin (Hb), WBC count,Platelet count, glutathione peroxidase (GSH-Px), glutathione reductase(GSH- R) and superoxide dismutase (SOD). The blood in the two part was allowed to clot for at least 10-15 min. at room temperature, centrifuged for (10) min. at (4000xg). Serum was removed for the measurement of biochemical parameters.

Total antioxidant capacity (TAC) in plasma samples was carried out according to Rice -Evans and Miller (Rice–Evans and Mille, 1994). Plasma malondialdehyde (MDA) was determined according to the modified method of Satoh (1978), Glutathione (GSH) was estimated by the method of Beutler's method (Beutler et al., 1963). Glutathione peroxidase (GSH-Px) activity was determined according to Pleban's method (Pleban et al., 1982). Glutathione reductase (GSH-Px) activity was determined according to a modified method of Lee et al. (1975). Superoxide dismutase(SOD) activity was determined according to the method of Misra HP and Fridovich I (Misra and Fridovich, 1972),Adenosine deaminase(ADA)activitywas measured by using Giusti's method (Giusti, 1974), protein electrophoresis by using cellulose acetate paper and ponceau S-stained and Advanced oxidation protein products (AOPPs) by Enzyme Linked Immunosorbent Assay (ELISA) (Demeditec Diagnostics GmbH, Germany).

All statistical analyses in studies were performed using SPSS version 17.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $P<0.05$=significant, $P>0.05$=non-significant.

Results

Three groups were included in this study: Group 1 consisted of thirty men suffering from MM before treatment (The mean age of the patients was 47.88±8.36 years), Group 2 consisted of thirty men suffering from MM after one month treatment with induction therapy (The mean age of the patients was 47.97±9.06 years), and the third group was the control men (The mean age of the patients was 48.16±6.95 years).

Hematological parameters (Hb, WBC, and Platelet count) and some antioxidant enzymes (GSH-R, GSH-Px, and SOD) levels in the three patients groups included in this study according to age were evaluated, (Table 1).

As the results in Table 2 show there are highly significant decreases ($P>0.001$) in the mean value of erythrocytes hemoglobin and the antioxidant enzymes levels between the both groups and the control group, in addition of WBC account between group 1 in comparison with control group. Meanwhile platelet count decreased significantly ($P>0.01$) in group 1, and group 2, when they were compared with control group, WBC account between group 2 in comparison with control group also decreased significantly.

The same table indicated highly significant increases ($P>0.01$) in hematological parameters with significant increases ($P>0.05$) in antioxidant enzymes except SOD between patients group before treatment (group 1) in comparison with patients group after treatment (group 2).

Levels of oxidation by products (AOPP and MDA) as

Table 1. Hematological Parameters and Some Antioxidant Enzymes Levels in the Three Patients Groups Included in This Study According to Age

Characteristic	Control group [n=30]	Group1 [N=30]	Group 2 [N=30]
Age(year)	48.16±6.95	47.88±8.36	47.97±9.06
Hb	13.88±3.44	7.05±2.76[d]	8.34±2.91[d,c]
WBC*10^3cell/ml	6.22±0.73	4.13±0.53[d]	5.00±0.66[a,c]
Platelet count	2.82±1.00	1.99±0.85[a]	2.25±0.92[a,c]
GSH-R [IU/gmHb]	13.92±4.21	7.39±3.55[d]	9.44±2.73[d,b]
GSH-Px [IU/gmHb]	38.21±4.21	18.31±3.43[d]	24.22±4.87[d,b]
SOD [IU/gmHb]	691.99±142.23	554.64±119.94[d]	568.40±102.32[d]

*Results were expressed as the mean±SD. (n=number of samples, SD=Standard Deviation, p=Test of significant). [a]P<0.01 compared with control group. [d]P<0.001 compared with control group. [b]P<0.01 compared with group 1. [c]P<0.05 compared with group 1

Table 2. Some Oxidation by Products and Antioxidant Levels in the Three Patients Groups Included in This Study

Characteristic	Control group [n=30]	Group1 [N=30]	Group 2 [N=30]
AOPP [ng/dl]	60.43±19.56	110.47±20.59[d]	96.33±16.44[d,c]
MDA [μ mol/L]	1.28+0.12	2.23+0.18[d]	1.89+0.15[d,b]
ADA [U/L]	19.88±4.17	28.38±6.22[a]	25.36±8.04[a,c]
TAC [μmol/L]	422.32±20.52	261.52±29.26[d]	300.00±24.32[d,b]
GSH [mg/dl]	62.21±8.33	31.66±8.04[d]	38.35±9.00[d,b]
Vitamin E (μmol/l)	20.96±4.54	15.26±5.33[a]	17.32±4.03[a,c]
VitaminC (mg/dl)	0.92±0.06	0.68±0.09[d]	0.74±0.06[d,b]

*Results were expressed as the mean±SD.(n=number of samples, SD=Standard Deviation, p=Test of significant).[a]P<0.01 compared with control group.[d]P<0.001 compared with control group.[b]P<0.01 compared with group 1.[c]P<0.05 compared with group 1

well as, ADA and some antioxidants (TAC, GSH, vitamin E,and vitamin C) were measured to study the oxidative stress status in the study subjects (Table 2).

The results in this table reveal the presence of highly significant increases (p<0.001) in both oxidation by products (AOPP and MDA) with significant increases (p<0.01) in ADA, in contrast there were of highly significant decreases (p<0.001) in antioxidants levels (TAC, GSH, and Vitamin C) with significant decreases (p<0.01) in Vitamin E of MM patients before and after therapy in comparison with control group.

In the group 2, the increases were highly significant (p>0.01) in TAC, with GSH and were significant (p>0.05) in Vitamin E & C,while the decrease was highly significant (p>0.01) in MDA levels and were significant (p>0.05) in AOPP and ADA levels in comparison to that of the group 1.

Cellulose acetate electrophoresis was carried out on sera samples of control and patients groups to detect the differences in total proteins present in the studied samples (to differentiate between protein patterns). After electrophoresis, the separated serum protein bands were detected using ponceau S-stain. The Figures 1 shows the results of electrophoretic profile of proteins present in crude sera of all studied groups.

Discussion

Multiple myeloma is characterized by plasma cells proliferation within the bone marrow and an excess of secreted monoclonal immunoglobulins. ROS are free radicals and other molecules with unpaired electrons (such as O_2. -and H_2O_2) that are highly reactive and can react with biologic macromolecules, peroxidise lipids to form mutagenic MDA, modify the structure and function of proteins, and cause oxidative damage to DNA (Marnett et al., 2003; Márquez et al., 2007; Ottaviano et al., 2008). Under normal conditions, intracellular ROS are maintained at a low level by various enzyme systems which maintain the in vivo redox homeostasis. Excessive production of ROS or inadequacy in a normal cell's antioxidant defense system (or both) can cause the cell to experience oxidative stress (Ishikawa et al., 2008).

Tumor cells usually have an imbalanced redox status resulting in the damage to DNA, proteins and lipids. Higher levels of DNA damage and deficient DNA repair may predispose individuals to cancer (Ishikawa et al., 2008). There are several approaches that may be used for the evaluation of oxidative stress status, these include measurement of the depletion of antioxidant reserves, changes in the activities of antioxidant enzymes, free radical production, and presence of protein, lipid, and DNA free radical adducts (Waris and Ahsan, 2006), and since he role of oxidant pathways may be disturbed in MM pathogenesis, therefore the present study was designed to assess the role of free radicals (in terms of byproducts generated during lipid peroxidation and DNA break), Adenosine deaminase in the redox imbalance as well as some antioxidants,and some antioxidant enzymes in MM patients before and after therapy, the results were observed among the age groups are shown in Tables 1 and 2.

In the present study, the mean AOPP and MDA levels and ADA activity in MM samples before and after therapy were higher than healthy control. The difference between these values for AOPP and MDA levels were highly significant (p<0.001), while for ADA activity was significant (p<0.01), in contrast the antioxidants levels and antioxidant enzymes specific activities are statistically high significant (except Vitamin E levels which decreased significantly). These results creates a picture of increased oxidative stress in the system (redox imbalance, i.e. pro-oxidants are higher than antioxidants) and the rise in the level of one of parameter is simultaneous to the rise in other suggest an association between them.

Therapy may altered pro-oxidant–antioxidant balance may lead to an increased oxidative damage and consequently play an important role in MM, this was evidenced by the statistical analysis of group 2 vs. group 1, Tables 1, 2 in which the antioxidant parameters and antioxidant enzymes increased significantly while the byproducts levels decreased significantly in MM patients after treatment than before treatment that mean that the studied patients responded the used therapy.

The specific immunoglobulin secreted by the malignant cell is the M-protein, named in reference to its monoclonal characteristics. It is found in the serum and/or urine of persons affected with MM (Alexander et al., 2007). In order to detect the differences in total proteins present in the studied groups Cellulose acetate electrophoresis was carried out on crude sera samples of control and the two groups of the patient, by which the separation of different proteins is based on the differences

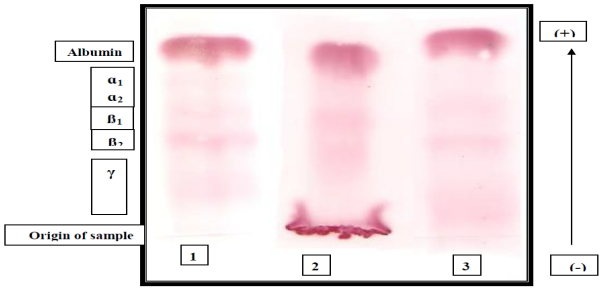

Figure 1. Electrogram of Proteins Profile Samples. The crude samples that applied were: 1: pooled crude sera (control); 2: pooled crude sera of multiple myeloma patients before treatment; 3: pooled crude sera of Multiple myeloma patients after treatment

of both molecular size and the charge of these proteins (Lenhinger, 2005).

Deep look at the electrogram of all studied group indicates that the sera was separated into distinct bands: albumin, α1- and α2-globulins, β1-and β2-globulins and γ-globulins and that the albumin had the maximum and gammaglobulin had the minimum mobility in the electrical field, that is due to the fact that albumin is a protein with the most negative charges and it contains the most acidic amino acids with COO- groups (Goljan and Sloka, 2008) and it has a molecular weight of 69 kDa (Chatterjea and Shinde, 2007), so it migrates furthest to the anode (positive pole). in contrast, globulins are a large group of proteins, larger in size than albumin whose molecular weights range between 90-1300 kDa as well as it contains proteins with the most positive charges (Goljan and Sloka, 2008) so it remain close to the point of application, which is near the cathode (negative pole).

From Figure 1, the decrease in albumin is not clearly observed, but the distinct differences in the intensity of the globulins bands in group 1 patient sample was obvious when compared with both group 2 and control samples. In the first group there was a slight increase in α-globulins and β-globulin bands, in contrast a slight reduction in the intensity of the γ region was indicated in comparison to that of both group 2 and control samples. In the second group there was a significant increase in the intensity of the γ region, with slight reduction in the intensity of the β1-globulins bands comparison to that of the first group samples.

The results may be because sometimes, IgG of MM may extend to the alpha-2 globulin area, because IgG M-protein may range from the slow gamma to the alpha-2 globulin region. However, in IgG multiple myeloma immunoglobulins may rarely migrate from gamma fraction to alpha-2 fraction (Kyle and Rajkumar, 2004).

In summary, the present result suggests that the increase in AOPP accompanied with decreased in TAC and high ADA activity in serum may be caused by MM, and may provide useful evidence for the diagnosis of MM.

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