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Original Research Article

Elevated neutrophil elastase and myeloperoxidase mRNA expressions in patients with metabolic syndrome: a cross-sectional study

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ABSTRACT

Background: Neutrophil-derived enzymes such as neutrophil elastase (NE) and myeloperoxidase (MPO) contribute to inflammation and vascular dysfunction. However, their expression in relation to metabolic syndrome (MetS) remains underexplored.
Methods: In this cross-sectional study, NE and MPO mRNA expression were evaluated in peripheral blood leukocytes of 44 adults. Among them 19 had MetS and 25 served as controls. Baseline metabolic parameters were compared between groups, and expression levels were analyzed using RT-qPCR.
Results: Individuals with MetS had significantly higher systolic blood pressure, waist circumference, triglycerides, and lipid ratios (TC: HDL-C, LDL-C: HDL-C), and lower HDL-C compared to controls. NE and MPO mRNA expression were significantly elevated in MetS (p=0.015 and p=0.029, respectively). Furthermore, both NE and MPO expression showed significant moderate positive correlations with MetS status.
Conclusion: Elevated NE and MPO gene expressions in peripheral blood leukocytes are associated with MetS, supporting their potential role as biomarkers of obesity-related dyslipidemia and inflammation. These findings highlight neutrophil activation as a possible molecular contributor to the pathogenesis of MetS.

Keywords: Neutrophil elastase, Myeloperoxidase, mRNA, Metabolic syndrome

INTRODUCTION

Being a growing global health concern, metabolic syndrome (MetS) affects about 25% of the world's adult population and contributes significantly to the burden of cardiovascular disease, type 2 diabetes, and their related complications.[1,2] MetS is defined by a cluster of metabolic abnormalities that often occur together, including central obesity, high blood pressure, high fasting plasma glucose, elevated serum triglycerides, and low levels of high-density lipoprotein cholesterol (HDL-C).[3] Different expert groups, including the International Diabetes Federation (IDF) and the National Cholesterol Education Program (NCEP), have set diagnostic cut-off values for these parameters, but the essential idea is that MetS represents a state of systemic metabolic imbalance that raises the risk of cardiovascular events and all-cause mortality.[1,4] Although the exact causes of MetS are complex, one key

factor that has drawn much attention is chronic low-grade inflammation.[5] A large body of research has shown that MetS is not only a metabolic condition but also an inflammatory one. Persistent low-level inflammation is known to drive insulin resistance, which is central to the development of type 2 diabetes and cardiovascular complications.[6] Among the many players in inflammation, neutrophils and their enzymes stand out as important but underexplored mediators in MetS. Neutrophils release a variety of proteolytic enzymes and oxidative agents that contribute to tissue damage, remodeling, and oxidative stress. Two major neutrophil enzymes are neutrophil elastase (NE) and myeloperoxidase (MPO). NE is a serine protease that breaks down extracellular matrix proteins and triggers local tissue injury, while MPO produces reactive oxygen species (ROS) that further damage cells and promote vascular inflammation.[7,8] Excessive NE activity can disrupt the balance of extracellular matrix remodeling, while MPO-generated oxidants modify lipids and proteins, fueling oxidative stress and vascular damage.[7,8] Therefore, the current study was designed to explore the mRNA expression levels of neutrophil elastase and myeloperoxidase in peripheral blood leukocytes in patients with MetS, which could provide new insight into the link between neutrophil-driven inflammation and metabolic vascular risk, potentially supporting the development and progression of MetS.

METHODS

This was a cross-sectional study conducted in the Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India, in collaboration with Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh, from January 2020 to January 2021. The study included a total of 44 subjects, consisting of 19 patients with metabolic syndrome and 24 age, sex and BMI matched apparently healthy individuals (controls) without metabolic syndrome.

Patients were recruited from the outpatient department of the Medicine Department. Inclusion criteria for cases were adults aged 20–40 years of either sex with a clinical diagnosis of metabolic syndrome based on the harmonized consensus definition, which requires the presence of at least three of the following five criteria: raised waist circumference (≥90 cm in men and ≥80 cm in women), fasting serum triglyceride ≥150 mg/dl or on specific treatment for this lipid abnormality, HDL cholesterol <40 mg/dl in men and <50 mg/dl in women or on specific treatment, blood pressure ≥130/85 mmHg or on antihypertensive therapy, and fasting plasma glucose ≥100 mg/dl or on treatment for diabetes.

Controls were healthy volunteers without metabolic syndrome and matched to cases for age, sex and BMI. Subjects with stroke, malignancy, ischemic heart disease, chronic kidney or liver disease, acute or chronic infections or inflammatory diseases, apparent causes that might cause alteration of total WBC count, and pregnancy were excluded.

After obtaining ethical clearance from the Institute Ethics Committee, AIIMS, New Delhi, India and informed written consent from all participants, demographic data were collected, anthropometric measurements including height, weight, and waist circumference, and blood pressure were recorded. BMI was calculated. Fasting blood samples (5 ml) were collected from each participant under aseptic conditions for biochemical and hematological tests and peripheral blood leukocytes isolation. Total RNA was extracted from leukocytes using the SV Total RNA Isolation System (Promega, Madison, WI, USA) according to the manufacturer's protocol.

First-strand complementary DNA (cDNA) was synthesized from RNA using the GoScript Reverse Transcription System (Promega). Primer sequences for neutrophil elastase (NE), myeloperoxidase (MPO), and β-actin (housekeeping gene) were designed and validated using conventional PCR prior to quantification. Real-time PCR was performed using the Rotor-Gene Q instrument (QIAGEN) with GoTaq qPCR Master Mix containing BRYT Green dye.

Gene expression levels were analyzed using the comparative Ct method (2-ΔCt), normalizing NE and MPO Ct values to β-actin Ct values for each sample. Relative expression was calculated as fold change compared to the mean expression in the control group, and fold change >2 was considered overexpression. Data were analyzed using statistical package for the social sciences (SPSS) version 21.0. Quantitative data were expressed as mean±standard deviation or median with interquartile range as adequate, and qualitative data as frequencies and percentages. Differences between groups were assessed using unpaired student's t-test or Mann-Whitney U test for continuous variables and Chi-square test for categorical variables. Correlations between NE and MPO mRNA expression levels and metabolic parameters were evaluated using Spearman's rho correlation. A p value of <0.05 was considered statistically significant.

RESULTS

Table 1 shows the comparison of baseline characteristics between two groups, subjects with (n=19) and without (n=25) MetS. Significant differences were found in terms of SBP, WC, serum HDL-C, serum TG, TC: HDL-C ratio and LDL-C: HDL-C ratio between the groups. A near-significant difference in DBP was observed between the groups.

Figures 1 shows a boxplot comparing neutrophil elastase (NE) and myeloperoxidase (MPO) mRNA expression of subjects with and without No MetS. The plot illustrates that both NE and MPO expression levels were significantly higher in the MetS compared to controls (p=0.015 and 0.029 respectively).

By Spearman's correlation by rank test, significant moderate positive correlation was found in terms of NE expression (r=0.40; p=0.011) as well as MPO expression (r=0.36, p=0.023) in peripheral blood leukocytes (Table 2).

Table 1: Baseline characteristics of study subjects (n=44).

Parameters	MetS (n=19)	No MetS (n=25)	P value
Age (in years)	33.68±4.59	33.00±5.47	0.66
Gender, male/female (n)	11/8	15/10	1.00[a]
Systolic blood pressure (SBP) (in mmHg)	122.89±14.65	114.60±9.00	0.025
Diastolic blood pressure (DBP) (in mmHg)	81.58±11.43	75.80±7.99	0.055
Body mass index (BMI) (kg/m^2)	29.76±9.99	25.64±4.09	0.068
Waist circumference (WC) (in cm)	102.03±16.12	92.82±10.33	0.026
Fasting plasma glucose (FBS) (in mmol/l)	4.57±1.47	4.55±0.54	0.93
Serum total cholesterol (in mg%)	189 (169-203)	176 (157-207)	0.33[b]
Serum LDL-C (in mg%)	116 (93-136)	111 (90-139)	0.87[b]
Serum HDL-C (in mg%)	34.70±3.74	44.17±9.11	<0.001
Serum triglyceride (in mg%)	184 (158-270)	108 (98-139)	<0.001[b]
TC:HDL-C ratio	5.58 (5.02-6.03)	4.14 (3.14-5.13)	<0.001[b]
LDL-C:HDL-C ratio	3.50 (2.75-4.24)	2.53 (1.69-3.36)	0.007[b]
Serum creatinine (in mg%)	0.80±0.11	0.74±0.12	0.17
SGPT (u/l)	36 (25-43)	30 (18-42)	0.22[b]
Total white blood cell count (in K/µl)	8.23±1.64	7.38±1.29	0.13
Neutrophil (in K/µl)	55.26±7.54	58.00±7.05	0.32
Lymphocye (in K/µl)	35.65±8.23	33.33±5.83	0.38
Monocyte (in K/µl)	4.56±1.84	5.27±2.73	0.42
Eosinophil (in K/µl)	4.21±1.58	3.40±1.68	0.19
ESR	17 (10-55)	25 (10-30)	0.81[b]

Continuous variables were expressed in mean±SD or median with interquartile range and categorical variable was expressed in absolute frequency; unpaired student's t test were done to measure the level of significance; [a]Chi square test was done to measure the level of significance; [b]Mann-Whitney U test were done to measure the level of significance

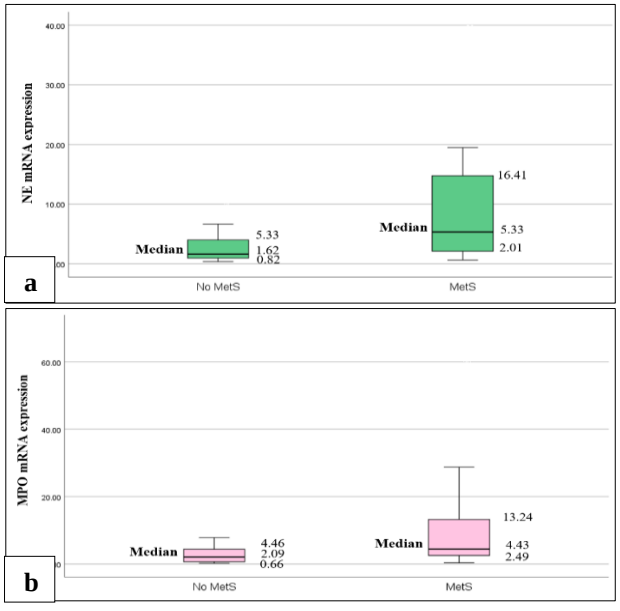

Figure 1: (a) Box plots showing neutrophil elastase (NE), and (b) myeloperoxidase (MPO). The line within the boxes represents the median, the bottom of each box represents the 25th percentile, and the top of the box represents the 75th percentile. The whiskers represent the 5th and 95th percentiles, [a]p=0.015; [b]p=0.029.

Table 2: Correlation of NE and MPO gene expression with MetS (n=44).

NE expression		MPO expression	
r value	**P value**	**r value**	**P value**
0.40	0.011	0.36	0.023

r value=Spearman's correlation coefficient

DISCUSSION

In this cross-sectional analytical study, expression of NE and MPO mRNA expressions were investigated in patients with MetS and explored their associations with key metabolic risk factors. SBP, WC, serum HDL-C, serum TG were found significantly higher in cases (with Mets) than control group (Table 1). This is evident as central obesity, hypertension, and dyslipidemias are the hallmarks of metabolic syndrome.[3,9,10] Again Bener et al and Gharipour et al showed that waist circumference reliably better predictor than other obesity indices to distinguish MetS from non-MetS populations regardless of age and sex.[11,12] Metabolic syndrome accompanies with chronic low grade inflammation which may be due to the fact that increase secretion of cytokines due to insulin resistance or from the adipose tissue of an obese person which ultimately leads to atherosclerosis and acute coronary syndrome.[5,6] Compared to separate matrices of lipid

profile, the ratios of TC/HDL and LDL/HDL cholesterol were recognized as cardiovascular risk markers with higher predictive value.[13] In this present study these lipid ratios were found significantly higher in MetS cases which is consistent with the theory that, metabolic syndrome increases the risk for cardiovascular disease (Table 1).

A major finding of this study was the significantly higher NE expression (p value 0.015; Figure 1a) and higher MPO expression (p value 0.029; Figure 1b) in the MetS group compared to controls. Different scientists all over the world, evidenced that excessive NE activity promotes adipocyte inflammation, insulin resistance as well as also linked to cardiovascular risk and vascular remodeling.[14-16] On the other hand, MPO was demonstrated as the mediator of oxidative stress and vascular inflammation and MPO protein and gene expression levels were found elevated in metabolic disorders.[17,18] All these evidences are providing a plausible mechanism linking NE and MPO overexpressions with metabolic dysfunction.

Neutrophils are activated by NE and MPO, responsible for oxidative stress and chronic inflammation.[7,8] Significant moderate positive correlations were found in terms of NE mRNA expression (r=0.40; p value 0.011) and MPO mRNA expression (r=0.36; p value 0.023) with cases (Table 2) which is consistent with previous studies where NE and MPO were found elevated in DM as well as in insulin resistance which is known to be the component of metabolic syndrome.[18-20]

In 2009, Nijhuis et al also detected an association between MPO and central obesity.[21] Ali et al also found association of obesity, TG and atherogenic indices of plasma with NE and MPO mRNA expressions in peripheral blood leukocytes in 2018; though failed to find any correlation with fasting plasma glucose and insulin resistance as well.[22] This may be due to the fact that concentrations of these intracellular enzymes (NE, MPO) may be affected by hyperglycemia, presence of inhibitors of proteolytic agents and renal excretion.[23]

Limitations

This study has some limitations. The sample size was moderate, and the cross-sectional design limits causal interpretation. Protein levels were not measured in parallel, which would have strengthened the link between gene activations and functional enzyme levels. Future longitudinal studies combining gene expression, enzyme activity assays, and clinical outcomes could provide more robust insight into the predictive value of NE and MPO in metabolic and vascular risk stratifications.

CONCLUSION

In this study, we demonstrated that neutrophil elastase and myeloperoxidase mRNA expression levels are significantly elevated in individuals with metabolic syndrome compared to controls. These elevations were accompanied by metabolic abnormalities including central obesity, dyslipidemia, and higher systolic blood pressure. Importantly, both NE and MPO expressions correlated positively with MetS, reinforcing the link between neutrophil-driven inflammation and metabolic risk.

Our findings suggest that NE and MPO may serve as early molecular markers of immune activation in MetS, providing additional insight into the interplay between inflammation and metabolic dysfunction. Future studies with larger cohorts and longitudinal designs, integrating protein activity measurements and clinical outcomes, are warranted to validate their utility as predictive biomarkers for cardiometabolic risk stratification.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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