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Myeloperoxidase was originally called verdoperoxidase by Agner (1941) who first purified this from purulent fluids of tubercular emphyema, and was to reflect both its green colour and ability to catalyze peroxidative reactions. In 1967, Klebanoff has demonstrated MPO system to be strongly bactericidal, and the enzyme an important component of the neutrophil antimicrobial armory.

In the next year, Klebanoff described the antibacterial effect of MPO, a halide (such as iodide, bromide or chloride ion) and H₂O₂ on *Escherichia coli* and *Lactobacillus acidophilus* and reported that activity decreased by catalase, cyanide, azide, tapazole and thiosulphate. Rosen and Klebanoff in 1976 investigated the role of superoxide anion- and myeloperoxidase-dependent reactions in the light emission (chemiluminescence) in phagocytosing polymorphonuclear leukocytes, using leukocytes that lack myeloperoxidase, inhibitors (azide, superoxide dismutase), and model systems. They suggested that light emission by phagocytosing polymorphonuclear leukocytes is dependent on both myeloperoxidase-catalyzed reactions and the superoxide anion, and involves in part the excitation of the ingested particle.

In 1976 Hansen et al., conducted sequential studies on changes in intraneutrophilic and plasma concentrations of the three antibacterial proteins- lysozyme, lactoferrin, and myeloperoxidase during acute bacterial infection in nine patients. Intraneutrophilic concentrations of the three proteins were decreased by more than 50 % during the 1st week of infection.

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followed by a slow increase over the following 2 weeks. The data suggests that neutrophilic granulocytes are deficient of these proteins during early bacterial infection, possibly because of deficient synthesis of antibacterial proteins in the bone marrow, and that neutrophil toxic granulation is the visual counterpart of this defect. While the plasma lysozyme did not show any sequential change, plasma myeloperoxidase was high at the start of infection and quickly decreased towards normal values, and plasma lactoferrin, high in the first samples, showed a secondary peak 1 week after the onset of the disease, before normalization was seen. These differences in enzymes may result from differences in the signals, which are specific for the individual antibacterial protein and not for the different types of neutrophil granules.

Christensen and Rothstein (1985) reported two situations in which the concentration of MPO/neutrophil was found to change; during the growth and development of animals and during bacterial infection. During fatal bacterial infection, MPO/neutrophil fell rapidly, often to undetectable levels, but during sublethal infections, following a 24-h lag period in adults and a 48-h lag in neonates, the concentration increased to twice the normal. For that they determined the quantity of myeloperoxidase in a volume of whole blood, and the neutrophil concentration in that same volume in experimental animals and the results expressed as units of MPO ($10^{-7}$/neutrophil).

Hofstra and Uetrecht (1993) studied the myeloperoxidase-mediated activation of xenobiotics by human leukocytes and reported that MPO- or MPO-generated oxidants are capable of oxidizing a wide variety of compounds and a broad range of functional groups, especially those that contain nitrogen and sulfur. The powerful oxidant is hypochlorous acid. Leukocytes have a role in immune response; therefore, reactive intermediates generated by leukocyte metabolism of xenobiotics may have a role in idiosyncratic drug reactions, particularly those that are immune-mediated such as drug-induced lupus or agranulocytosis.

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In 1995 Lincoln demonstrated that the exogenously added MPO at physiological levels, enhance both phagocytosis and killing of *Escherichia coli* by macrophages. Both superoxide dismutase and catalase ablated MPO-induced bactericidal activity. They suggested that soluble MPO, released from neutrophils at a site of infection or inflammation, could enhance both phagocytosis and killing of microorganisms.

Marquez and Dunford (1995) found that myeloperoxidase is the most efficient catalyst of tyrosine oxidation at physiological pH, when compared with horseradish peroxidase, thyroid peroxidase, and lactoperoxidase. Although chloride is considered as the major myeloperoxidase substrate, tyrosine is able to compete effectively for compound I (one of activated state of MPO). Steady state inhibition studies demonstrate that chloride binds very weakly to the tyrosine-binding site of the enzyme. Both Heinecke et al., (1993) and Marquez and Dunford (1995) reported the dityrosine, the highly fluorescent stable compound, as a possible marker for proteins oxidatively damaged by myeloperoxidase in phagocyte-rich inflammatory lesions.

The neutrophil myeloperoxidase uses H$_2$O$_2$ to oxidize chloride, bromide, iodide and thiocyanate to their respective hypohalous acids. In 1997 Van Dalen et al. analyzed whether myeloperoxidase oxidizes thiocyanate in the presence of chloride at physiological concentrations of these substrates and determined the relative specificity constants for chloride, bromide and thiocyanate as 1:60:730 respectively, indicating that thiocyanate is by far the most favoured substrate for myeloperoxidase. Regardless of where the enzyme acts, thiocyanate is a major physiological substrate of myeloperoxidase.

Kabutomori et al. (1999) measured the mean myeloperoxidase index (MPXI) of neutrophils in normal subjects, with an automated hematology system, which differentiates white blood cells. MPO activity was represented by the MPO staining intensity. They found that the MPXI in

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normal women was significantly higher than that in normal men and the menstrual cycle affects the MPXI. This sex difference suggested that some microbicidal activity may be stronger in women than in men. The MPXI may be useful as a partial index for microbicidal activity of neutrophils.

MPO has several roles in biological functions. Since it is an inevitable factor, its deficiency causes many problems like increased infection susceptibility, decreased tumor surveillance etc. The MPO deficiency can occur in two forms, hereditary and acquired. It may be partial or complete. In 1977 Cech et al.,\textsuperscript{90} reported of hereditary MPO deficiency in the granulocytes of patients with diabetes mellitus suffering from Candida albicans hepatic abscess. Their functional granulocyte studies had revealed normal chemotactic and phagocytic activity although the bactericidal activity is partially diminished with regard to Staphylococcus aureus and almost nil with regard to Candida albicans. After two years (1979), they studied a case of hereditary myeloperoxidase deficiency in a diabetic patient suffering from Candida albicans liver abscess and found that peroxidase activity is completely absent in the neutrophils and monocytes although it is present in the eosinophils.\textsuperscript{91}

In the study on ‘MPO deficiency and its prevalence and clinical significance’, by Parry et al.,\textsuperscript{92} (1981) noted only minor defects in killing of Staphylococcus aureus by MPO deficient leukocytes, where as candidicidal activity was much more impaired. In the same year (1981), the study on hereditary MPO deficiency was conducted by Kitahara et al.,\textsuperscript{93} and revealed that partial deficiency have impaired bactericidal activity against S.aureus. Their findings have supported the concept of multiple leukocyte bacterial killing systems. Another work supporting the multiple leukocytic bactericidal systems, was done by Lanza\textsuperscript{94} (1998) on the clinical manifestations of MPO deficiency and reported that the total or partial deficient individuals does not have an increased frequency of infections.
Stendahl, et al.,55 (1984) opined in support of their finding in a patient with complete MPO deficiency suffering from generalized pustular psoriasis, do not usually show any increased susceptibility to infection or altered inflammatory response, in contrast to several other biochemical defects in polymorphonuclear neutrophils. They found that the MPO-deficient neutrophils showed enhanced phagocytosis than in normal persons and prolonged stimulation of superoxide production. When MPO was added to the hyperactive MPO-deficient cells, phagocytosis was reduced more rapidly. Catalase, azide, and methionine eliminated the inhibitory effect, and catalase and methionine, in fact, enhanced the phagocytic activity of adherent neutrophils. Apart from being a potent antimicrobial system, the oxidizing activity of the MPO- H₂O₂-halide system may modulate the inflammatory response by impairing certain receptor-mediated recognition mechanisms of phagocytic cells, which otherwise could elicit inflammatory reactions and tissue injury.

3.1 Myeloperoxidase and diabetes mellitus

In 1975 Niethammer et al.,95 had reported the impairment of granulocyte function in juvenile diabetes. They did not find a phagocytic defect in the ingestion of particles, but the capacity of intracellular killing of Staphylococcus aureus was impaired. Chemotaxis was also reduced whereas intracellular killing of Candida albicans were normal. Better control of diabetes led to an improvement of bactericidal killing capacity.

Kaneshige et al.,83 (1982) also examined the phagocytosis and intracellular killing of Staphylococcus aureus by granulocytes in diabetic patients. There was no significant difference in the phagocytic activity of granulocytes between control and diabetic-subjects. However, intracellular killing by granulocytes was significantly reduced in insulin-treated diabetic patients compared with control subjects. No significant difference observed between controls and diet-treated diabetic patients. It is suggested that decreased activity of intracellular killing of bacteria in granulocytes is one of

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the mechanisms of increased susceptibility to infection in patients with advanced stages of diabetes mellitus.

In 1993, Wykretowicz et al.,\textsuperscript{96} noticed normal phagocytosis to staphylococci, decreased intracellular killing, impaired stimulated superoxide production and H$_2$O$_2$ production and low intracellular MPO activity. Their data indicated that the decreased bacterial killing by PMNs isolated from diabetics is partly at least related to an impairment of oxygen dependent bactericidal mechanism. They opined that impairment of the oxygen-dependent microbicidal mechanisms of polymorphonuclear neutrophils in patients with type 2 diabetes is not associated with increased susceptibility to infection. A previous observation of Nauseef\textsuperscript{53} (1988) also revealed that though MPO activity is critical for optimal microbicidal activity of normal PMNs, in the absence of MPO, auxiliary mechanisms protect most MPO deficient hosts from clinically significant sequence except for some persons with diabetes mellitus who suffered from severe candidial disease.

In 1996 Zozulinska, et al.,\textsuperscript{97} reported that toxic oxygen species production might be influenced by disease duration in patients with insulin-dependent diabetes mellitus (IDDM). The production of H$_2$O$_2$ by unstimulated PMNs is increased in diabetic patients while generation of O$_2^-$ superoxide anions by stimulated neutrophils is markedly impaired.

In 1977, De Toni et al.,\textsuperscript{81} observed that PMN function in diabetic patients is multifactorial in origin and is probably correlated to the glucose level and to glycation of PMN protein such as NADPH oxidase or MPO. In diabetic condition, the competition for NADPH, the coenzyme for the respiratory burst, reduces the superoxide production.

Kemona et al.,\textsuperscript{98} (1985) determined the cytochemical indices of leukocytes in patients with diabetes mellitus in the period of uncontrolled and controlled. In unbalanced diabetics an evident decrease in the activity of acid phosphatase and MPO could be noted as well as a decrease of
glycogen content and an increase of lipid content. Balancing this disease induced the increase of all parameters in granulocytes except MPO activity. Their findings clearly indicate the role of metabolic disorders in diabetes mellitus on the activity of some neutrophilic enzymes and the glycogen and the content of lipids in neutrophils.

Sato et al.,84 (1992) have reported the decreased MPO activity in poorly controlled diabetes mellitus patients and there is a significant correlation between glycosylated hemoglobin (HbA1c) levels. They demonstrated that every step in leukocytic generation of reactive oxygen intermediates should be reduced in the leukocytes from poorly controlled diabetes patients.

In 1997, Delamaire et al.,82 evaluated polymorphonuclear neutrophil (PMN) cell performance in type 1 and type 2 diabetic patients free of infection, using tests that explore all the functional steps of PMN: adherence, chemotaxis, phagocytosis, and bactericidal activity. PMN chemotaxis was significantly lower in patients than in the healthy control group (p < 0.001) and associated with spontaneous adherence and increased expression of adhesion molecules. There is spontaneous activation of PMN cells and increased free radical production; after stimulation, response was lower than in the control group. The type of diabetes, the age of patients, HbA1C level and disease duration did not affect the responses. Chemotaxis was further reduced in patients with vascular complications and hyperglycaemia. They concluded that all steps of PMN functioning are altered in diabetic patients, which may increase the risk of vascular complications and infectious episodes.

In the same year Sato et al.,90 opined that in poorly controlled non insulin dependent diabetes mellitus (NIDDM , Type 2) patients, granulocyte-colony stimulating factor (G-CSF, a growth factor that stimulate the bone marrow for production and function of granulocytes) improves the impaired production of oxygen derived free radicals by neutrophils. They also found a
positive correlation between HbA1 and improving the effect of G-CSF on MPO activity. They suggested that G-CSF may be useful as a drug to prevent the aggravation of bacterial infections by improving neutrophil function, especially through $H_2O_2 - MPO - OC_1^-$ mechanism in poorly controlled diabetic patients.

Uchimura et al.,\textsuperscript{85} (1999) evaluated the changes in superoxide dismutase activities and concentrations and myeloperoxidase activities in leukocytes from patients with diabetes mellitus than the control group. Myeloperoxidase activity in leukocytes was significantly reduced in NIDDM patients whereas Cu-SOD and Zn-SOD (types of superoxide dismutase) showed no change than those in control group. These findings suggested that changes in these enzymes may affect the susceptibility to infection and immunocompetence of patients with diabetes.

In 2000, Dodds et al.,\textsuperscript{100} (2000) observed a similar pattern of decreased saliva flow rates and increased concentrations of saliva proteins (stimulated parotid lactoferrin, myeloperoxidase, and salivary peroxidase, as well as submandibular/sublingual total protein, albumin, lactoferrin and secretory IgA) in three groups of diabetics, healthy control and hypertensives. But this decreased flow rates and increased protein concentrations consistently greater in diabetics than hypertensives, which suggested that disease-specific mechanisms may be responsible. They also found diabetics are more prone to oral dryness and infections than non-diabetics.

The prevalence of pathogens in diabetic foot infections was determined by Viswanathan\textsuperscript{101} (2002), in relation to parameters like Wagner's grading, duration of diabetes and healing time. Diabetic foot infection is polymicrobial in nature. The healing time of wound infected with anaerobic pathogens was higher than those infected with aerobic pathogens. Neuropathy was common in diabetic patients infected with both
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aerobic and anaerobic pathogens. Presence of neuropathy increased the risk of foot infection.

The inhibition of MPO activity may happen in many ways. In 1998 Saeed et al.,\textsuperscript{102} reported the inhibition of neutrophil chemiluminescence during phagocytosis by abnormally elevated acetoacetate. The inhibition of MPO activity and chemiluminescence implicate metabolic ketosis in the inhibition of neutrophil microbicidal activity and thus increased susceptibility to infections. According to Mario Allegra et al.,\textsuperscript{103} (2001) melatonin also can inhibit the chlorination activity of MPO in the reaction between human MPO and melatonin at both pH 7 and pH 5.

The study of Accardo-Palumbo et al.,\textsuperscript{104} (1996) revealed the occurrence of anti-myeloperoxidase (anti-MPO) antibodies in 34 out of 88 (38\%) patients with type 1 diabetes mellitus but in only 3 of 55 (5.7\%) healthy subjects and in 4 of 20 patients with autoimmune disease. Specificity of anti-MPO antibodies was assessed by MPO inhibition studies. A state of chronic neutrophil activation has been described in diabetes mellitus. As anti-MPO antibodies can stimulate neutrophils to damage endothelial cells in systemic vasculitis, this suggests that a similar mechanism may be operative in the development of diabetic angiopathy.

Oldenborg\textsuperscript{105} (1999) suggested that elevated levels of insulin do not affect the NADPH-oxidase activity but, together with superoxide anions, interfere with myeloperoxidase availability and a subsequent myeloperoxidase-dependent generation of reactive oxygen metabolites in fMet-Leu-Phe-stimulated normal human neutrophils. The insulin-induced reduction of the chemiluminescence response was reversed by the addition of exogenous peroxidase and was also paralleled by a reduced myeloperoxidase activity, with no effect on the elastase activity, in cell-free supernatants from fMet-Leu-Phe-stimulated neutrophils.
An increased incidence of heart problems was found in diabetic patients. The role of myeloperoxidase in heart diseases has been reported in many works. In 2000 Anitra et al.,\textsuperscript{106} reported the property of MPO to bind with low density lipoprotein (LDL) and its potential implication in atherosclerosis. MPO catalyzes the formation of a number of reactive oxygen species that modify LDL to a form that converts macrophages in 'lipid laden' or 'foam' cells, the hallmark of atherosclerosis.

In 2003, Baldus et al.,\textsuperscript{107} observed that patients with elevated MPO levels experienced a markedly increased cardiac risk. They found that in patients with acute coronary syndrome (ACS), MPO serum levels powerfully predict an increased risk for subsequent cardiovascular events and extend the prognostic information gained from traditional biochemical markers. Given its proinflammatory properties, MPO may serve as both a marker and a mediator of vascular inflammation and further points towards the significance of PMN activation in the pathophysiology of ACS.

According to Zhang, et al.,\textsuperscript{108} (2004), vascular non-leukocyte-derived reactive oxygen species (ROS), such as superoxide and H$_2$O$_2$, have emerged as important molecules in diabetic endothelial dysfunction. In addition, leukocyte-derived myeloperoxidase has been implicated in vascular injury, and its injury response is H$_2$O$_2$ dependent. It is well known that MPO can use leukocyte-derived H$_2$O$_2$; however, it is unknown whether the vascular-bound MPO can use high-glucose-stimulated, vascular non-leukocyte-derived H$_2$O$_2$ to induce diabetic endothelial dysfunction. They demonstrated that MPO activity is increased in vessels from diabetic rats and suggest that vascular-bound MPO could use high-glucose-stimulated H$_2$O$_2$ to amplify high-glucose-induced injury in the vascular wall. MPO/H$_2$O$_2$/HOCl/chlorinating species may represent an important pathway in diabetes complications and a new mechanism in phagocyte- and systemic infection-induced exacerbation of diabetic vascular diseases.
In 2004, when Buraczynska, et al., determined the tumor necrosis factor (TNF) and MPO levels in plasma, found that, in diabetic nephropathy patients molecular variants of TNF are more frequent than in nondiabetic patients with chronic renal failure and this change might be associated with altered ability to TNF synthesis. Analysis of the myeloperoxidase genotypes showed significant difference in genotype distribution in dialyzed patients with diabetic nephropathy. Chronic renal failure patients show a significant reduction in the intracellular myeloperoxidase level.

According to Moutschen (2005), polymorphonuclear neutrophils are clearly influenced by the diabetic state. On one hand, their antimicrobial function is inhibited by hyperglycaemia, due to inhibition of glucose-6-phosphate dehydrogenase or diversion of NADPH in the polyol pathway; on the other hand, the pathogenesis of chronic complications of diabetes amplify inflammatory systemic manifestations associated with infections and play a role in the higher mortality rate observed in diabetic subjects with severe infections. These observations argue for the systematic vaccination of all diabetic patients against influenza and Streptococcus pneumoniae, for the reappraisal of diabetes as a significant pejorative risk factor in community acquired pneumonia and for intensive insulin therapy in all diabetic patients with severe infection.

In addition to antimicrobial activity of myeloperoxidase system, or its main product HOCl by itself, it readily abrogate the ability of α 1-proteinase inhibitor to inhibit elastase. In 1979, Matheson et al. reported this inactivation property of MPO in presence of H₂O₂ and Cl⁻. In 1981 they demonstrated that there is a direct dependence on the concentration of α-1-PI and H₂O₂ and Cl⁻ ion. In 1987, Borregaard described that this inactivation of alpha 1-proteinase inhibitor was effectively prevented by N-acetyl cysteine, methionine and reduced glutathione. These results indicated that the sulphhydryl compounds work as scavengers of the products of the

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myeloperoxidase system, and might be useful in inflammatory disorders, to prevent tissue damage inflicted by this system.

Vissers and Winterbourn\(^{114}\) (1991) demonstrated that the primary and tertiary structures of purified human plasma fibronectin extensively modified on exposure to the myeloperoxidase- \(\text{H}_2\text{O}_2-\text{Cl}^-\) system of neutrophils or to reagent HOCl. It has implications on the mechanism of tissue injury by neutrophils in inflammation, since a loss of functional fibronectin would result in cell detachment and a distortion of normal tissue organization.

Ottonello et al.,\(^{115}\) (1993) took a rational approach to the pharmacological control of neutrophil-mediated tissue injury and focused on the histoprotective potential of an anti-inflammatory drug, Nimesulide as a down regulator of the activity of the neutrophil myeloperoxidase pathway during inflammatory processes. They investigated that this agent reduced the function by MPO pathway, responsible for HOCl production, by exerting a cell-directed inhibitory activity, as shown by measurement of superoxide anion and hydrogen peroxide production. Nimesulide also inactivated hypochlorous acid directly and protected alpha 1-antitrypsin from the neutrophil-mediated oxidation. These data suggested that nimesulide may prevent tissue injury at sites of inflammation by maintaining natural host protective systems.

According to Vijayalingam\(^{116}\) (1996) antioxidant status is poor in both impaired glucose tolerant (IGT) and NIDDM. Antioxidant enzymes - superoxide dismutase and catalase were significantly lower in red blood cells obtained from IGT and early hyperglycaemic groups. They were closer to the levels showed in NIDDM confirming that antioxidant deficiency is already present in IGT subjects. Lipid peroxidation product in plasma, erythrocyte, and erythrocyte cell membrane was found to be significantly elevated \((p<0.001)\) in IGT, early hyperglycaemia and diabetes mellitus while glycosylated haemoglobin was also higher. Among the antioxidant
scavengers, reduced glutathione and ascorbic acid are reduced by 15% and 20% in IGT and NIDDM, respectively.

Muchova et al., (1999) examined the effect of type 2 diabetes mellitus on enzymes of importance for oxygen-dependent killing of microorganisms by leucocyte. The superoxide dismutase activity was lower by 41% in polymorphonuclear leucocytes from patients with Type 2 DM than in control group. Glutathione peroxidase and glutathione reductase activities of type 2 DM patients were 73.04% and 81.12% respectively of control values. The catalase activity showed no significant difference. A significant increase in the concentration of thiobarbituric acid reactive products was observed. A positive correlation between thiobarbituric acid reactive products and glucose, glycosylated haemoglobin and fructosamine was observed in the serum of diabetic patients. These findings may explain some of the mechanisms underlying the increased susceptibility to certain infections in patients with type 2 diabetes mellitus.

In 2001 Majchrzak et al., found reduced glutathione level and markedly lower dismutase activity in well metabolically controlled diabetic patients, compared to the control group. The levels of glutathione peroxidase did not differ significantly from values obtained in healthy subjects. They did not observe any correlation between the analysed parameters and duration of diabetes, HbA1c or presence of chronic complications of the disease. Their results indicated that antioxidative systems in the state of good metabolic control of diabetes have adaptive properties.

Galeano et al., in 2001 observed a delayed wound healing together with low collagen content, breaking strength, and increased malondialdehyde (MDA) levels and MPO activity in diabetic mice when compared with their healthy littermates. They found that the raxofelast, a protective membrane antioxidant, restores wound healing nearly to normal levels in experimental diabetes-impaired wounds in diabetic mice through the stimulation of angiogenesis, reepithelialization, synthesis, and maturation.
of extracellular matrix. Furthermore, raxofelast treatment significantly reduced MDA levels, MPO activity, and increased the breaking strength and collagen content.

Diegelmann et al.\textsuperscript{120} (2003) has reported that chronic ulcers contain the persistence of neutrophils and their destructive enzymes, especially neutrophil-derived matrix metalloproteinase-8 and elastase which appears responsible for the extensive matrix dissociation and thus contributes to the chronicity of these ulcers. They also found that a significant correlation of myeloperoxidase activity with actual neutrophil counts in the ulcer biopsies further confirm the dense presence of neutrophils with occasional large macrophages actively phagocytosing depleted neutrophils. Their studies directly showed that there is extensive neutrophil infiltration in chronic pressure ulcer granulation tissue.

In 2005, Jantschko et al.\textsuperscript{20} designed reversible mechanism of HOCl production mediated by MPO, for the development of drugs against HOCl-dependent tissue damage, with respect to the enzymology of MPO. It was based on the extraordinary and MPO-specific redox properties of its intermediates compound I and compound II.