Original Article

The Use of Neutrophil Elastase and Neutrophil Myeloperoxidase as Biomarker in Hemarthrosis

Rusul Qasim Mohammed[1], Abeer Anwer Ahmed[2]

[1]Department of Pathology, Faculty of Medicine, Mustansiriyah University, Baghdad, Iraq, [2]Department of Pathology and Forensic Medicine, Faculty of Medicine, Mustansiriyah University, Baghdad, Iraq

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Abstract

Background: Hemophilia is an inherited bleeding disorder that could cause many complications one of them is hemarthrosis. Neutrophils are the predominant immune cells that infiltrate joints after hemorrhage. Tissue injury is often accompanied by the productions of neutrophil extracellular traps (NETs), which are DNA constructs containing attached granular enzymes. **Aims of Study:** Measuring the circulating NETs, neutrophil elastase (NE), and myeloperoxidase (MPO) in the plasma of hemophilia A patients with hemarthrosis, for an understanding of hemarthrosis' underlying pathology. **Subjects and Methods:** Fifty persons were recruited in this study, during a period of 8 months from November 2022 to June 2023: 25 patients were diagnosed as hemophilia A with hemarthrosis and another 25 individuals served as a control group who were unrelated, apparently healthy, and were age and sex matched. Plasma NE and MPO were measured by ELIZA technique. **Results:** The plasma MPO and NE levels were significantly higher in hemophilia A patients than controls: 12.714 ± 19.439 and 1535.34 ± 2059.87 ng/mL, respectively, at ($P < 0.05$) compared to control group 3.672 ± 3.623 and 235.26 ± 274.61 ng/mL. **Conclusions and Recommendations:** Patients with hemarthrosis had a considerably higher level of NETs in their plasma than healthy individuals. These findings may indicate the function of NETs in the pathology of hemophilia A with hemarthrosis, and the identification of high NETs might serve as a biomarker, as well as a possible prognostic and therapeutic target for such individuals.

Keywords: Hemarthrosis, myeloperoxidase, neutrophil elastase, neutrophil extracellular traps

Introduction

Hemophilia is x-linked disorder in which the affected patients bleed heavily for prolonged period of time after injuries,surgery,tooth extraction,or circumcision. Constant bleeding after minimal trauma or spontaneous bleeding is an indicator of severe hemophilia who defined that factor VIII <1%.[1] It is characterized by deficiency of clotting factor FVIII, which results from distinct gene variations or mutations.[2] Hemophilia A affects around 1 in 5000 males, which representing 8%–85% of hemophilia cases.[3] The severity of hemophilia may be further classified by examining the correlation between bleeding symptoms and the amount of residual factor. Patients who have moderate hemophilia, in which afflicted person have 1-5% of normal factor VIII clotting activity,sometimes show sign of bleeding after substional trauma or undergoing surgery.[4] One of the complications that causes synovial hyperplasia, proliferation of macrophage-like synoviocytes and fibroblast-like synoviocytes is recurrent hemarthrosis. These synoviocytes produce for angiogenesis vascular endothelial growth factor and vascular remodeling, as well as pro-inflammatory cytokines like tumor necrosis factor alpha, IL 6 and IL 1-beta.These factors lead to an increase in the number of fibroblast-like synoviocytes (FLS) and the death of chondrocytes.[5] The relase of erythrocytes and damage-associated molecular pattern (DAMP) may be triggered by joint bleeding, which may lead to sterile inflammation in the joints and production of neutrophil extracellular traps(NETs).[6] This is just one of many molecular mechanisms that lead to the injury and causes of hemophilic arthropathy. Neutrophil net-like structures called extracellular traps are produced from

Address for correspondence: Dr. Rusul Qasim Mohammed, Department of Pathology, Faculty of Medicine, Mustansiriyah University, Baghdad, Iraq.
E-mail: rusulq2017@gmail.com

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active neutrophils and are made of DNA-histone complexes and proteins.[7] The cause of this occurring can be attributed to ongoing inflammatory process triggered by the occurrence of joint bleeding. This process leads to the release of several cytokines,including tumor necrosis factor alpha, interleukin-6 ,interleukin-1 beta, as well as pro-angiogenic chemicals into bloodstream.Consequently,these substances drive the development of neutrophil extracelluar traps.[8]

Subjects and Methods

Subjects and design study

This case–control study was done in the Department of Pathology and Forensic Medicine/Faculty of Medicine/ Mustansiriyah University in cooperation with Al-Karama Teaching Hospital in Wasit and Children Welfare Teaching Hospital in Baghdad, during the period from November 2022 to June 2023. This study has a total of 50 samples,of which 25 were identified as individuals with sever hemophilia A who factor VIII less than 1% and the presence of persistent hemarthrosis. The clinical diagnosis of hemophilia A patients was carried out by hematologist specialist physician based on history ,clinical examination,additionally the confirmation of this diagnosis was achieved by the use of a factor VIII assay. Patients were directly interviewed and the data were taken using the questionnaire that includes age, gender, length, weight, and family history. The other 25 samples were from apparently healthy subjects who had no pathological state at the time of this study; all of these individuals were matched to patients for age and sex.

Methods

All participants gave their informed permission after being given a thorough explanation of the study's goals and any potential risks to their health, each participant in the research project provided their informed written consent. We took 5 mL of venous blood from each patient and healthy control and let it clot at room temperature and undisturbed for 10–20 min. The clot was extracted after a centrifugation process lasting 20 minutes,the serum was transferred into eppendorf tubes.

Enzyme-linked immunosorbent assay(ELISAs) are used for quantifying of neutrophil myeloperoxidase(MPO) level and the neutrophil elastase (NE) level for each sample,according the company(Snulong biotech.,Ltd).

Statistical analysis

IBM SPSS-29 (IBM Statistical Packages for the Social Sciences, version 29, Chicago, Illinois, United States) was used to analyze the data. The data were shown using a mean, standard deviation (SD). The significance of a difference in means between two groups was tested using the Students' *t*-test, Paired *t*-test was used to determine statistical significance between paired observations (or two dependent means), and analysis of variance was used to determine statistical significance between three or more independent means. When the probability level was <0.05, it was judged to be statistically significant.[9-12]

Results

In Tables 1 and 2, the results showed a significant rise ($P < 0.05$) in the level of MPO and NE for hemophilia patients compared to healthy subjects. Table 1 shows the results (mean ± SD) of MPO (12.714 ± 19.439) compared to controls (3.672 ± 3.623) and Table 2 shows the results of NE (1535.34 ± 2059.87) of hemophilia patients compared to controls (235.26 ± 274.61).

Discussion

Identifying the process of inflammation that causes joint damage in hemophilia patients is important for treating their major complications. This is because neutrophils make up a large portion of human leucocytes and has major role in the body's immune system and inflammatory processes. As shown in Tables 1 and 2, the results showed that the levels of NE and MPO in the plasma of patients are significantly higher than those of healthy subjects. Our study agreed with Oneto *et al.,* 2022,[13] van Vulpen *et al.,* 2021,[14] and Kaminski *et al.,* 2023.[6] Melchiorre *et al*., 2017,[15] confirmed that the pathogenesis of arthropathy has several aspects between rheumatoid arthritis (RA) and osteoarthritis (OA) of both clinical and biological features, such as synovitis, bone resorption, and articular cartilage deterioration in RA and OA, respectively. According to studies by Camona *et al.,* 2020,[16] Clarke *et al.,* 2020[17] and Song *et al.,* 2020,[18] considering the widely accepted evidence that neutrophil extracellular traps (NETs) play a vitol role as synovial structures in corrdinating aberrant immune responses,inflammation and joint deterioation, it is probable that interaction between synovial neutrophil and fibroblast-like synoviocytes will lead to the generation of inflammatory signals that promote the development of pathogenic immune reaction. Synovial NE may directly degrade cartilage components in addition to promoting inflammation through FLS and macrophages.

Table 1: Comparison of myeloperoxidase level between patients and control

Type subject	Parameter MPO (ng/mL), mean±SD	SEM	*P*
Hemophilia	12.714±19.439	3.888	0.027#
Control	3.672±3.623	0.725	

#There is a statistically significant difference seen between two independent mean when doing student's t -test at a significance level of 0.05. SEM: Standard error of mean, MPO: Myeloperoxidase, SD: Standard deviation

Table 2: Comparison of neutrophil elastase level between patients and control

Type subject	Parameter NE (ng/mL), mean±SD	SEM	*P*
Hemophilia	1535.34±2059.87	411.974	0.003#
Control	235.26±274.61	54.922	

#There is a statistically significant difference seen between two independent mean when doing student's t-test at a significance level 0.05 NE: Neutrophil elastase, SEM: Standard error of mean, SD: Standard deviation

Conclusions

Hemophilic patients who suffering from hemarthrosis had considerably higher plasma NET levels compared to healthy controls.

Recommendations

Patients with hemophilia A who are experiencing hemarthrosis may benefit from using NETs as a biomarker for the disease, as well as a prognostic and therapeutic target.

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Conflicts of interest

There are no conflicts of interest.

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