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Original article

Increased myeloperoxidase activity as an indicator of neutrophil-induced inflammation and sepsis in neonates

Background: *MPO is an enzyme that contains heme secreted by phagocytic cells after the respiratory burst system activation. MPO is expressed mainly by neutrophils and monocytes in small quantities and it is very important to determine further process of hydrogen peroxide.* ***Objective:*** *to evaluate neutrophils activation and the MPO enzyme activity in plasma as an indicator of sepsis as well as sepsis severity in neonates with sepsis with correlation to their clinical and laboratory findings.* ***Methods:*** *were classified into 2 groups: sepsis group: included 45 neonates with gestational ages 28-40 weeks with sepsis, 15 of them had been subjected to follow up samples, control group: included 30 neonates proved to be free of sepsis. All neonates were subjected to history taking, clinical examination and measurement of plasma MPO enzyme.* ***Results:*** *this study revealed that MPO activity and neutrophil cell count are increased in sepsis group compared to the non-septic neonates. The ROC curve showed that the best cut off for MPO in prediction of septic patients and mortality was found >54 mu/ml and >83 mu/ml respectively. There was positive correlation between MPO enzyme activity and the total leukocyte count and neutrophil count. By following up some of sepsis group neonates there was significant decrease in MPO activity goes along with improvement in clinical state of neonates with sepsis. MPO enzyme activity was found to be low in septic shock patients who also have pancytopenia compared to septic patients without shock.* ***Conclusion:*** *plasma MPO enzyme proved to be a good marker of sepsis in neonates, with a good prognostic value in severe cases.*

Keywords: MPO, inflammatory response, neonates, sepsis.

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INTRODUCTION

Neutrophil plays a role as one of the frontline of body's defense against infection. These cells use bactericidal pathways that are oxygen dependent or independent as a weapon to eliminate infectious agents. Oxygen-dependent mechanism involves production of bactericidal reactive oxygen compounds. The mechanism oxygen-independent involves chemotaxis, phagocytosis, degranulation, and the release of lysis enzyme and bactericidal peptides[1].

MPO is an enzyme that contains heme secreted by phagocytic cells after the activation of the respiratory burst system. MPO is expressed mainly by neutrophils and monocytes in small quantities and it is very important to determine further process of hydrogen peroxide[2]. Most of the hydrogen peroxide produced by neutrophils will be consumed by the MPO. Hydrogen peroxide substrate is also derived from in vivo variety sources, including the leukocyte NADPH oxidase, xanthine oxidase, nitric oxide synthase (NOS) and various isoenzymes[3].

MPO has been implicated in promoting tissue damage in various inflammatory diseases, including acute respiratory distress syndrome, ischemia-reperfusion injury, rheumatoid arthritis, bronchial asthma, and glomerulonephritis [4].

The MPO level also increases in the lung during sepsis, which confirms the infiltration of lung parenchyma by neutrophils and reflects the severity of neutrophil-mediated organ damage[5]. MPO is usually used as a marker of neutrophil accumulation in tissues and a marker of neutrophil activity when it is measured in plasma[6].

The aim of our study is to evaluate neutrophil activation and the MPO enzyme activity in plasma as an indicator of sepsis as well as sepsis severity in neonates with sepsis with correlation to their clinical and laboratory findings.

METHODS

The current study was approved by the Ethical Committee of Pediatrics department, Ain Shams University. A written informed consent was taken

from parents before enrollment of neonates in the study.

Myeloperoxidase enzyme activity was compared in 45 preterm and full term neonates to 30 healthy neonates with comparable gestation and birth weight. Out of 45 neonates with sepsis the severest 15 were subjected to follow up sample to assess MPO serum level at 48-72 hours after the initial sample, to see if the marker has an early prognostic value (time when most neonates responding to antibiotics will show clinical and laboratory improvement).#All neonates with gestational age of e 28 weeks and up to 40 weeks admitted to NICU of Maternity Hospital of Ain Shams University between January and December 2013 with proved sepsis (i.e. positive blood culture) or clinical sepsis (clinical signs of sepsis prompting five or more days of antibiotics, but the blood culture was negative) [7] were included. Neonates with Severe congenital anomalies, Chromosomal abnormalities and inborn errors of metabolism were excluded.

All neonates were subjected to full history taking, thorough clinical examination, laboratory investigations which included complete blood count (CBC) using blood samples collected in EDTA tubes by Coulter counter T660 (USA) with differential leucocytic count using Leishman-stained blood smear, C-reactive protein (CRP) estimated by latex agglutination assay (UK), and blood culture using a signal blood culture system (Oxoid, England). Myeloperoxidase enzyme activity was determined in the serum by Enzyme Linked Immunosorbent Assay (ELISA) (MPO Activity Assay Kit, BioVision Research Products, Mountain View, CA, USA).

Statistical Methods

Analysis of data was done by IBM computer using SPSS (Statistical Program for Social Sciences version 12). Quantitative variables were described as mean, SD, range, median and interquartile range. Qualitative variables were expressed as number and percentage. Chi-square test was used to compare qualitative variables between groups. Unpaired t-test used to compare quantitative variables, in parametric data. Mann-Whitney test was used in non-parametric data. Spearman Correlation co-efficient test used to correlate variables versus each other positively or inversely. ROC (receiver operator characteristic curve) was used to find out the best cut off to test the validity of certain variables (sensitivity, specificity, PPV and NPV). $P<0.05$ was considered significant.

RESULTS

The present study included 45 newborns with sepsis; 24 males (53.3%) and 21 females (46.7%), and 30 healthy controls; 15 males (50%) and 15 females (50%). Demographic characteristics of studied neonates are shown in table 1.

Sixty percent of neonates in the sepsis group were delivered by CS as compared to 53.3% in the control group. Neonates in sepsis group had many risk factors for sepsis including PROM (33.3%), maternal UTI (44.4%), maternal fever (22.2%) and chorioamnionitis (15.5%). Fourteen neonates had septic shock (31.1%). Most of the neonates had late onset sepsis (73.3%) (sepsis starting in 2nd week of life). Most of our septic neonates were ventilated (68.9%), 15.6% needed CPAP and 11.1% needed oxygen by nasal cannula (one liter/minute or less) while 4.4% did not need any respiratory support. APGAR score at 1min and 5 min were significantly decreased in sepsis group compared to control group; p=0.00 and 0.02 respectively.

The current study showed that there was significant decrease in segmented cell count and platelet count, while significant increase in I/T ratio and staff count, hematology and clinical septic score in sepsis compared to the control group. Also there was significant decrease in hemoglobin in sepsis group compared to control group; table 1. But we found no statistical difference in absolute neutrophil, lymphocyte count and serum creatinine between the two groups.

There was significant higher in MPO level, CRP titre and direct billirubin, as well as significant increase in total billirubin levels, RBS and bleeding profile (PT, PTT and INR) in sepsis group compared to control group; table I.

Forty two neonates (out of 45 neonates in sepsis group) had positive blood culture; *gram positive cocci* were detected in 17 patients, *pseudomonas* in 5, *coagulase negative staph*. (CONS) in 2, *klebsiella* in 9 and *E-coli* in 9. Blood culture was negative in 3 patients in sepsis group. We found that there was significant positive correlation between the MPO levels and the APGAR score at 1 minute, while there was no correlation with weight, G.A or APGAR score at 5 minute. Our study showed that there was highly significant positive correlation between the MPO level with WBCs; p=0.00, absolute and relative neutrophil count; p=0.00, and 0.00, staff count; p=0.00, segmented cell count; p=0.02, I/T ratio; p=0.00, Hematology septic score; p=0.01 and Clinical septic score; 0.00. But there was no correlation between the MPO level with lymphocytic cell count or platelet count or CRP titer in sepsis group.

As regard the follow up samples in 15 neonates with sepsis we found highly significant increase in HB, RBCs, abs. neutrophil count and WBCs in the follow up sample compared to the first sample. There was highly significant decrease in clinical septic score, staff count, I/T ratio and hematology sepsis score in follow up sample in comparison to first sample table 2.

Table 2 showed also, the significant statistical difference between the first and follow up sample as there was significant decrease in lactate level, also significant increase in PH level and HCO_3 level in follow up sample compared to first sample. There was no significant difference as regard PO_2 or PCO_2 level in arterial blood gas. MPO levels decreased as the septic neonates improved in the follow up sample. Same finding were detected in CRP level.

There was also a significant statistical difference as regard MPO level; P=0.02, as it was decreased in septic neonates with septic shock in comparison to septic neonates without septic shock median 67 vs 149 mu/ml respectively.

Of the 45 neonates in sepsis group 19 died, they had lower mean gestational age and lighter mean birth weight (31.1weeks and 1.6 kg respectively) compared to neonates who survived (36.1 weeks and 2.6 kg respectively) ($p<0.05$). Babies who died had lower mean total white blood cell count and lower mean serum MPO ($p<0.05$). Most of the neonates who died were suffering from severe shock or severe pneumonia. The ROC Curve (figure 1) defined the best cutoff value for MPO in the diagnosis of sepsis was found to be > 54 mu/ml with a sensitivity of 100%, specificity of 62.2%, area under the curve of 77.7 and accuracy of 81.11%.

The ROC Curve of figure (2) showed that the best cut off point for MPO in prediction of mortality was found to be > 83 mu/ml with sensitivity of 53.33% and specificity of 88.46% area under the curve 71.9 and accuracy of 78.2%.

Table 1. Comparison of laboratory parameters of the studied groups.

	Sepsis group (n=45)	**Control group (n=30)**	**t/z***	**p-value**
Clinical septic score(Mean±SD)	6.5±2.5	1.4±0.8	7.20	0.00
Hematology sepsis score (Mean±SD)	5.7±1.6	0.7±1.2	6.33	0.00
HB(gm/dl) (Mean±SD)	13.2±2.8	14.5±1.3	2.23	0.02
WBC(10^3/mm) (Mean±SD)	11.5±6.5	10±2.5	-1.13	0.26
Absolute nuetrophelic count (10^3/mm) (Mean±SD)	6.6±4.2	5.8±1.7	-1.07	0.28
Segmented% (Mean±SD)	34.3±5.7	54±7.5	12.92	0.00
staff %(median IQR)	20 (18 - 23)	2 (1 - 4)	7.31*	0.00
I/T (median IQR)	0.35 (0.3 - 0.4)	0.04 (0.01 – 0.06)	7.30*	0.00
Plt (10^3/mm) (Mean ±SD)	114.7±83.9	234.1±59.9	6.71	0.00
MPO (mU/ml) Median (IQR)	112 (75.7- 175.5)	29 (18 - 44)	-5.94	0.00
CRP (mg/L) Median (IQR)	96 (96 - 192)	0 (0 - 0)	-7.93	0.00
RBS (mg/dl) Median (IQR)	112 (78-214)	89 (79 - 105)	-2.06	0.03
Total bilirubin (mg/dl) Median (IQR)	10 (6.2 - 13.9)	6.65 (4.1- 10)	-2.18	0.02
Prothrombin time(sec.) PT Median (IQR)	20 (15 – 28.2)	14 (12 – 15)	-4.52	0.00
Partial thromboplastin time (sec.) PTT Median (IQR)	45 (35.5 – 55)	35 (32 – 39)	-4.62	0.00
INR Median (IQR)	1.60 (1 – 2)	0.85 (0.6 – 1)	-5.22	0.00

Z*: Data are presented as median (IQR) and compared by Mann-Whitney test RBS = random blood sugar WBC_S = white blood cells HB=hemoglobin plt. = platelets count CRP= C-reactive protein I/T ratio = immature to total neutrophil count IQR= Interquartile Range SD= standard deviation MPO = myeloperoxidase enzyme INR= International normalizing ratio

Table 2. Comparison between newborns with clinical sepsis at start and at three days later.

Variable	First sample n (15) Mean±SD/ Median (IQR)	Follow up sample n (15) Mean±SD/ Median (IQR)	t/Z*	p-value
Clinical septic score Mean±SD	7.8±1	6.2±2.1	2.35	0.00
Hematology sepsis score Mean±SD	6.2±1.5	5.1±1.8	1.09	0.02
HB(gm/dl) Mean±SD	10.5±2.4	15±1.7	8.50	0.00
Staff % Median (IQR)	18 (15 – 22)	16 (3 – 19)	2.53*	0.02
WBC(10^3/mm) Mean±SD	6.4±3.1	13.4±5.6	5.94	0.00
abs.count(10^3/mm) Mean±SD	3.6±2.2	7.6±3.6	4.88	0.00
I.T Median (IQR)	0.3 (0.3 - 0.4)	0.2 (0.2 - 0.3)	2.52*	0.02
Plt(10^3/mm) Mean±SD	123±62	106.3±92.2	0.56	0.58
PH Median (IQR)	7.2(7 - 7.4)	7.3 (7.2 - 7.3)	2.02*	0.04
HCO_3(mmol/l) Mean±SD	17.6±3.7	19.3±2	2.39	0.03
Lactic (mmol/l) Median (IQR)	4.7 (1.3-5.8)	2.4 (1.3-3.8)	2.28*	0.02
MPO (MU/ML) Median (IQR)	163 (108 – 213)	41 (31 - 49.5)	3.05*	0.00
CRP (mg/dl) Median (IQR)	96 (96 – 192)	48 (0 – 48)	2.45*	0.01
RBS (mg/dl) Median (IQR)	194(87.5-271.7)	76 (49 - 86.7)	2.10*	0.03

WBCS = white blood cells HB=hemoglobin Abs.count = absolute neutrophil count plt. = platelets count I/T ratio = immature to total neutrophil count t: Data were presented as mean±SD and compared using paired t-test Z*: Data are presented as median and IQR and compared using Wilcoxon rank test. HCO_3 = bicarb in blood MPO = myeloperoxidase enzyme CRP = C-reactive protein RBS = random blood sugar bil. Level = bilirubin level

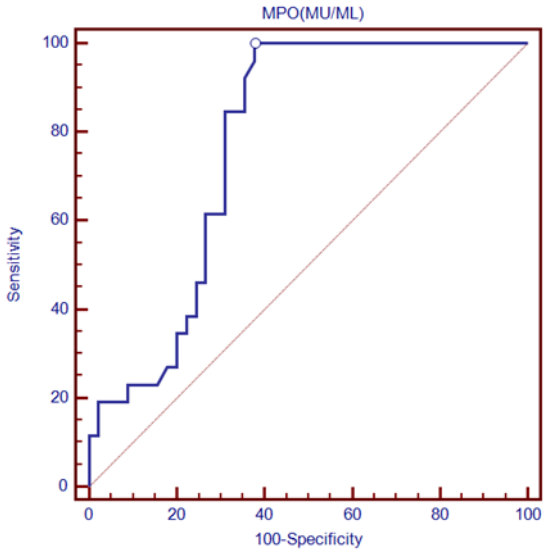

Figure 1. Receiver operating characteristic curve (ROC) for MPO in prediction of sepsis.

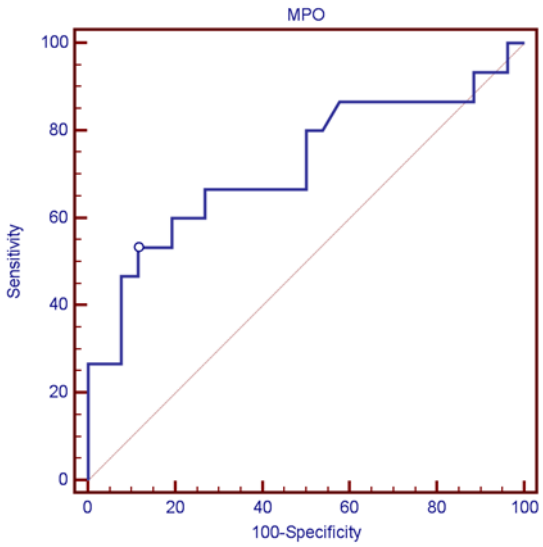

Figure 2. Receiver operating characteristic curve (ROC) for MPO in prediction of mortality.

DISCUSSION

Many studies have shown that in neonates signs of sepsis are nonspecific and are observed with other noninfectious conditions. Clinical criteria were unable to distinguish infected infants from uninfected infants[7-9].

According to Christensen[10] leukocytosis and neutrophilia are unreliable indicators of neonatal sepsis. The reference range for these parameters changes daily, and may be influenced by many factor as gestational age, type of delivery and maternal hypertension. Neutropenia might even be better marker for sepsis[11,12], if we can exclude other causes of neutropenia like preeclampsia, asphyxia and hemolytic disease of newborn.

Serum C-reactive protein rises secondary to macrophage, T-cells or adipocyte production[13]. But not all neonates with systemic bacterial infections have elevated CRP with sensitivity anywhere between 50-90%[14]. Da Silva et al.[15] showed in a comprehensive systematic review of the literature evaluating leukocyte ratios and CRP, a wide range of sensitivity (17% to 90%) and specificity (31% to 100%) for leukocyte count and ratios. Similarly

CRP measurements showed variable accuracy, though better accuracy than the leukocyte parameters. Ng and Lam[13] suggested that The CRP level is not recommended as a sole indicator of neonatal sepsis but may be used as part of a sepsis workup or as a serial study during infection to assess the response to antibiotics.

According to Kothari et al.[16], increased plasma MPO level is a marker of neutrophil proliferation and degranulation in humans as MPO constitutes 5% of human PMN by weight and believed to represent a major pathway for oxygen dependent microbicidal activity. MPO catalyzes the formation of hypochlorous acid, a potent oxidant that has been implicated in killing bacteria[17] and tissue destruction through induction of necrosis and apoptosis[18].

Our study shows high significance increase in MPO level in neonates with proven sepsis compared to healthy neonates, ($p < 0.01$). This is in agreement with Nahm et al.[19] and Basher et al.[20] who's results of MPO studies in patients with sepsis and septic shock demonstrate that MPO blood level has a clear correlation with the severity of these pathological processes. This goes with Yunanto et al.[21] study on neonates with early onset sepsis (EOS). They found significant increase in MPO blood level in septic neonates (623.52 ± 97.55 ng/ml) compared with healthy control (360.95 ± 142.72 ng/ml). The Gustafsson et al.[22] study on adult septic patient admitted to ICU revealed higher levels of serum IL-6, IL-10, MPO and PCT in patients with severe sepsis compared to healthy controls ($P = 0.001$).

In our study, there was significant correlation between MPO levels and previously described hematological marker of sepsis: total leukocytic count, absolute and relative neutrophil count, I/T ratio, staff count and with segmented cell ($p = .022$). This could be explained by Nicholls and Hazen[3] study which stated that MPO, through formation of secondary oxidants and nitration of protein tyrosine residues, could modulate intercellular signaling in the vasculature. Lau et al.[23] found that MPO affect the activation state of neutrophils. Also, Yunanto et al.[21] study showed significant positive correlation with neutrophil count in blood as well as saliva samples in neonates with EOS.

Leviton et al.[24] also stated that the concentrations of many inflammatory proteins including MPO: are higher in newborns exposed to inflammation with positive correlation to CRP. Similarly Kothari et al.[16] study proved that MPO levels correlated linearly with sepsis severity except in advanced stages of septic shock. While Carr[25] stated that MPO is reduced in premature babies. Leviton et al.[24] have found higher levels in premature babies which decrease as the baby matures. They explained that as in most previous studies of protein concentrations in preterm newborns, term newborns served as the comparison group. They, however, have evaluated protein concentrations within a narrow gestational age range, 23 to 27 6/7 weeks. They are reluctant to extrapolate the significance of their findings beyond this narrow range.

In our study we found that patients with severe septic shock suffering from severe neutropenia usually have significant decrease in MPO ($p < 0.01$) level up to being undetectable. Also MPO levels were positively correlated with neutrophil count. Both MPO and neutrophil count correlate inversely with septic shock severity as MPO plasma level is usually derived from neutrophils; its levels will decrease with the decrease of neutrophil count which goes with Kothari et al.[16] study.

Also we found marked decline in MPO level in follow up sample in comparison with first sample which could be explained by decrease in number of neutrophils with less degranulation and less MPO in serum which implicate that MPO has good prognostic value in sepsis in neonates. Kothari et al.[16] study concluded that the plasma MPO concentration may be a marker of the neutrophil proliferation and severity of inflammation which is consisting enough with our results. In study by Vrba and Modriansky[6] it was proved that MPO is usually used as a marker of accumulation of neutrophils in tissue and it is a marker of neutrophil activity measured in plasma which supports our study.

In our study, the ROC curve and interactive dot diagram show that the best cut off point for MPO to prove sepsis in ill neonates was found to be >54 mu/ml with a sensitivity of 100%, specificity, of 62.2%, area under the curve of 77.7% and accuracy of 81.11%. Yunanto et al.[21] have similar findings in saliva and blood sample of neonates with EOS. Also in our study, the ROC curve and interactive dot diagram showed that the best cut off point for MPO in prediction of mortality was found to be > 83mu/ml with sensitivity of 53.33% and specificity of 88.46%.

In conclusion we demonstrated that plasma MPO enzyme activity is a good biomarker of inflammatory response in neonates with sepsis regardless of weight or the gestational age, as well as sepsis severity, with cut off point for MPO >54 mu/ml with a sensitivity of 100%, specificity of 62.2% in prediction of septic patients. However

levels of MPO decrease in sepsis shock due to neutropenia associated with severe toxemia. The best cut off point for MPO in prediction of mortality was found >83 mu/ml with sensitivity of 53.33% and specificity of 88.46%.

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