Assessment of Oxidative Stress and Inflammatory Process in Patients of Multiple Myeloma

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Abstract Multiple myeloma is a disseminated malignancy of monoclonal plasma cells that accounts for 15 % of all hematological cancers. The present study was conducted to evaluate the role of inflammation and oxidant-antioxidant dynamics in the etiology of this disease. The study population comprised of 20 cases of multiple myeloma and 20 healthy controls. The parameters evaluated were serum malondialdehyde (MDA), superoxide dismutase (SOD) and ferritin levels. The serum MDA levels were 1.9 ± 0.96 nmol/ml in cases as compared to 0.98 ± 0.55 nmol/ml in the controls. Similarly, a statistically significant difference was noted in the SOD and ferritin levels between the cases and controls (93.2 ± 23.8 vs. 210.1 ± 190.5 U/ml and 285.8 ± 216.4 vs. 131.8 ± 30.1 ng/ml respectively). Our study highlights the imbalance in the

oxidant-anti oxidant mechanism and the role of smoldering inflammation in the etiology of multiple myeloma.

Keywords Multiple myeloma · Oxidative stress · Thiobarbitone reactive substances (TBARS) · Antioxidants · Superoxide dismutase (SOD) · Inflammation · Ferritin

Introduction

Multiple myeloma is a malignant neoplasm of plasma cells characterized by a myriad of signs such as pathological fractures as a consequence of osteolytic lesions, anemia and end-organ damage due to monoclonal immunoglobulin secretion. The reported incidence of multiple myeloma in India ranges from 0.5–1.2 per 100,000 [1].

The various mechanisms that may have a role in multifactorial etiology of multiple myeloma include oxidative stress, smoldering inflammatory milieu, genetic mutations and polymorphisms among others [2]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated during cellular events such as oxidative phosphorylation, enzymatic reactions, leukocyte action as well as during environmental insults (ionizing radiation) [3]. These are involved in the reaction cascade known to have deleterious effects on protein, DNA architecture and genetic fidelity. All these perturbations initiate the processes underlying carcinogenesis [4]. Malondialdehyde (MDA), the end product of lipid peroxidation, is an important marker for assessment of oxidative status.

The body has a protective mechanism against these free radicals in the form of antioxidant scavenging system which comprises of enzymes and non enzymatic defense mechanisms. These include superoxide dismutase (SOD),

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catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), vitamins E, C and A, and β carotene. The imbalance between oxidative stress and antioxidant defense mechanism underlies the etiopathogenesis of various diseases [5].

Lately, low grade chronic inflammation has been implicated in carcinogenesis. Cells that incur genetic damage (initiated cells) proliferate in an inflammatory micro environment due to the growth factors and cytokines released by the inflammatory cells. The molecular interplay between inflammatory cytokines with the other neoplastic processes through the NF- κ B pathway also adds to the problem [6].

Free iron is associated with cytotoxicity due to its role in initiating pro oxidant damage through Fenton and Haber–Weiss reactions [7, 8]. It has also been seen that iron can modulate the activity of various signaling pathways such as NF- κ B. Ferritin level is a reliable indicator of iron status of the body and hence it helps us to evaluate the oxidative and pro inflammatory role of iron in carcinogenesis [9].

The present study was undertaken to evaluate the association of MDA, SOD and ferritin levels with the clinico-biochemical profile in patients with multiple myeloma.

Materials and Methods

The study was conducted in the XXX. The study comprised of 20 cases of clinically diagnosed multiple myeloma and 20 healthy age and sex matched controls. The controls were chosen from hospital staff and healthy volunteers. Subjects with a history of chronic inflammatory disorders, surgery or trauma in the past 3 months, anemia, renal or hepatic disorders were excluded from the study population. The study was commenced after taking detailed consent from the subjects. The study protocol was passed by the institutional ethical committee.

Routine hematological and biochemical assessments were carried out. The various parameters under study include MDA, SOD, ferritin, bone marrow aspiration analysis, serum and urine electrophoresis followed by immunofixation. Radiological skeletal survey was carried out to assess skeletal involvement.

Single blood samples were taken from patients, before start of any therapy and controls. Blood was collected by venous arm puncture in heparinized tubes. Half the sample was used for superoxide dismutase estimation. From the other half, the plasma was separated by centrifuging at 1,000 *g* for 15 min. After separating buffy coat and the plasma, the packed cells (erythrocytes) were washed thrice with physiological saline. To determine the activity of RBC antioxidant enzymes, hemolysate was prepared by lysing a known volume of erythrocytes with cold hypotonic

phosphate buffer, pH 7.4. The hemolysate was separated by centrifuging at 2,500 *g* for 15 min at 4 °C. Biochemical estimations were carried out immediately.

Lipid peroxidation was estimated by measurement of thiobarbituric acid reacting substance (TBARS) in plasma by the method of Yagi [10]. Whole blood SOD levels were measured using commercially available kits from Randox, UK. Serum ferritin levels were estimated by enzyme-linked immunosorbent assay (ELISA) using commercially available kits from Diaclone Research, France.

Serum electrophoresis was carried out on Sebia automated electrophoresis system followed by immunofixation and gel documentation. The presence of Bence Jones proteins in urine was determined by the heat coagulation method.

Statistical Analysis

The data were expressed as the mean $\pm$ standard deviation. Mann–Whitney test was used to compare the values between the patients with multiple myeloma and controls. Spearman's correlation analysis was used to find the association between the various parameters of our study. A *p* value of <0.05 was accepted as statistically significant. All statistical analyses were performed with the program Statistical Package for the Social Science 17.0 (SPSS Inc, Chicago, Illinois).

Results

Table 1 depicts the clinical profile of the multiple myeloma patients. Majority of the patients (80 %) presented in stage III. Skull lytic lesions followed by humerus and vertebral fractures were the most frequent radiological finding in these patients. Bone marrow analysis demonstrated increasing plasma cell population ranging from 15 to 85 % and altered myeloid:erythropoietic (M:E) ratio. Immunoglobulin G kappa light chain was the most common monoclonal antibody detected by immunofixation technique.

Table 2 highlights the comparison of the different biochemical and haematological findings in the cases and the controls. A significant difference was observed in the hemoglobin and total leukocyte count between the cases and controls. The albumin: globulin ratio was significantly higher in the cases as compared to the controls. Kidney function tests were deranged in the cases with significantly raised urea and creatinine levels. No significant difference was observed in serum LDH, phosphorus and uric acid levels between the cases and controls.

Upon comparing the markers of oxidant- antioxidant dynamics (MDA & SOD) and inflammatory marker (ferritin), the levels of ferritin and MDA were significantly

Table 1 Clinical profile of the multiple myeloma patients

Characteristics	Number of patients
Stage	
I	0
II	4
III	16
IV	0
Skeletal survey	
Skull lytic lesions	6
Humerus fracture	5
Vertebral fracture	5
Rib fracture	4
Bone marrow analysis	
Plasma cells	13–85 %
M:E ratio	3:1–4:1
Immunofixation pattern	
IgG Lambda	11
IgG kappa	5
Misc light chain gammopathy	4
Bence Jones protein in urine	10/20
Urine electrophoresis	10/20

Table 2 Demographic profile of the study population

	Cases	Controls	<i>p</i> value
Age	52.12 ± 12.82	53.3 ± 12.2	0.45
Male/Female	11/9	11/9	0.87
Hb	7.7 ± 3.4	14.7 ± 4.9	<0.001
TLC	12,250 ± 7,253	9540 ± 2754	0.02
Platelet count	2.32 ± 0.92	1.9 ± 916	0.66
A:G ratio	0.692 ± 0.294	1.15 ± 0.3	0.01
Urea	138 ± 65.1	35 ± 5.34	<0.001
Creatinine	6.93 ± 6.04	1.1 ± 0.9	<0.001
Uric Acid	5.3 ± 1.4	4.6 ± 1.4	0.77
Calcium	10.09 ± 1.74	8.9 ± 1.25	0.04
Phosphorus	4.9 ± 2.17	4.1 ± 1.9	0.89
LDH	566.2 ± 211	332 ± 101	0.65
MDA (nanomoles per millilitre)	1.9 ± 0.96	0.98 ± 0.55	0.01
SOD (U/ml)	93.2 ± 23.8	210.1 ± 190.5	<0.001
Ferritin (ng/ml)	285.8 ± 216.4	131.8 ± 30.1	0.014

higher in the cases as compared to the controls. On the other hand, the SOD levels were significantly lower in the cases thereby highlighting the imbalance. Multivariate analysis highlighted the superior discriminatory role of MDA in the etiology of multiple myeloma (Table 3). Table 4 illustrates the correlation analysis between the different parameters with multiple myeloma. MDA demonstrated a pearson's coefficient of -0.660 with coefficient of

Table 3 Multivariate analysis of the study variables

Parameters	β coefficient	<i>p</i> value
SOD	0.214	0.079
MDA	0.541	0.000*
Ferritin	0.107	0.429
T. protein	0.329	0.007*

* *p* value <0.05 considered as statistically significant

Table 4 Coefficient of correlation and coefficient of determination of various parameters with multiple myeloma

Parameters	Pearson coefficient of correlation	Coefficient of determination
SOD	-0.413	0.170
MDA	-0.660	0.436
Ferritin	-0.462	0.213
T. protein	0.410	0.168

The correlation analysis has been done between the incidence of multiple myeloma with the various study parameters

The contribution of various parameters on total variation in multiple myeloma is as follows: MDA > FERRITIN > SOD > TOTAL PROTEIN

63 % of total variation is contributed by all these parameters together as coefficient of determination is 0.630

determination of 0.436 proving its superiority in the etiopathogenesis of multiple myeloma. The coefficient of determination is used for the prediction of future outcomes on the basis of other related information. It provides a measure of how well future outcomes are likely to be predicted by the model.

Discussion

Carcinogenesis has now been identified as a pathology arising out of perturbations in the pathways involved in regulation of cell cycle and cellular/genetic integrity and maintenance of homeostasis of the factors involved in the same. Some of these etiological pathways include smoldering inflammation and disturbances in redox potential. The clinical manifestations may also be explained in view of these alterations [2].

We evaluated the role of disturbances in redox potential as one of the factors involved in the etiopathogenesis of multiple myeloma. Malondialdehyde (MDA) levels were evaluated as the marker of lipid peroxidation. The MDA levels were significantly elevated in the cases as compared to the controls thereby substantiating the evidence implicating increased oxidative state as the cause behind genotypic and phenotypic modifications in plasma cells leading

to neoplastic transformation. Our findings are corroborated by the findings of Sharma et al. [11] and Zima et al. [12]. Cieslar et al. [13] establishes a correlation between MDA levels and platelet dysfunction manifested as bleeding diathesis in multiple myeloma patients. Multivariate analysis proved the superiority of MDA in the risk profiling of patients with multiple myeloma.

The antioxidant system for the scavenging of free radicals include both enzymes such as Superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and non enzymatic factors such as Vitamins E & C, uric acid, and bilirubin etc. We evaluated the serum levels of SOD in both cases as well as controls as a measure of the anti oxidant defense mechanism. The levels were significantly lower in the cases as compared to the controls. Our findings are similar to that reported by Bakan et al. [14] and Paul et al. [15]. These findings highlight the imbalance between the oxidant antioxidant systems leading to impairment of anti oxidant defense mechanism.

The serum ferritin level is a reflection of the pro inflammatory state and it constitutes an important acute phase reactant in the body. The ferritin levels in the body are modulated by inflammatory cytokines and immune regulatory pathways. The serum ferritin levels were significantly higher in the cases as compared to the controls. Our findings are similar to those observed by other researchers [7].

Conclusions

Multiple myeloma is malignant neoplasm of plasma cells. The role of inflammatory and oxidant pathways has been implicated in its pathogenesis. Better understanding of the etiopathogenesis will aid in effective patient management and reduction of morbidity and mortality due to multiple myeloma.

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