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Assessment of Serum Nitric Oxide and Some Essential Trace Elements Levels in Patients of Multiple Myeloma

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Abstract

Background and objective: Multiple myeloma is the most common malignant plasma cell dyscrasia and ranks second among primary haematological malignancies. Multiple Myeloma (MM) is a neoplasm of B cell lineage characterized by excessive proliferation of abnormal plasma cells. The principle goal of this study was to evaluate the effect of serum essential Trace elements such as: levels of copper (Cu), zinc (Zn), selenium (Se) and molybdenum (Mo) in serum of patients with Multiple Myeloma and compared to healthy individuals

Material and Methods: The studied consist of 100 volunteers with age range 40-65 years, 50 healthy individuals compared with 50 patients with Multiple Myeloma in order to investigate the following serum parameters: nitric oxide and some essential Trace elements (copper, zinc, selenium, and molybdenum) and compared to healthy individuals. NO levels were determined spectrophotometrically using the Griess reaction method. The serum levels of copper (Cu), zinc (Zn), selenium (Se) and molybdenum (Mo) were established using Flame Atomic Absorption Spectrometry.

Results: The results of this study showed significant increase in the level of nitric oxide ($p<0.001$) in all MM patients as compared to controls. Circulating levels of zinc and selenium, molybdenum ($p<0.001$were significantly lower in patients with MM than in healthy controls, while serum copper level was raised significantly in patients (MM) compared with normal control.

Conclusion : Estimation of nitric oxide and essential Trace elements may aid in diagnosis, assessment of severity and monitoring of : Multiple Myeloma (MM) .

Keywords: Nitric Oxide; Multiple Myeloma; Essential Trace elements

Introduction

Multiple Myeloma (MM) is a neoplasm of B cell lineage which is characterized by excessive proliferation of abnormal plasma cells. These abnormal plasma cells secrete abnormal immunoglobulin that produces a condition called monoclonal gammopathy, which can be detected by the presence of M protein in serum and urine electrophoresis [1]. It accounts for 10% of the haematological malignancies [2]. A different feature of MM is the accumulation of malignant cells in the bone marrow, where they lead to osteolytic bone devastation and impaired hematopoiesis [3,4]. The etiology of multiple myeloma (MM) is unknown, but one of the suspects is the oxidative stress, as is the case in many other malignant diseases.

Nitric oxide (NO) is a multifunctional molecule produced in a variety of mammalian cells. At physiological levels, NO is associated with neurotransmission and vasodilatation. At higher levels, NO has tumoricidal and bacteriocidal effects [5,6]. In the cell-mediated immune responses, NO is produced in macrophages, neutrophils and lymphocytes [7-9]. Several lines of evidence indicate that NO has cytotoxic effects on human cell lines from patients with leukemia or lymphoma [9-13].

Nitric oxide is a pleiotropic ancestral molecule, which elicits beneficial effect in many physiological settings but is also tenaciously expressed in numerous pathological conditions. NO• plays

multiple roles in both intracellular and extracellular signaling mechanisms [14]. This highly reactive molecule is produced in the body by the three iso enzymes of nitric oxide synthase (NOS) using L-arginine as a substrate. NO• is either cytostatic or cytotoxic, interacting with a number of molecular targets within cells. Cells within different tissues display varying responses to NO•. Chronic inflammation causes over expression of NOS leading to genotoxicity. NO• may mediate DNA damage through the formation of carcinogenic nitrosamines, generation of RNS and inhibition of DNA damage repair mechanism. It can thus be considered as a tumor initiating agent. However, NO• may also have an impact on other stages of cancer development ranging from cellular transformation and formation of neoplastic lesions to the regulation of various other aspects of tumor biology [15]. NO• plays an important role in host defense and homeostasis when generated at a low level for a brief period of time, but becomes genotoxic and mutagenic when generated at higher concentrations for prolonged periods of time. Thus, the biological outcome of the NO• mediated effects is complex and depends on the internal and external environment of the target and generation sites of the cells as well as the concentration of NO• generated [16].

Trace elements play an important role in the structure of proteins, enzymes and complex carbohydrates to participate in biochemical reactions. Essential trace elements are involved in a number of metabolic activities, including neuro conduction, transport, excretory processes and serving as cofactors for enzymes. Some of the trace elements like Selenium, Zinc, Manganese, Magnesium and Copper are cofactors or structural components of antioxidant enzymes. Moreover, selenium and glutathione peroxidase play an important role in protecting cell membranes from oxidative damage and decreased blood selenium and are common in chronic renal failure patients. Zinc and Copper are the intensively and metabolically important trace metal for nutrients [17]. Although the trace elements are the essential components of biological structures, they may show toxic effect when they are more concentrated than the amount that are required for biological functions. In addition, the toxicity can be spread to other non-essential elements of very similar atomic characteristics that can mimic the reactivity of a trace element [18]. Trace element determinations in blood serum have become important to investigate their vital role in human metabolism, as well as to obtain information regarding the health status of individuals [19].

Material and methods

All the chemicals and reagents used in the study were of analytical grade and pure. Doubly distilled demineralized water was used for all the washings and preparation of solution. In this study 100 samples were collected. The sampling procedure was done in 50 patients (61.45+12 years) with MM before start of any therapy comparing with 50 healthy persons (48.16±6.95) years. None of these patients received medicines. Patients were chosen from the patients referred to the Nanakaly Hospital in (Erbil-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 vacationer tubes, one The blood in the tube 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. The study was conducted after obtaining approval from local ethics committee of Haw'ler Medical University (HMU).

- Estimation of nitric oxide (NO), concentration: NO was estimated colorimetrically by Griess reaction [20].
- Estimation of Essential trace elements concentration: Atomic absorption spectrophotometer method was used to determine (Cu, Zn, Se and Mo) in serum samples. The serum samples were digestion first. And then Stock standard solutions of each trace elements were prepared (1000 µg/ ml).

Statistical analysis

All results were expressed as means ± S.E. The significance of difference was evaluated by ANOVA test. A probability of $P < 0.05$ was considered statistically significant.

Results

Nitric oxide concentration in blood serum of Multiple Myeloma (MM) .The results in (Figure 1) (Table 1) showed that there was a significant increase ($p < 0.001$) in serum nitric oxide concentration in Multiple Myeloma (MM) compared to the control group.

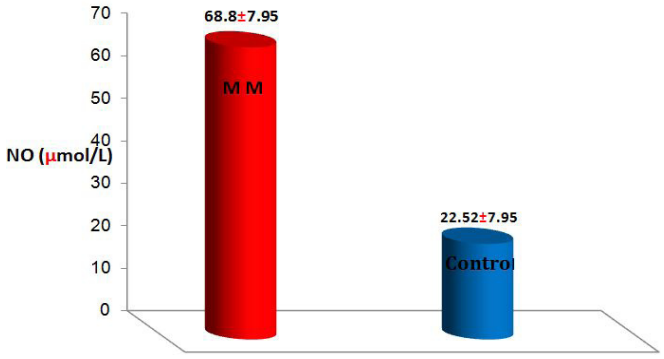

Figure 1 : Serum Nitric Oxide level in the control and pateints groups with MM

Parameter	Mean ±SD		P-value
	Controls (n=50)	MM patients (n=50)	
NO (µmol/L	22.52±7.95	68.8±67.1	p<0.001

Table 1: Serum NO (mean ± SD) in healthy controls (group I) and patients group with MM

Trace elements concentration in blood serum of Multiple Myeloma

(Figure 2) (Table 2) show the results of the levels of some serum Trace elements such as: Zn, Se, Cu, and Mo of one hundred participants, 50 healthy volunteers and 50 patients with MM with age range of 40-65 years. The results shows that the levels of serum Zn (P<0.001), Se (P<0.001), Mo (P<0.007), were significantly decreased in patient with MM as compared with control groups as shown in Figure 1. The analyses results of the serum levels of Cu, show that the level of the serum Cu was significantly (P<0.001) decreased in patient with MM as compared with control groups as shown in (Figure 2) (Table 2).

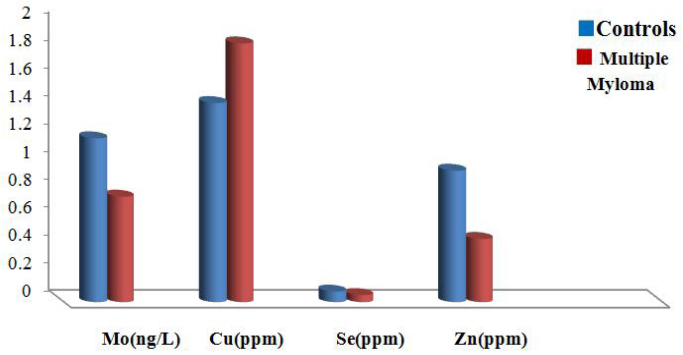

Figure 2: Serum Trace elements concentration in the control and patients group with MM

Serum metals	Mean ±SD		P-value
	Controls (n=50)	MM patients (n=50)	
Zn (ppm)	0.9468± 0.1148	0.4553± 0.08649	p<0.001
Se (ppm)	0.076428± 0.008234	0.051310± 0.004723	p<0.001
Co (ppm))	1.4341± 0.16675	1.8634± 0.09017	p<0.001
Mo (ng/mL)	1.18±0.46	0.76±0.23	p<0.007

Table 2: Serum Trace elements concentration (mean ± SD) in healthy controls (group I) and patients group with MM

Discussion

The levels of nitric oxide were found raised (p<0.001) in Multiple Myeloma (M.M) as compared to control group. The difference in NO levels between control group and patient group was statistically significant (p<0.001). NO influences various aspects of tumor biology like modulation of cell growth, apoptosis, differentiation, angiogenesis and metastatic capability. Mechanism of action of nitric oxide involves inhibition of DNA synthesis, mitochondrial respiration and enhancement of cellular oxidative injury. This increased oxidative stress may be implicated in increasing ferritin secretion by breast tissue [21]. Hypoxia, often associated with neoplastic tissues, is another factor which may induce NO production. NO, acting as an oxidant, may induce oxidative stress [22]. Thus NO is sufficiently interlinked in malignant conditions including bone carcinoma.

Role of nitric oxide in cancer biology is complex. Several lines of investigation suggest that NO• is involved in the initiation of numerous cancers. High levels of NO• may modify DNA directly or indirectly by inhibiting DNA repair activities, modifying the proteins by the nitration of phenolic amino acids [23,15]. A body of evidence indicates a definite role for NO• in tumor growth. One of the causes of high concentrations of total NO• in the serum of patients in the advanced stage of the disease could be secretion of NO• by cells of the immune system [24] demonstrated higher expression and concentration of inducible nitric oxide synthase (iNOS), the enzyme responsible for the generation of NO•, in polymorphonuclear cells and peripheral blood mononuclear cells in oral cancer patients. NO• generated by NOS (located either within the tumor or in the surrounding stroma) may promote new blood vessel formation by up-regulating VEGF. This neovascularization not only enhances the ability of the tumor to grow, but also increases its invasiveness and metastatic ability.

The results showed that the levels of trace elements (Zn, Mg, Se, and Mo) of patients significantly decreased compared with control. In-vivo antioxidant nutrients which include vitamin C, trace elements such as Se, Zn and Cu play a crucial role in defending against oxidant damage [25], thus The lowering in these values was reflected the principle function of these elements acts as antioxidants (as free ion of elements or bounding with enzyme) in biological system [26]. The decrease in zinc level and its concomitant effect on copper level may affect the activity of some antioxidant enzyme that use these elements as cofactor within its structure. Hence those enzymes lose some of their activity and ability to remove free radicals [27].

Human body uses selenium to produce glutathione peroxidase, which works with vitamin E to protect cell membranes from damage caused by dangerous, naturally occurring substances known as free radicals produced by oxidative metabolism, Selenium is taking center stage as a potential anticancer agent by promoting formation of white blood cells which destroys the cancer cells and is an essential component of more than ten selenoproteins with multiple biochemical functions. Moreover, it boosts the

immune system by increasing the activity and number of white blood cells and prevents premature aging, degenerative diseases, cardiovascular diseases, inflammatory diseases, stroke, cataracts, and rheumatoid arthritis [28].

Mo is a cofactor of enzyme xanthine oxidase which catalyze the reduction of oxygen quinines [29,30]. This enzyme interacts with compounds containing aromatic nitro group reducing them to hydroxyamino derivatives, which prevents the formation of N-nitroso compounds that have high degree of carcinogenicity [31]. In present study the mean serum Mo content was 0.76 and 1.18 ng/ml in, cancer and normal groups respectively. Serum levels were significantly increased in MM. The results suggest a linkage between lowered Mo levels in serum and MM. Highly significant increase in copper level was observed in the sera of patients of compared with control. Among the cationic ligands, copper deserve particular consideration because it act as transition metal, it is very potent to generate ROS after a reaction with oxygen. Free Cu (II) ion can interact with hydrogen peroxide (H2O2) leading to the formation of the deleterious hydroxyl radical via the Fenton reaction. Bound to proteins, copper is generally less susceptible to participate in the Fenton reaction [32].

Conclusion

In this study, a significant increase in serum nitric oxide was observed with significant decrease in serum essential Trace elements for the Multiple Myeloma patients, and it was concluded that both could be important for the diagnosis of Multiple Myeloma and for its treatment.

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