Increased Myloperoxidase level is a biomarker of inflammation and oxidative stress in diabetic foot ulcers

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Abstract

Background: myeloperoxidase (MPO) a leukocyte enzyme acts as a first-line defender against microorganisms. However, increased MPO levels have been found to be associated with different diseases. Therefore, this study aimed to investigate whether MPO, a biomarker of inflammation and oxidative stress ,increased in patients with diabetic foot ulcers (DFU)

Methods: Plasma MPO antioxidant enzyme activity superoxide dismutase (SOD), and the inflammatory ,marker iterlukin-6 (IL-6) were determined in 30 patients with DFU and 30 healthy subjects as control group using ELISA. All results were statistically analyzed.

Results A highly significant increase was found in the plasma level MPO in patients with DFU compared to control ($P < 0.05$). Plasma levels of antioxidants (SOD) were significantly decreased in the patient group ($P < 0.05$) compared to control. However, cytokines (IL-6) levels demonstrated a significant increase in DFU patients ($P < 0.01$).

Conclusion The results of the present study provide evidence that MPO, oxidative stress, inflammation are associated with risk of DFU that may have future facilitate the development better management of DFU.
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Diabetes is of metabolic diseases in which high levels of sugar found in the

KEYWORD: Myeloperoxidase, inflammation, oxidative stress, diabetic foot ulcers

Introduction:-

Diabetes mellitus is a metabolic disease with hyperglycemia either to the absolute deficiency of insulin secretion in b-cell of pancreas or insulin resistance. The long term effect of hyperglycemia may induce inflammation and oxidative damage to other organs and result in many complications such as cardiovascular disease, blindness, amputations and renal failure [1]. Diabetic foot is the foot of a patient with diabetes type II, which are susceptible to infections and gangrene due to a variety of neurological disorders and arterial muscle and that caused by diabetes, which affects all members of the body and its impact on the feet of more complications frequent and dangerous, causing the amputation of the lower limbs in whole or in part.

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. High glucose level can stimulate free radical production, when it increased to the highest level, the defense system of the body becomes unable to counteract the enhanced ROS generation and as a result an imbalance between reactive oxygen species (ROS) and their protection occurs which leads to the condition known as oxidative stress [2, 3]. For the normal metabolic processes a certain amount of oxidative stress is necessary, since ROS play various regulatory roles in cells [4]. ROS are produced by inflammatory cells such as neutrophils and macrophages during the process of the respiratory burst in order to eliminate pathogen [5]. Superoxide dismutase (SOD) is one of antioxidant enzymes that catalyzes the

dismutation of superoxide anion ($O_2^{\bullet-}$) into hydrogen peroxide and molecular oxygen [6,7]. SOD has an important protective role against oxidative stress that is produced cellular and histological damages.. Myeloperoxidase (MPO (EC 1.11.1.7)), is a mammalian heme peroxidase, that produced from neutrophils and monocytes at sites of inflammation [8] When released as part of the innate host defense, MPO reacts with halides, thiocyanate and nitrite and generates ROS that play an important role in antimicrobial activity for phagocytic cells [8,9.] However, persistent activation of MPO promotes oxidative damage of host tissue at sites of inflammation, including neurodegenerative disorders, carcinogenesis, lung disease and respiratory damage, rheumatoid arthritis, kidney damage and atherosclerosis [8, ,10,11] MPO reacts with different biological molecules, thus enhancing inflammatory tissue damage in diabetic patient. Research regarding MPO activity in diabetes is inconsistent, indicating both higher and lower levels of MPO in different tissues and clinical condition [12,13].Previous studies strived to identify the mechanisms of a possible relation between MPO and diabetes mellitus. One study demonstrated that adhered neutrophils, evaluated by MPO activity in vitro are enhanced by insulin treatment and the authors, therefore, speculated that high MPO activity in diabetes might be related to hyperinsulinemia [14]. Proinflammatory cytokines play a key role in the development of diabetes by enhancing insulin resistance in diabetes type II disease as high blood sugar stimulates the connective cells (macrophages) to produce kinematics cellular inflammatory such as IL-6, IL-1,

IL-18, TNF-α [15]. However, understanding of inflammation and MPO, especially with their pathophysiological role in DFU, is still unclear and further investigations will facilitate the development better management of DFU.

In this study, we hypothesized that levels of MPO as a marker of inflammation and generation of ROS increase in diabetic patients. To test our hypothesis, we compared baseline MPO levels in diabetic and nondiabetic patients.

Experimental

Subjects

Plasma MPO, IL-6, SOD levels were measured in 30 (12 males and 18 females) patients with a diabetic foot ulcer (DFU), and 30 healthy subjects (10 males and 20 females) as a control group. The mean age of control (48.5±0.25) and in the patient group (53.33±0.261 y) which were randomly selected from patients with DFU from April to October 2016. Information regarding the medical history of each subject was obtained, including age, sex, diseases suffered and duration of illness with their daily diet and occupation. None of the patients had consumed alcohol, nor was there any history of surgery

Methods

All groups were subjected to thorough clinical history, examination and specific DFU investigation. Venous blood samples (5 ml) were collected from the patient and control groups. Plasma was separated by centrifugation (Gallenkamp, Germany) at 3000 RPM for 10 min and stored in capped plastic tubes at −20°C

until analysis. Glucose, total cholesterol, and urea were measured by commercial kits. MPO,IL-6, SOD levels in the plasma were measured by ELISA Absorbance was measured in duplicates with a micro plate reader (Bioneer-Korea, Cloud-clone Corp-U. S. A). The final concentration was expressed in pg/ml

Statistical analysis:-

Data are expressed as mean ± SEM. Statistical analysis was carried out using a design, statistical package for social science (SPSS), the significant differences between control and the patient groups were determined by using a Student's t-test. The probability of ($P < 0.05$) is considered significant throughout.

Results

Clinical characteristics about patients' age, blood glucose, total cholesterol and urea and so forth were summarized in (Table 1). The levels of total cholesterol, glucose and urea were significantly higher than those of controls ($P < 0.05$, table 1). Plasma MPO levels were found to be significantly higher in DFU patients compared to control ($P < 0.05$ Fig .1). A significant decrease was found in the activity of antioxidant enzyme SOD ($P < 0.05$, Fig. 2,) in the DFU patients compared to the control. Serum levels of IL-6 were measured in patients with DFU using ELISA. IL-6 was significantly increased in the plasma of DFU patients compared to control ($P < 0.01$, Fig. 3)

Table (1): Samples ,plasma glucose, cholesterol and urea levels in study groups

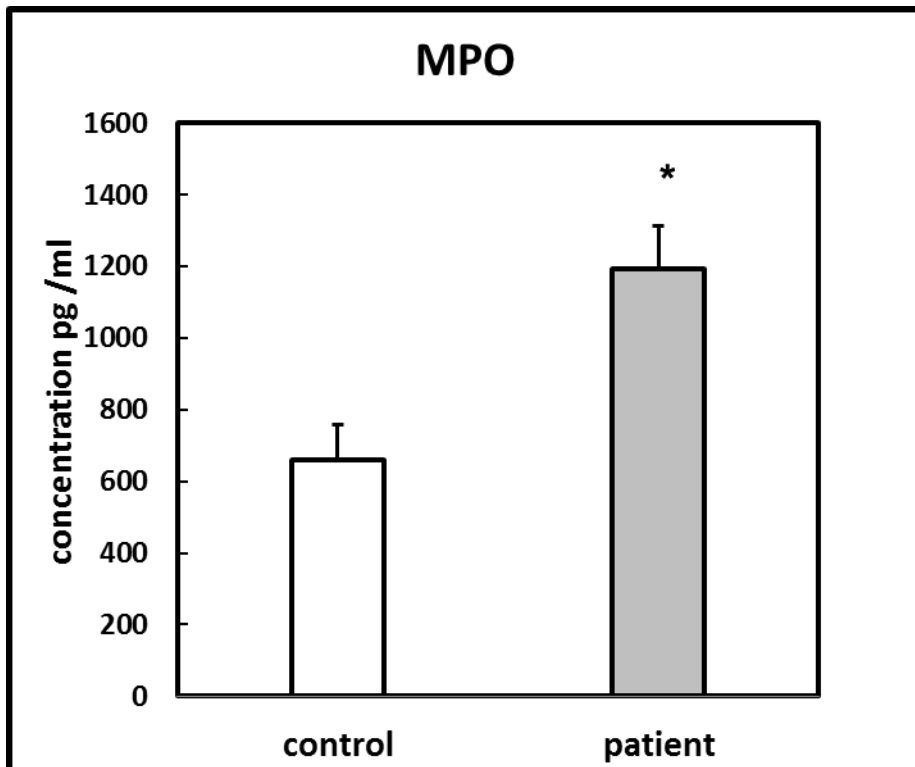| Groups	Patients	Control
Number of samples	30	30
Mean age	53.33±0.261	48.5±0.253
Glucose level mg/dl	265.1053±0.628*	105.10±1.228
Cholesterol level mg/dl	161±0.455*	123±0.852
Urea level mg/dl	40.47059±0.253*	19.370±0.43

Fig1 Serum MPO levels in patients with DFU. Plasma samples were isolated from the blood of patients with DFU. MPO was assessed by ELISA. Data are expressed as means ± SEM, for 30 patients, $n = 30$ with duplicate measurements. **indicates significant differences compared to the control (Student's t-test, $P < 0.05$).
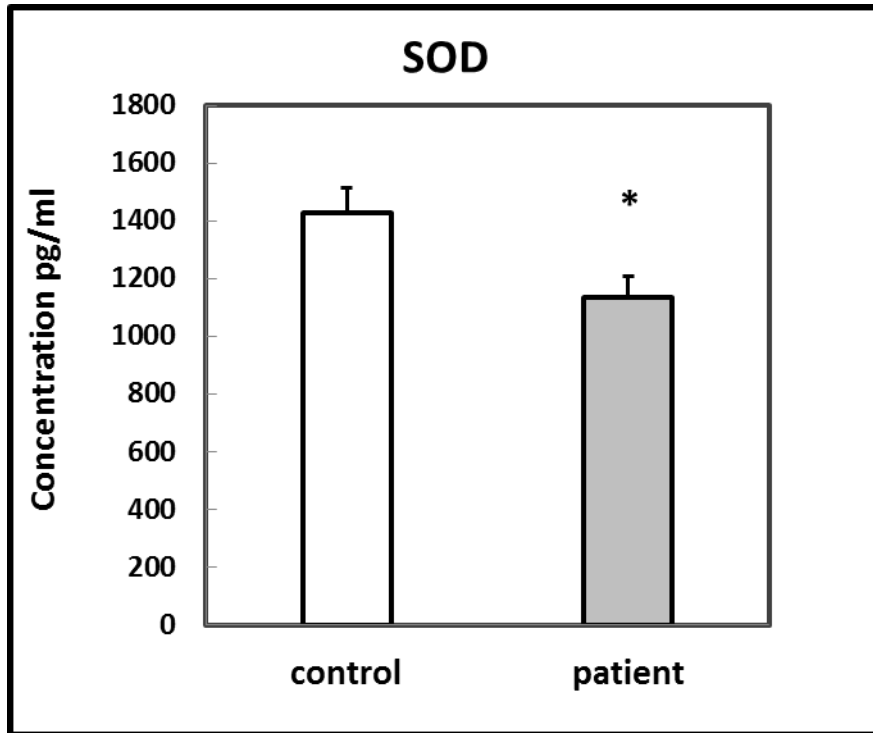
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Fig. 2 Plasma SOD activity in patients with DFU. Plasma samples were isolated from the blood of patients with DFU. SOD activity was assessed by ELISA. Data are expressed as means ± SEM, for 30 patients, $n = 30$ with duplicate measurements. *indicates significant differences compared to the control (Student's t-test, $P < 0.05$)

Fig.3 Plasma pro-inflammatory cytokine levels in the patients with DFU. Plasma serums were isolated from blood of patients with DFU and control. IL-6 levels were analyzed by ELISA. Data are expressed as the means ± SEM, for 30 patients, $n = 30$ with duplicate measurements. *indicates significant differences compared to the control (Student's t-test, $P < 0.01$).
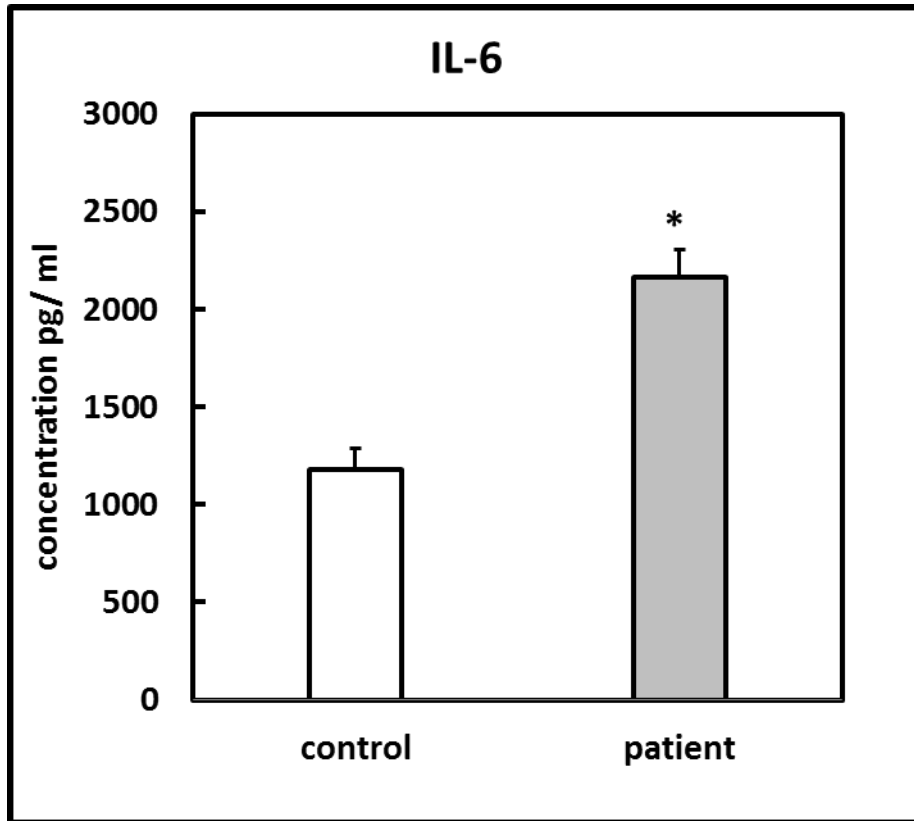
Discussion

In the present study we observed a significant increase in MPO levels in patients with DFU compared with control. A few studies have interested in the relation between MPO levels and the presence of diabetes. Three clinical studies found significantly higher MPO levels in diabetics twice measured in serum and once in plasma samples), whereas four clinical studies found no correlation [16,17,18,19,20].This may be explained in part, by differences in populations, methods, and examined tissues . Some studies have attempted to identify the mechanisms of possible relations between MPO and diabetes mellitus . One study demonstrated that adhered neutrophils, evaluated by MPO activity *in vitro*, are enhanced by insulin treatment, and therefore it speculated that high MPO activity in diabetes might be related to hyperinsulinemia[21]. Another study suggested that vascular bound MPO could use high glucose-stimulated hydrogen peroxide to amplify high glucose-induced injury to the vascular wall [22]. The findings in the present study are in accordance with those studies demonstrating higher levels of MPO in diabetes. It has been demonstrated that elevated plasma myeloperoxidase levels correlate with high circulating inflammatory markers in patients with several diseases like coronary artery disease [23]

Diabetic oxidative stress associated with a decrease in the antioxidant status, which can further enhance the deleterious effects of free radicals [24]. In the present study, SOD activity was significantly decreased in DFU patients (Figs. 2). The results of this study are in agreement with those reported that an increase in glucose concentration can lead to oxidative stress due to tissue damage [25].An increase in oxidative stress, as well as the reduction in antioxidant capacity could be related to the oxidative DNA damage and insulin resistance as an complications in patients with diabetes [26].This attributed to the decrease in antioxidant potential of plasma, complications of diabetes increase which include DFU, nerve damage, blindness [27].

The specific relationship between MPO and inflammation hasn't been completely understood. However, based on the present study observations, it is possible that the interaction between MPO, inflammation might be mediated by MPO that enhanced oxidation of cholesterol esters, iron and cellular debris. Another potential mechanism could be by the presence of advanced glycation end products (AGE) that might enhance the inflammatory content in the atheroma leading to increase MPO levels [28,29]. Furthermore, hyperglycemia and diabetes mellitus have been shown to be associated with activation of leukocyte counts [30,31]. The present study clearly supports this concept and shows that circulating

MPO levels, derived predominantly from leukocytes, are higher in diabetics compared with controls. Additionally, we observed associations of MPO

concentration with markers of systemic inflammation, i.e. IL-6. This result is in line with previous studies reporting correlations of MPO with CRP and/or fibrinogen [32,33]. The relationship of MPO levels with pro inflammatory markers may be due to a reverse causation. Macrophages in diabetes released cytokines, which in turn enhanced the synthesis of pro-inflammatory proteins that may prefer recruitment and activation of MPO-containing leukocytes. The increased release of inflammatory cytokines such as IL-6 during high blood pressure and the activation of the renin-angiotensin system could lead to neutrophil activation and respiratory burst [34,35] which might promote the release of MPO from neutrophils and resulted in the increase of MPO in the blood. Diabetic patients often have abnormal lipoprotein metabolism. HDL-C not only can reduce blood cholesterol level, but also exhibit antioxidant and anti-inflammatory effects. Studies have reported that HDL-C can inhibit the neutrophil activation [36,37]. Furthermore, diabetes mellitus has been shown to be related to activation of leukocyte counts [38,39],[40].The present study clearly supports this concept and shows that circulating MPO levels, released from leukocytes, are higher in diabetics compared with controls.

Conclusions:-

In the present study, MPO level has been consistently demonstrated to be elevated in patients with DFU. Increase the effectiveness of MPO in diabetic foot leads to oxidative damage, tissue damage, and chronic inflammation that play an important role in the development of diabetic foot disease. However, the

data so far available are relatively few; therefore, more studies are requested to investigate the role of the MPO.

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