Nat. Volatiles & Essent. Oils, 2021; 8(4): 14084-14092

NVEO
Natural Volatiles &
Essential Oils

Serum Myeloperoxidase Activity Association With Cardiovascular And Kidney Disease Risk Factors In Pre-Diabetic And Type 2 Diabetic Patients

Suha Abdu Sala Moneam[1] , Hassan H . Al-Saeed[2] , Mahmood S Hakir. Khudair[3]

[1,2,3] College of Medicine/Al-Nahrain University/Iraq

Abstract

Type 2 diabetes mellitus (T2DM) is one of the most common kinds of diabetes. Insulin resistance and insulin insufficiency are the causes. Myeloperoxidase (MPO) is a pro-oxidant enzyme produced by activated neutrophils, monocytes, and some tissue macrophages in their granules. While one of MPO's main biological functions is to protect the body from infections by producing antimicrobial oxidants, free radicals, and other reactive oxidant species, this activity can also cause endothelial and vascular oxidative damage.**Materials and Methods:** By using an ELISA kit, serum levels of human myeloperoxidase was measured in 30 patients type2 diabetic ,30 patients Prediabetic and 30 subjects as control group. The age range (30-65) years. Fasting blood sugar (FBS), HbA1c, CRP and Lipid profile by enzymatic method. Also, body mass index (BMI) measured .Blood samples were talked after fasting. **Results:** The serum MPO levels were significantly higher in Type2 diabetes Mean ±SD (13.10±3.4) and significantly increased MPO was prediabetic subjects Mean ±SD (9.57±2.80) as compared to controls. MPO was found to be significantly and positively correlated with all the cardiovascular disease risk factors i.e. age, gender ,body mass index (BMI), lipid parameters except high density lipoprotein (HDL) to which it was negatively correlated. The serum MPO levels were positively correlated with the albumin creatinine ratio (ACR), glycated hemoglobin (HbA1C) and FBG.**Conclusions:** Serum MPO levels were linked to ACR, HbA1c, LDL, and FBG in type 2 diabetic patients, suggesting that elevated levels in these patients could be used to measure vascular dysfunction. In pre-diabetes, MPO has a strong relationship with cardiovascular disease risk factors. As a result, MPO may be utilized to assess cardiovascular risk in pre-diabetic individuals.

I. Introduction

Diabetes is a chronic condition characterized by a rise in blood glucose levels. One of the leading causes of death worldwide is a condition that causes major damage to the heart, blood vessels, eyes, kidneys,

and nerves. **(H. Wu, S. Yang, Z. Huang, J, el at. 2017)**. Diabetes is a type of chronic hyperglycemia that, through a variety of processes, can cause a pro-oxidative shift in the glutathione redox state in the blood. The creation of glycated proteins and the production of superoxide are linked to glucose auto-oxidation; interactions of glycated proteins with cell surface receptors drive ROS production and decrease intracellular glutathione levels. Hyperglycemia also increases endothelial cell-mediated LDL peroxidation, which can contribute to the formation of atherosclerotic lesions in the artery walls.**(SIES, Helmut;et al,2017).** Type 2 diabetes (T2D) is caused by a gradual decrease in the pancreas' capacity to generate adequate insulin, as well as insulin resistance in insulin-sensitive tissues. Excessive ectopic fat buildup in the liver, pancreas, and skeletal muscles is a hallmark of T2D pathogenesis, which finally manifests as insulin resistance in these tissues and pancreatic beta-cell failure, leading to hyperglycemia. **(Skyler JS, et al.2017).** Myeloperoxidase (EC 1.11.1.7) belongs to the peroxidase subfamily. It is produced in other bodily cells and is most abundantly expressed in immune cells such as neutrophilic polymorph nuclear leukocytes (neutrophils) and lymphocytes, monocytes, and macrophages. Myeloperoxidase is kept in cytoplasmic membrane-bound azurophilic granules, which are released into the extracellular space via degranulation or exocytosis during stimulation. Although the exact molecular mechanism of neutrophil degranulation is unknown, oxidative stress is thought to have a role in the release of MPO from these cells. **(Khan AA, Alsahli MA, Rahmani AH,2018).** The MPO gene is found on chromosome 17's long arm section q12–24, and its principal transcriptional product consists of 11 introns and 12 exons. It produces apopro-MPO after some changes such as the removal of the signal peptide and glycosylation with mannose-rich side chains. n the endoplasmic reticulum, this protein product is enzymatically inactive and forms complexes with chaperons such as calreticulin and calnexin. The insertion of a heme moiety into apoproMPO results in the formation of proMPO, which is enzyme inactive. Furthermore, by removing certain N-terminal amino acids, a 72–75 kDa protein is produced, which is then further cleaved to create and subunits. The -subunit is hefty, weighing in at 57 kDa and containing 467 amino acids, while the -subunit is light, weighing in at 12 kDa and containing 112 amino acids. MPO is a cationic, dimeric protein with a mass of 146 kDa that is made up of two 73 kDa monomers joined by a cystine bridge at Cys153. Each monomer is made up of two parts: a heavy chain with a molecular mass of 58.5 kDa and a light chain with a molecular mass of 14.5 kDa and 106 amino acids. The former is glycosylated and contains the active site of the modified iron protoporphyrin IX. The heme group is located at the bottom of a deep cleft, preventing most elements from reaching the iron atom: only H2O2 and tiny anions have easy access. **(Davies, Michael J.2010).** MPO can be produced

outside of neutrophils during inflammation, causing oxidative damage to the host tissues. **(Klebanoff SJ ,2005).**

Two processes can cause these damages:

- producing HOCl, which oxidizes bio macromolecules like DNA, RNA, proteins, and lipoproteins;
- MPO directly oxidizes certain amino acids and hormones, which can act as substrates (ex: tyrosine and serotonin).

MPO's HOCl has the ability to oxidize a wide range of biomolecules by chlorination and/or oxidation. It inactivates proteins by oxidizing their sulfhydryl groups. This oxidation can result in the formation of disulfide linkages, which can cause protein crosslinking. Cys is oxidized by HOCl to produce cystic acid and cysteine. A chloramine molecule is formed when hypochlorous acid reacts with amino acids that have an amine side chain (for example, Lys). Tyr can be oxidized by MPO to produce either o,o'-dityrosine (di-tyr) or chlorotyrosine (Cl-tyr) via HOCl, with Cltyr serving as a marker of MPO oxidation.**MongirdienėA, Laukaitienė J, Skipskis V, et al,2019).**

II. Methods

Al-Nahrain University's Department of Chemistry and Biochemistry, College of Medicine, undertook this case control study. The research was carried out between December 2020 and March 2021. 90 individuals, ranging in age from 30 to 65, were placed into three groups as follows:

Group I consisted of 30 diabetic patients.

According to the ADA guidelines, the following are the criteria for diagnosing diabetes: (ADA 2020).

126 mg/dL (7.0 mmol/L) fasting plasma glucose level Fasting is defined as consuming no calories for at least 8 hours.**a)** or 2-h PG ≥ 200 mg/dL (11 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load.

b) Consisting of the anhydrous glucose equivalent of 75 g dissolved in water.

c) Or an HbA1C level of less than 6.5 percent (48 mmol/mol). The test should be done in a lab with an NGSP-certified method that is standardized to the DCCT assay.

d) Or a random plasma glucose of 200 mg/dL (11 mmol/L) in a patient with typical hyperglycemia or hyperglycemic crisis symptoms

Group IIconsisted of 30 pre-diabetic patients.

All samples taken from participants who were fasting during their visit were tested for prediabetes and type 2 diabetes mellitus according to the American Diabetes Association's (ADA) recommendations.

Group III: There were 30 people in this group**.**

Ethics committee:

All of the patients at Al-Imamain Al Kadhimain Medical City's Diabetes Center and outpatient diabetes clinic volunteered. The study was approved by the Al- Nahraine University College of Medicine's ethical committee. Each subject gave their informed consent.

Blood samples were collected in seven milliliters from patients and controls and processed as follows:

1. For the HBA1c test, two milliliters of blood will be taken in EDTA tubes.

2. Five milliliters of blood will be collected in a gel tube and kept at room temperature for 20 minutes. Serum was separated by centrifugation at 2000 xg for 10 minutes after coagulation and will be split into tiny aliquotsfor:-

a. Using suitable enzymatic and colorimetric methods, immediate measurements of serum sugar, lipid profile, creatinine, urea, and C- reactive protein were made.

b. The remainder will be kept at -20°C until the concentration of serum human MPO is determined. It will be determined using an ELISA kit (enzyme-linked immunosorbent assay).

Statistical research:

SPSS version 18 and Microsoft Excel 2010 were used to evaluate the data obtained. Numeric data was reported as mean standard deviation. The individual p-value of different groups between control, pre-DM, and type 2DM was calculated using the Student's F test, and the level of serum MPO was correlated with the exogenous factor (body mass index (BMI)) in patients with Type 2 diabetes and pre diabetes using the Pearson correlation test. P values of less than 0.05 were deemed significant**.**

III.Results

When compared to controls, serum MPO levels were considerably higher in Type 2 diabetes participants Mean ±SD (13.10±3.4), and significantly elevated MPO in prediabetic subjects Mean±SD (9.58±2.80). MPO was found to be significantly and favourably connected with all cardiovascular disease risk factors, including age, gender, BMI, and lipid parameters, with the exception of high density lipoprotein (HDL), with which it was found to be negatively correlated. The albumin creatinine ratio (ACR), glycatedhemoglobin (HbA1C), and FBG were all favourably linked with serum MPO levels.

Table (1) Comparison concentration of serum MPO between study groups:

Note:- P value of less than 0.05 is considered statistically significant.

A significant elevation was shown in MPO concentration in sera of patients with T2 DM in comparison with MPO concentration in sera of control group and patients with Pre-DM fig (3.1).

Tested value	Control(n=30)	Pre Diabetic (n=30)	Diabetic (n=30)	P

Tested value	Control(n=30)	Pre Diabetic (n=30)	T2-Diabetic (n=30)	F	P
	Mean ±SD	Mean ±SD	Mean ±SD		
MPO (ng/ml))	6.22±1.91	9.58±2.80	13.10±3.4	46.06	2.27E-14 *
*: Significant difference					

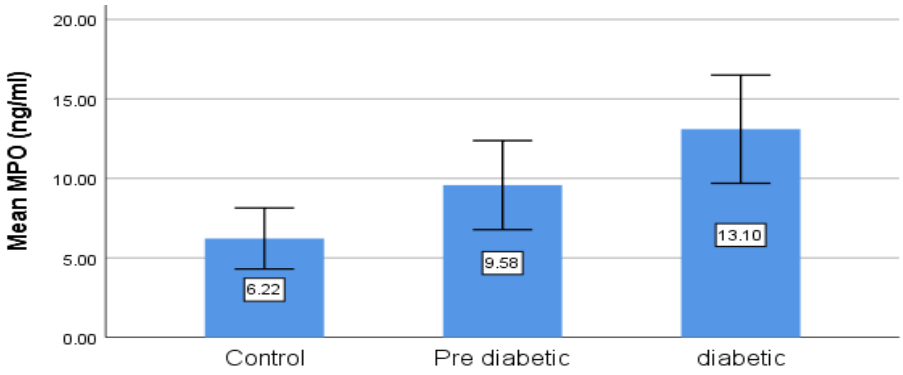

Figure (3.1): mean and standard deviation of concentration MPO in Control, P-

DM andT2 DM

In this study, urea, creatinine, ACR, AI, CRI, FBS, and lipid profile were assessed in both patients and controls. The mean and standard deviation (Mean± SD) as well as the p value for each biochemical test are listed in

Table:(2)

	Mean ±SD	Mean ±SD	Mean ±SD	
Age (years)	45.63±8.81	46.23±11.11	49.90±9.71	0.2 NS
BMI(kg/m²)	24.79±1.62	29.94±6.4	32.66±5.92	8.73E-9*
vLDL (mg/dl)	20.79±4.68	31.34±12.43	30.88±10.57	0.1E-4*
Cholesterol(mg/dl)	157.15±18.44	164.36±29.04	176.04±53.29	0.137 NS
T.G(mg/dl)	105.71±25.46	157.0±61.89	152.52±52.46	0.12E-3*
HDL(mg/dl)	48.16±4.98	39.36±9.89	36.71±11.16	0.3E-4*
HbA1c (%)	4.84±0.29	5.93±0.23	8.87±1.9	8.7E-24*
Glucose (mg/dl)	92.03±6.75	99.22±15.45	246.49±78.72	5.4E-24*
AI	0.23±0.33	0.54±0.28	0.61±0.2	0.1E-5*
CRI	2.01±0.38	4.5±2.67	4.64±2.26	0.5E-5*
ACR	6.75±2.76	7.71±5.43	50.59±63.21	1.2E-19*
LDL (mg/dl)	89.85±18.23	92.82±31.87	129.6±61.0	0.36E-3*
CRP (mg/l)	2.02±1.27	2.48±1.41	3.07±1.11	0.008*
Urea(mg/dl)	27.18±6.77	24.62±5.98	25.24±5.8	0.25 NS
Creatinine(mg/dl)	0.72±0.14	0.68±0.11	0.65±0.15	0.06NS
*: ($p<0.05$)				

Table (2): General Serum Biochemical Tests in the Study Groups:

Table (3): Correlation between Myeloperoxidase &measured parameters in type 2 diabetic'spatients:

Parameters	**Correlation Coefficient(r)**	**P**
HbA1c (%)	0.76	0.1E-5*
FBG (mg/dl)	0.63	0.15E-3*
BMI(kg/m²)	0.5	0.004*
Cholesterol(mg/dl)	0.69	0.24E-4*
T.G(mg/dl)	0.52	0.003*
HDL(mg/dl)	-0.48	0.006*
VLDL (mg/dl)	0.51	0.003*
LDL (mg/dl)	0.66	0.6E-4*

ACR	0.47	0.0001*
CRI	0.50	0.005*
AI	0.67	0.00004*

Table(4): Correlation between Myeloperoxidase &measured parameters in prediabetic patients:

Parameters	**Correlation Coefficient(r)**	**P**
HbA1c (%)	0.477	0.008*
FBG (mg/dl)	0.43	0.01*
BMI(kg/m²)	0.45	0.01*
Cholesterol(mg/dl)	0.40	0.029*
T.G(mg/dl)	0.72	0.6E-6*
HDL(mg/dl)	-0.58	0.001
VLDL (mg/dl)	0.71	0.8E-5*
LDL (mg/dl)	0.38	0.037*
ACR	0.55	0.001*
CRI	0.70	0.1E-4*
AI	0.55	0.001*

IV.DISCUSSION

As indicated in (Table1) and figure (1), the level of MPO was higher in the type 2 DM group as compared to the other groups, with a statistically significant difference between the control and pre diabetic groups. The p-value of MPO was (2.27E-14*). Because diabetes mellitus hyperglycemia with its associated metabolic syndromes, hypertension, obesity, and dyslipidemia these factors increase the production of free radicals and weaken the anti-oxidant defense system, resulting in oxidative stress. As a result, greater oxidative stress and an increase in MPO are expected, which explains why MPO levels in T2DM are higher.**,(Kusuma KS, Vasudha KC, Vanitha Gowda MN,2009**).The findings of this investigation corroborate previous research. (**Shiu, Sammy WM, et al, 2014)** and **(Song, Ping, et al,2015).** As indicated in (Table 2), the HbA1c p-value was 8.7E-24*, and the FBG was also statistically significant, indicating that there was evidence for the development of diabetes in the type 2 diabetes group. The findings of this study corroborate those of (**Qaddoumi, Mohammad G., et al.2020)** study This finding contradicts

the findings of (**(Song, Ping, et al,2015)** Because of the study's several types of groups, all of the participants were type 2 diabetes. MPO levels were found to have a strong positive link with HbA1C, FBG, BMI, cholesterol, TG, VLDL, and LDL, as well as a negative correlation with HDL cholesterol. Dyslipidemia is also thought to play a function in the pathogenesis of atherosclerosis and is linked to an elevated risk of cardiovascular disease (CVD). These findings corroborate findings from a previous study. **(Qaddoumi,Mohammad G., et al ,2020) (Mahat RK ,et al , 2018).** MPO is positively correlated with AI and CRIIn type 2 diabetes mellitus and pre-diabetic patients, atherogenic indices and CRI were found to be significantly different, and the ratios contribute significantly to the assessment of CVD risk. The main feature of lipoprotein abnormalities in diabetes patients is diabetic dyslipidemia, which is characterized by an elevated TG level and a decreased HDL-C value. This affects the results of CRI and AI, which leads to a rise in risk factors for developing CVD. These findings are consistent with previous research. **(Domingo O, et al, 2017)** and **(Harini D, et al, 2016).**We discovered that serum myeloperoxidase levels were significantly higher in type 2 diabetes patients, and that they were also strongly connected with ACR, implying that it is useful for vascular dysfunction diagnosis. These findings are in line with the findings of a previous study **(Song P, et al, 2015).**

REFERENCES:

1. **American Diabetes Association**. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020 Jan;43(Suppl 1):S14.)

2. **Shiu, Sammy WM, et al**. Carbamylation of LDL and its relationship with myeloperoxidase in type 2 diabetes mellitus. Clinical science, 2014, 126.2: 175-181 .

3. **Harini D, Ambika D,** Prabath K, Vani N. Lipid ratios, atherogenic coefficient and atherogenicindexof plasma as parameters in assessing cardiovascular risk in type 2 diabetes mellitus. IJRMS, 2016;4(7).

4. **Domingo O Beltran,** Vicente F Gil-Guillen,R, Jose M Martin-Moreno, Vicente P Carratala et al., Lipid profile, cardiovascular disease and mortality in a Mediterranean high-risk population: The ESCARVAL-RISK study. PLoS One. 2017;12(10): e0186196.

5. **.Klebanoff SJ.** Myeloperoxidase: friend and foe. J. Leukoc. Biol. Comprehensive review on the role of MPO in pathology, 2005;77:598–625.

6. **Mongirdienė A, Laukaitienė J, Skipskis V, et al.** The Effect of Oxidant Hypochlorous Acid on Platelet Aggregation and Dityrosine Concentration in Chronic Heart Failure Patients and Healthy Controls. Medicina (Kaunas). 2019;55

7. **DAVIES, Michael J**. Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention. Journal of clinical biochemistry and nutrition, 2010, 48.1: 8-19.

8. **H. Wu, S. Yang, Z. Huang, J. He, X. Wang**, "Type 2 diabetes mellitus prediction model based on data mining," Informatics in Medicine Unlocked, vol. 10, pp. 100-107, 2018. DOI: 10.1016/j.imu.2017.12.006 .

9. **Khan AA, Alsahli MA, Rahmani AH**. Myeloperoxidase as an active disease biomarker: recent biochemical and pathological perspectives. Medical sciences. 2018 Jun;6(2):33.

10.**Kusuma KS, Vasudha KC, Vanitha Gowda MN**: A Comparative Study of Serum Myeloperoxidase Activity in Type 2 Diabetes and Diabetic Nephropathy. Biomedical Research 2009; 20 (3): 198-204.

11.Mahat RK, Singh N, Rathore V:Association of myeloperoxidase with cardiovascular disease risk factors in prediabetic subjects. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2018.

12.Qaddoumi, Mohammad G., et al. Investigating the role of myeloperoxidase and angiopoietin-like protein 6 in obesity and diabetes. Scientific reports, 2020, 10.1: 1-10.

13.SIES, Helmut; BERNDT, Carsten; JONES, Dean P. Oxidative stress. Annual review of biochemistry, 2017, 86: 715-748

14.Skyler JS, et al. Differentiation of Diabetes by Pathophysiology, Natural History, and Prognosis. ImmunolMetabEndocrinol. 2017;66:10–12.

15,Song, Ping, et al. Association of plasma myeloperoxidase level with risk of coronary artery disease in patients with type 2 diabetes. Disease Markers, 2015,

16. **Lata, . M. ., &Jamali, M. . (2021).** Immunity Boosting Medicinal Plants to Beat Covid -19 in Seraj Block of Mandi District, Himachal Pradesh. Journal of Scientific Research in Medical and Biological Sciences, 2(4), 44-56. https://doi.org/10.47631/jsrmbs.v2i4.337

20. Zebari, H. M. (2021). The Comparison Between Reproductive Performance in Synchronized and Spontaneous Estrus Sheep. Journal of Scientific Research in Medical and Biological Sciences, 2(4), 30-37. https://doi.org/10.47631/jsrmbs.v2i4.332