Original Article

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.1053773
J Health Sci Med 2022; 5(2): 429-433

Myeloperoxidase deficiency: a single center experience

Özlem Arman Bilir, Namık Yaşar Özbek
Ankara City Hospital, Department of Pediatric Hematology and Oncology, Ankara, Turkey

Cite this article as: Arman Bilir Ö, Özbek NY. Myeloperoxidase deficiency: a single center experience. J Health Sci Med 2022; 5(2): 429-433.

ABSTRACT

Aim: Myeloperoxidase (MPO) deficiency is the most common inherited defect of phagocytes. In this article, we aimed to reveal clinical characteristics of our patients with primary MPO deficiency.

Material and Method: In our study, patients aged 0-18 years, who were consulted to Ankara City Hospital Pediatric Hematology Department between 1 October 2019 and 1 December 2021 due to neutropenia, were retrospectively examined. If a patient had neutropenia in the complete blood count and inconsistently normal neutrophil count in the peripheral blood smear formula it was accepted as pseudoneutropenia. Patients with pseuduneutropenia were included in the study.

Results: Fifteen patients diagnosed with MPO deficiency were analyzed in the study. Nine of the patients were female, 6 were male, median age of the patients was 7 (0 – 17.5) years. The mean white blood cell (WBC) count of the patients was reported as 8219±2879/mm³, and the mean neutrophil count and percentage in the complete blood count printout was 33.30±15.88/mm³ and 0,74%±0, 94% respectively. The mean neutrophil count and percentage counted in the peripheral blood smear were 5186±1710 and 63.8%±10.59%, respectively. The mean LUC value on the complete blood count printout was 54.35%±19.47% (Normal range, 0-4%). In the flow cytometry evaluation of peripheral blood samples of the patients, it was observed that neutrophils were stained with CD33, CD13, CD16, CD11b monoclonal antibodies but not with MPO.

Conclusion: Peripheral smear evaluation is important when investigating the etiology of neutropenia. Many hematology analyzers using the MPO staining technique are indicative of MPO deficiency by identifying large unstained cells that do not stain with MPO. In patients who present with recurrent infections and MPO deficiency, other reasons that may predispose to infections should be investigated.

Keywords: Myeloperoxidase deficiency, children, pseudoneutropenia, hematology analyzer, flowcytometry

The study received the 2nd oral presentation award at the 13th National Pediatric Hematology Congress held in Pine Beach Hotel-Antalya between 14-17 October 2021 by the Turkish Pediatric Hematology Association

INTRODUCTION

Myeloperoxidase (MPO) is an iron-containing hemoprotein expressed in azurophilic granules of neutrophils and lysosomes of monocytes. The enzyme has strong antibacterial properties, producing strong bactericidal compounds such as hypochlorous acid from hydrogen peroxide and the halide, chloride (1-3). It is the most common inherited defect of phagocytes. It plays a role in killing various micro-organisms and foreign cells, including bacteria, fungi, viruses, malignant and non-malignant cells (4). Myeloperoxidase deficiency was first described in 1954 and is an autosomal recessive disease caused by mutations in the MPO gene on chromosome 17q23 (5,6). Its incidence was reported as 1:2000-4000 in Europe and America, while it was reported as 1:55000 in Japan (7). Microbial killing is impaired in patients with MPO deficiency, but most patients are asymptomatic, except for the diabetic patients (6). In this article, we aimed to show clinical characteristics of our patients with primary MPO deficiency.

MATERIAL AND METHOD

For all the records examined, the study was carried out with the permission of the Ankara City Hospital Ethics Committee (Date: 22.12.2021, Decision No: E2-21-1178). All procedures were carried out in accordance with ethical rules and the principles of the Declaration of Helsinki.

In our study, patients under the age of 18 years, who were consulted to Ankara City Hospital Pediatric Hematology Department between 1 October 2019 and 1 December 2021 due to neutropenia, were retrospectively examined. If a patient had neutropenia in the complete blood count and inconsistently normal neutrophil count in the peripheral blood smear formula it was accepted as

Corresponding Author: Özlem Arman Bilir, ozlemarman@gmail.com
Received: 05.01.2022 **Accepted:** 17.01.2022

pseudoneutropenia. Our study was performed on patients with pseudoneutropenia. To define myeloperoxidase deficiency, a peripheral blood smear prepared using Wright's stain was evaluated and a leukocyte formula was made. The diagnosis of MPO deficiency was primarily considered by confirming a normal population of neutrophils and monocytes in the absence of any atypical or blastic white cells in the peripheral blood smear. To make the definitive diagnosis of the patients, 5 cc blood samples were taken from the peripheral blood to the tube with EDTA and intracytoplasmic MPO staining was performed by flow cytometry. Whether the neutrophils have MPO expression in peripheral blood was evaluated in our hospital by flow cytometry (Beckman Coulter, USA) and using Kaluza software. MPO expressions of cells in the area of neutrophils were examined in the forward scatter / side scatter dot plot. In addition, to differentiate these cells from other cells in peripheral blood, CD45(KO), MPO (PE), CD33 (PC5), CD13 (ECD), CD16 (PB), CD11b (FITC), CD3 (A700), CD19 (A750) by preparing a tube consisting of antibodies, neutrophils and other blood cells were identified, and other surface markers expressed by neutrophils were also examined. In our center, a complete blood count (CBC) is performed from the peripheral blood sample taken into an EDTA tube with the ADVIA 2120i hematology analyzer. The ADVIA 2120i hematology analyzer utilizes a combination of two unique cytochemical methodologies termed collectively as peroxidase activity and nuclear density analysis. In addition, cells larger than normal and unstained on the analyzer output; given as the large unstained cells (LUC) value.

Statistical Analysis

Descriptive statistics were presented as median value (minimum-maximum) for quantitative variables and frequency (percent) for categorical variables. Calculations for descriptive statistics were performed with Statistical Package for Social Sciences (SPSS Version 18.0, Chicago, IL).

RESULTS

Between October 2019 and December 2021, 15 patients were diagnosed with MPO deficiency in the Pediatric Hematology Department of Ankara City Hospital. Nine of the patients were female, 6 were male, median age of the patients was 7 (0–7.5) years. While seven patients were under follow-up due to any chronic disease, neutropenia was detected incidentally in 8 patients at the time of admission to the hospital for any reason. In two patients, neutropenia was detected in the neonatal period. The mean white blood cell (WBC) count of the patients was reported as 8219±2879/mm³, and the mean neutrophil count and percentage in the complete blood count printout was 33.30±15.88/mm³ and 0,74%±0, 94% respectively. The mean neutrophil count and percentage counted in the peripheral blood smear were 5186±1710 and 63.8%±10.59%, respectively. The mean LUC value on the complete blood count printout is 54.35%±19.47% (Normal range, 0-4%). It was observed that the neutrophil counts in the CBCs of all patients were inconsistent with the neutrophil counts counted in the peripheral blood smear. It was determined by peripheral smear examination that none of the patients were neutropenic. In addition, the LUC values of all patients were found to be higher than normal as a result of the hemogram. The laboratory and demographic characteristics of the cases are summarized in **Table**. In the flow cytometry evaluation of the patients' peripheral blood samples, it was observed that neutrophils were stained with CD33, CD13, CD16, CD11b monoclonal antibodies, but not with MPO, and the patients were diagnosed with MPO deficiency.

Table. The laboratory and demographic characteristics of the patients

Patient No	Age (Year)	Gender	Diagnosis of Chronic Disease	Analyzer counts (ADVIA 2120i)				Peripheral blood smear manual count	
				WBC (/mm³)	Neutrophil Count (/mm³)	Neutrophil (%)	LUC (%)	Neutrophil Count (/mm³)	Neutrophil (%)
1	0	K	None	16000	10	0.6	54.4	8800	55
2	6.5	K	ANA +	8830	20	0.2	50.8	4944	56
3	18	K	Granulamatöz Polianjiitis	8180	60	0.7	66.2	7034	86
4	16	K	None	7640	10	0.2	65.4	5195	68
5	11	K	Hypothyroidism	5480	60	1.1	41.8	3836	70
6	1.75	K	Retinoblastoma	4400	20	4	92.4	3432	78
7	7	K	None	10320	30	0.3	71.2	6604	64
8	17	E	None	8050	40	0.5	13	5313	66
9	5	E	Developmental Retardation	5110	50	1	36.3	2350	46
10	3	E	None	9340	30	0.3	56	6351	68
11	15.75	E	Hypertension	8590	40	0.4	63.5	4638	54
12	10	E	None	7560	30	0.4	57.7	5594	74
13	17.5	K	Familial Mediterranean Fever	5030	40	0.8	56.7	2917	58
14	0.16	K	None	7970	40	0.5	24.6	4303	54
15	0	E	None	10790	20	0.2	65.3	6474	60

DISCUSSION

Neutrophils perform their roles in host defense by producing hydrogen peroxide by the oxygen-dependent respiratory burst system, via the myeloperoxidase they contain. Although myeloperoxidase deficiency is one of the most common inherited defects of phagocytosis that can impair microbial killing, it has been rarely reported to be associated with clinical symptoms (4,8). Several point germline mutations cause primary MPO deficiency, such as defective post-translational processing of the myeloperoxidase precursor protein and pre-translational defects caused by mutations in the regulatory part of the MPO gene. Most of the mutations associated with the inherited form are R569W (most common), Y173C, M251T, G501S, and R499C, and deletions of 14 bases (D14) in exon 9 (6). Secondary MPO deficiency is rarer than the inherited form but may develop due to somatic mutations of the MPO gene. In most cases, the deficiency is partial and affects only a portion of the neutrophils, is usually transient, and usually resolves when the underlying condition improves. A variety of disorders, including heavy metal poisoning, severe infections, diabetes mellitus, myelodysplastic syndrome, acute and chronic myeloid leukemia, and Hodgkin lymphoma, are causes of secondary or acquired MPO deficiency (6,9). Secondary MPO deficiency is always accompanied by a disease, and neutrophils have different MPO activity that varies from cell to cell. In the flow cytometric examination of our patients, there was no MPO expression in all neutrophils and monocytes, including those with accompanying chronic diseases. Therefore, we accepted our patients with primary MPO deficiency. While 6 of our patients were under follow-up due to chronic disease, 9 patients did not have an underlying chronic disease and neutropenia was detected at the time of admission to the hospital for any reason. One of our patients had received multiple immunosuppressive therapies for his rheumatological disease, therefore secondary MPO deficiency might have developed. However, we also detected MPO deficiency in the patient's healthy sibling, and the secondary deficiency was excluded. Because, primary MPO deficiency has a genetic origin, occurs with varying degrees of severity in more than one family member, and involves both the neutrophil and monocyte lineages (7,10).

Most patients with MPO deficiency are asymptomatic without an increase in infection, serious infections have been reported in up to 5% of patients (8). Recurrent severe infections with Candida Albicans have been observed in individuals with co-morbidities such as diabetes mellitus (11). It is unclear whether the infections in these patients are solely the result of MPO deficiency or whether other MPO-independent mechanisms are also responsible. Severe infectious complications requiring hospitalization were not observed in any of our patients. Therefore, it is important to diagnose MPO deficiency. It helps us to prevent unnecessary examination and treatment in the follow-up of patients. In the literature in a case series of 4 patients with MPO deficiency, it was reported that one of the patients presented with pneumonia and neutropenia was detected according to the result of the hematology analyzer output, and granulocyte-colony stimulant factor (G-CSF) was administered to the patient. They reported that they subsequently evaluated the patient's peripheral blood smear and found that the patient was not neutropenic (12). One of the main purposes of our article is to emphasize that abnormal results in the hematology analyzer output of the patients should be confirmed by peripheral blood smear. Hematology analyzer ADVIA2120i (Siemens AG), used in our hospital laboratory, separates leukocytes by peroxidase activity and nuclear density. Neutrophil counts are reported to be very low in hemogram results of patients with myeloperoxidase deficiency. The mean neutrophil counts in the laboratory printout of our patients were 33.30±15.88/mm³, while the mean neutrophil counts counted in the peripheral blood smear were 5186±1710/mm³. It gives the LUC value by counting the neutrophils and monocytes that are not stained with myeloperoxidase in the large unstained cell group. Therefore, LUC values in the hemogram printout of patients with MPO deficiency are found to be quite high (12,13). The mean LUC values of our patients were found to be 54.35%±19.47%. These values were seen well above the normal range. Large unstained cells identify blasts, variant, and atypical lymphocytes as well as MPO deficient patients show large neutrophils with reduced MPO activity (14). The increased proportion of large unstained cells should alert the clinician and require peripheral blood smear evaluation to distinguish a pathological condition from normal variants. Myeloperoxidase deficiency can be easily diagnosed in clinical hematology laboratories with flow cytometric examination by evaluating the peroxidase activity of neutrophils from peripheral blood samples (12). We also performed flow cytometric analysis from peripheral blood samples of all our patients. We observed that the neutrophils of the patients were stained with CD33, CD13, CD16, CD11b monoclonal antibodies, but not with MPO. One of our patients was being followed up with a diagnosis of retinoblastoma. However, it is controversial in the literature whether there is a relationship between MPO deficiency and cancer susceptibility (15,16). Our patient number two was being followed up because of anti-nuclear antibody positivity, although he had no clinical findings. Interestingly, in a study, they reported statistically higher MPO plasma levels in SLE patients compared to healthy patients,

but they could not show its relationship with disease severity (17). Our patient number three was diagnosed with granulomatous polyangiitis, and patient number 4 was selected by the pediatric rheumatology department because she was the sister of patient number 3. The patient, who was being followed up with the diagnosis of granulomatous polyangiitis, had received multiple immunosuppressive treatments. It has been reported in the literature that there is a relationship between MPO deficiency and autoimmunity (18). In a study in mice, it was shown that MPO can limit the adaptive immune response by reducing dendritic cell activation, thus reducing both the migration of dendritic cells to the lymph node and the antigen presentation capacity (18). While long-term overproduction of MPO causes tissue damage, it is thought to play an anti-inflammatory role in some cases, depending on the type of inflammation (19).

Because most people with MPO deficiency do not suffer from infections and are typically asymptomatic, prophylactic antibiotics are not recommended. In people with MPO deficiency without comorbidities, specific treatment of infections is sufficient, and no additional treatment is required. The fact that none of our patients had severe infections that required hospitalization also supports this. However, since the incidence of localized and systemic infections is high in patients with diabetes mellitus, rapid and aggressive treatment with antimicrobials is usually required to control infections.

CONCLUSION

Peripheral smear evaluation is important when investigating the etiology of neutropenia. Many hematology analyzers using the MPO staining technique are indicative of MPO deficiency by identifying large unstained cells that do not stain with MPO. In patients who present with recurrent infections and MPO deficiency, other reasons that may predispose to infection should be investigated. Usually, simple laboratory tests and/or clinical presentation and history can distinguish the underlying cause. In patients with MPO deficiency, specific treatment of infections is sufficient, and no additional treatment is required.

The limited number of our patients and the short-term follow-up timeare the limitations of our study.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital Ethics Committee (Date: 22.12.2021, Decision No: E2-21-1178).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Xiao X, Saha P, Yeoh BS, et al. Myeloperoxidase deficiency attenuates systemic and dietary iron-induced adverse effects. J Nutr Biochem 2018; 62: 28-34.
2. Domingues-Ferreira M, Levy A, Barros NC, et al. Case report of myeloperoxidase deficiency associated with disseminated paracoccidioidomycosis and peritoneal tuberculosis. Rev Soc Bras Med Trop 2017; 50: 568-70.
3. Albrett AM, Ashby LV, Dickerhof N, et al. Heterogeneity of hypochlorous acid production in individual neutrophil phagosomes revealed by a rhodamine-based probe. J Biol Chem 2018; 293: 15715-24.
4. Yonezawa K, Horie O, Yoshioka A, et al. Association between the neutrophil myeloperoxidase index and subsets of bacterial infections. Int J Lab Hematol 2010; 32: 598-605.
5. Marchetti C, Patriarca P, Solero GP, et al. Genetic characterization of myeloperoxidase deficiency in Italy. Hum Mutat 2004; 23: 496-505.
6. Pahwa R, Modi P, Jialal I. Myeloperoxidase deficiency. 2021 Sep 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID: 29262241. Bookshelf ID: NBK470278,
7. Nauseef WM. Diagnostic assays for myeloperoxidase and myeloperoxidase deficiency. Methods Mol Biol 2014; 1124: 537-46.
8. Lanza F. Clinical manifestation of myeloperoxidase deficiency. J Mol Med 1998; 76: 676-81.
9. Patıroğlu T, Güngör HE, Belohradsky JS, et al. Myeloperoxidase deficiency: the secret under the flag of unstained cell. Turkish J Hematol 2013; 3: 232
10. Larrocha C, De Castro MF, Fontan G, et al. Hereditary myeloperoxidase deficiency: study of 12 cases. Scand J Haematol 1982; 29: 389–97.
11. Cech P, Stalder H, Widmann JJ, et al. Leukocyte myeloperoxidase deficiency and diabetes mellitus associated with *Candida albicans* liver abscess. Am J Med 1979; 66: 149-53.
12. Roh S, Ham JY, Song KE, et al. Myeloperoxidase deficiency manifesting as pseudoneutropenia with low mean peroxidase index and high monocyte count in 4 adult patients. Lab Med 2020; 51: 16-9.
13. Gleghorn DM, Thomas W. Prevalence of myeloperoxidase deficiency determined using an ADVIA 2120i. Int J Lab Hematol 2020; 42: 220-3.
14. Cakir I, Cakir N, Atalay MA, et al. Large unstained cells are correlated with inflammatory biomarkers in patients with invasive aspergillosis. Turkish J Biochem 2018; 43: 306–11.
15. Kutter D, Devaquet P, Vanderstocken G, et al. Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit? Acta Haematol 2000; 104: 10–5.
16. Lanza F, Fietta A, Spisani S, et al. Does a relationship exist between neutrophil myeloperoxidase deficiency and the occurrence of neoplasms? J Clin Lab Immunol 1987; 22: 175-80.

17. Telles RW, Ferreira GA, da Silva NP, et al. Increased plasma myeloperoxidase levels in systemic lupus erythematosus. Rheumatol Int 2010; 30: 779-84.
18. Odobasic D, Kitching AR, Yang Y, et al. Neutrophil myeloperoxidase regulates T-cell-driven tissue inflammation in mice by inhibiting dendritic cell function. Blood 2013; 121: 4195–204.
19. Aratani Y. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. Arch Biochem Biophys 2018; 640: 47-52