Comparison of the Volumetric Parameters of Platelets in Diabetic Children with and without Diabetic Ketoacidosis: A Case-Control Study in the North of Iran

Fatemeh Mirdoosti MD¹, Hassan Mahmoodi Nesheli MD², Hoda Shirafkan PhD³, Sajedeh Hajipour Msc⁴, Pouria Alijanpour Msc⁵, Mohammad Pournasrollah MD⁴, Morteza Alijanpour MD^{*2}

1. Student Research Committee, Babol University of Medical Sciences, Babol

2. Non-Communicable Pediatrics Diseases Research center, Health research Institute, Babol University of Medical Sciences, Babol, Iran

3. Social Determinants of Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

4. Clinical Research Development Unit of Amirkola Children's Hospital, Babol University of Medical Sciences, Babol, IR Iran

5. Student of Research Committee, School of Health Management & Information Science, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding author: Dr. Morteza Alijanpour, Non-Communicable Pediatrics Diseases Research center, Health research Institute, Babol University of Medical Sciences, Babol, Iran. Email: m.alijanpour@yahoo.com. ORCID ID: 0000-0002-0031-4718.

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Abstract

Background: This study aimed to determine the volumetric parameters of platelets including MPV (Mean Platelet Volume) and PDW (Platelet Distribution Width) in diabetic children with and without diabetic ketoacidosis (DKA) and to evaluate their relationship with the severity of DKA.

Materials and Methods: A case-control study was conducted on 112 children with type 1 diabetes mellitus (T1DM), with and without DKA, who referred to Amirkola Children's Hospital, Iran, in 2016-2021. Of them, 43 were boys, and 69 were girls. The diagnosis of diabetes was based on clinical symptoms and diagnostic criteria, and DKA was diagnosed through the analysis of arterial blood gas. The patients' MPV and PDW were also evaluated, and the P values less than 0.05 were considered significant.

Results: Out of 112 children with diabetes (with the mean age of 8.71 ± 3.22 years), 56 persons had DKA. In terms of DKA severity, 17 patients (30.4%) had mild, 29 (51.8%) had moderate, and 10 (17.8%) had severe DKA. The children with DKA had the mean blood glucose of 490.98±46.11mg/dL, MPV of 10.02± 1.19fl), and PDW of 13.07± 1.18flwere significantly higher (P<0.001). It was found that, an increase in the severity of DKA would raise the mean MPV (P = 0.006) and PDW(P<0.001) significantly.

Conclusion: Based on the results of this study, as the severity of DKA increases in diabetic children, the mean levels of MPV and PDW increase significantly. Therefore, it is suggested that MPV and PDW be used to predict the severity of DKA in diabetic children.

Keywords: Diabetes mellitus type 1, Diabetic ketoacidosis, Mean platelet volume

Introduction

Diabetes mellitus refers to hyperglycemia and glucosuria, which are diabetes of types of 1 and 2, respectively. Type 1 diabetes mellitus (T1DM) is associated with the autoimmune destruction of pancreatic beta cells and, as a result, insulin deficiency. It is also the most common type of diabetes in children. Type 2 diabetes mellitus occurs due to insulin resistance and the relative deficiency of insulin. In some cases, the cause of diabetes can be due to some drugs such as chemotherapy drugs (1-4). The incidence of T1DM at the age of less than 14 years in different countries varies significantly from 0.1 to 36.8 per 100,000 people per year (3). T1DM includes approximately 10% of all diabetic patients, and its incidence is increasing in the world. The annual incidence of T1DM worldwide, affecting children under the age of 14, ranges from 1 to 35 cases per hundred thousand people. In Iran, it is estimated to be 3.7 cases per hundred thousand people, providing a comparative perspective on the specific incidence rates in different countries (5). Diabetes has short-term and long-term complications (6-9). The long-term complications of T1DM include cardiovascular diseases, retinopathy, nephropathy, and peripheral neuropathy, common short-term and the most complication is hypoglycemia (6). Another acute complication of this disease is diabetic ketoacidosis (DKA), which occurs in diabetic patients who have not yet been diagnosed and are not receiving any treatment or in patients with T1DM who do not receive enough insulin. It has been found that 20-40% of children with T1DM are hospitalized for the first time with DKA, although this frequency varies in different studies (7, 8). DKA manifests itself with symptoms such as nausea, vomiting, abdominal pain, drowsiness, and coma, and even death is possible if the disease is not treated properly (9). The conducted studies indicate that the volumetric parameters of platelets can be used as a marker for infectious diseases (10). One of the factors that leads to the activation of platelets is chronic hyperglycemia (11). The activation of platelets often leads to changes in their shape and swelling, as the mean platelet volume (MPV) and platelet distribution width (PDW) increase (11). In a study, the mean values of MPV and PDW in diabetic people with DKA were significantly higher than those in diabetic people without DKA and healthy people (12). However, in another study, the people with DKA and the diabetic ones without DKA were not significantly different in terms of mean MPV and PDW (13). Considering the conflicting results in the existing research and the fact that very few studies

have investigated the volumetric parameters of platelets in clinical conditions, this study was conducted with a unique focus on the morphological parameters of platelets, including MPV and PDW, in diabetic children with and without DKA. The studied cases referred to Amirkola Children's Hospital from 2016 to 2021.

Materials and Methods Study design and participants

This case-control study was conducted on 112 T1DM child patients with and without DKA who had referred to Amirkola Children's Hospital, Iran, from 2016 to 2021. The inclusion criteria were the consent to participate in the study, 2 to 18 years of age, type 1 diabetes with DKA in the case group, and type 1 diabetes without DKA in the control group. The exclusion criteria included coagulation disorders, known cardiovascular diseases, trauma, blood diseases. liver diseases. and receiving anticoagulants or steroids.

Sample size

The sample size was estimated according to a previous study (14). Considering the means and standard deviations of the MPV values in the children with and without DKA (11.00 \pm 0.8 and 10.6 \pm 0.7, respectively), the power of 80%, the error level of 0.05, and the group ratio of 1 to 1 were found for 112 people. The studied people were selected through convenient sampling.

Data collection

The children diagnosed with TIDM were included if they met the inclusion criteria. The diagnosis of diabetes was performed based on clinical symptoms and the corresponding criteria (Table I) (15). The study design involved a retrospective analysis of the medical records of the children diagnosed with diabetes with and without DKA.

Examination of chemical parameters

The children's complete blood count was used to measure their (CBC) volumetric platelet parameters (MPV and PDW). For this purpose, 2 ml of venous blood was mixed with anticoagulant ethylenediaminetetraacetic acid (EDTA), and the sample was evaluated on the very day of sampling (up to 4 hours after sampling). The tests were performed with the Seismix instrument (KX), a state-ofthe-art device designed for accurate and efficient blood analysis, at the laboratory unit of Amirkola Children's Hospital. No additional sampling was done. The mean value of MPV was 7-12, and PDW was considered in the range of 9-14 femtoliters. In this study, the children's data were extracted and analyzed. The data covered demographic features (age, sex), laboratory parameters, MPV, PDW, existence of DKA, severity of DKA, pH value and HCO3 quantity in ABG, clinical status, clinical outcome (discharge or death), and duration of hospitalization.

Data analysis

The data were statistically analyzed using the SPSS version 26 software. The descriptive results of the analysis were presented in terms of mean ± standard deviation (SD), frequencies and percentages. Independent t-tests and analysis of variance (ANOVA) were used to compare the groups in terms of the quantitative variables. Also, Chi-square tests served to check the relationships qualitative variables. among the Furthermore, the relationships of PDW, MPV, and the other factors with the severity of ketoacidosis were investigated through multinomial logistic regression. In this model, the severity of ketoacidosis (normal ABG in the control group as well as mild, moderate, and severe degrees) was considered as the dependent variable, and PDW, MPV, HCO3, and BS as covariates. Normal ABG was deemed to be the

reference category. A P-value less than 0.05 were considered significant.

Results

Among the studied 112 children with diabetes, 56 were diagnosed with DKA, while the other 56 had no DKA. Of them, 69 individuals (61.6%) were girls, and 43 (38.4%) were boys. The mean age of the children was 8.71 ± 3.22 years. Also, the mean age of the children with and without DKA was 8.43 ± 3.11 and 9.00 ± 3.33 years, respectively. Notably, the age difference between the two groups was statistically insignificant (P = 0.47). In terms of DKA severity, 17 patients (30.4%) had mild, 29 (51.8%) had moderate, and 10 (17.8%) had severe DKA. The mean blood glucose of the children with DKA was 490.98 ± 46.11 mg/dL; for the children without DKA, it was $274.07 \pm 60.88 \text{ mg/dL}$. This was significantly higher in the DKA patients (P < 0.001) (Table III). The mean MPV and PDW in the children without DKA were 9.44 ± 0.94 and 11.69 ± 1.29 femtoliters, respectively. In the children with DKA, those mean values were 10.59 ± 1.15 and 13.07 ± 1.18 femtoliters, respectively. They were significantly higher in the patients with DKA (P < 0.001). The mean MPV and PDW in the diabetic girls with DKA were considerably higher than those in the girls without DKA (P < 0.001). In boys, however, this difference was not statistically significant (P = 0.05 and 0.06, respectively). As also observed, with the increase in the severity of DKA, the mean MPV (P = 0.006) and PDW (P < 0.001) increased significantly (Table IV). The average duration of hospitalization was 1.20 ± 0.93 days for the children without DKA and 3.89 ± 1.04 days for the children with DKA. It was significantly higher for those with DKA (P < 0.001). As also found, with the increase of MPV and PDW, the duration of hospitalization increased significantly (P < 0.001). The

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mean levels of bicarbonate and pH in the children without DKA were 24.34 ± 3.55 and 7.41 ± 0.02 mmol/litre, respectively. In the children with DKA, those mean values were 11.35 ± 4.66 and 7.21 ± 0.07 mmol/litre, respectively; they were significantly lower in the patients with DKA (P < 0.001). The results of the multinomial logistic regression indicated that PDW is a risk factor for all severity levels of ketoacidosis. The odds ratios

were 1.47, 3.28, and 3.42 for mild, moderate. and severe ketoacidosis, respectively. This means that, with each unit of increase in PDW, the chance of mild ketoacidosis increases by 47% compared normal children. to Additionally, with each unit of increase in PDW, the chance of moderate and severe ketoacidosis is three times higher than that in normal children (Table V).

Table I: Diagnostic	criteria for	· dysglycemia	and diabetes	mellitus

Dysglycemia	Diabetes mellitus
Impaired fasting glucose: Fasting (at least 8 hr) plasma glucose 100-125 mg/dl (5.6- 7.0 mmol/l)	Fasting (at least 8 hr) plasma glucose \geq 126 mg/dl (7.0 mmol) or
Impaired fasting tolerance: 2-hr plasma glucose during OGTT ≥ 140 mg/dl (7.8 mmol), but 200 mg/dl (11.1 mmol)	2-hr plasma glucose during OGTT $\ge 200 \text{ mg/dl} (11.1 \text{ mmol})$ or
Prediabetes: Hemoglobin A1c 5.7-6.4% (39-47 mmol/mol)	$\begin{array}{l} Hemoglobin \ A1c \geq 6.5\% \ (48 \ mmol/mol) \\ or \\ Symptoms \ of \ diabetes \ mellitus \ plus \ random \ or \ plasma \ glucose \geq \\ 200 \ mg/dl \ (11.1 \ mmol/l) \end{array}$

	Normal	Mild	Moderate	Severe
CO2 (mEq/l, venous)	20-28	16-20	10-15	<10
PH (venous)	7.35-7.45	7.25-7.7.35	7.15-7.25	<7.15
Clinical	No change	Oriented, alert but fatigued	Kussmaul respiration; oriented but sleepy; arousable	Kussmaul or depressed respiration; sleepy to depressed sensorium to coma

Table Π :Classification of diabetic ketoacidosis

		a the other parameters	
Variable	With DKA Mean ± SD	Without DKA Mean ± SD	P-value ^a
BS* (mg/dL)	490.98 ± 46.11	274.07 ± 60.88	< 0.001
HCO3**(mEq/l,)	11.35 ± 4.66	24.34 ± 3.55	< 0.001
MPV*** (fl)	10.59 ± 1.15	9.44 ± 0.94	< 0.001
PDW****(fl)	13.07 ± 1.18	11.69 ± 1.29	< 0.001
рН	7.21 ± 0.07	7.41 ± 0.02	< 0.001
Hospitalization duration (in days)	3.89 ± 1.04	1.00 ± 1.00	< 0.001

 Table III: Comparison of the diabetic children with and without diabetic ketoacidosis in terms of MPV, PDW and the other parameters

* Blood sugar

** Bicarbonate

*** Mean Platelet Volume

**** Platelet Distribution Width

a: Using independent t-test

Table IV: Comparison of the diabetic children at different DKD levels in terms of MPV, PDW and the other parameters

Variable	Mild of DKA Moderate of DKA Sever of DKA Mean ± SD Mean ± SD Mean ± SD		Sever of DKA Mean ± SD	P-value ^a	
BS* (mg/dL)	476.05 ± 24.59	483.00 ± 31.14	539.50 ± 75.22	0.007	
HCO3** (mEq/l)	13.71 ± 3.85	11.36 ± 4.85	7.35 ± 2.16	< 0.001	
MPV***(fl)	9.90 ± 1.03	10.87 ± 1.07	10.97 ± 1.12	0.006	
PDW****(fl)	12.21 ± 1.05	13.43 ± 0.79	13.49 ± 1.62	< 0.001	
рН	7.29 ± 0.02	7.20 ± 0.02	7.10 ± 0.03	< 0.001	
Hospitalization duration (in days)	3.12 ± 0.60	3.83 ± 0.66	5.40 ± 0.97	< 0.001	

* Blood sugar

** Bicarbonate

*** Mean Platelet Volume

**** Platelet Distribution Width

a: using ANOVA test

Table V: Evaluation of the risk factors of ketoacidosis severity using multinomial logistic regression

	Mild DKA		Moder	Moderate DKA			Severe DKA		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
PDW	1.47	(0.92, 2.34)	0.105	3.28	(2.04, 5.30)	< 0.001	3.42	(1.81, 6.44)	< 0.001
MPV	1.68	(0.94, 2.99)	0.075	3.90	(2.22, 6.86)	< 0.001	4.23	(3.04, 8.81)	< 0.001
HCO3	0.69	(0.60, 0.81)	< 0.001	0.62	(0.52, 0.74)	< 0.001	0.25	(0.14, 0.45)	< 0.001
BS	6.25	(6.09, 6.42)	< 0.001	6.29	(6.15, 6.47)	< 0.001	6.48	(6.38, 6.65)	< 0.001

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Discussion

This study. conducted Amirkola at Children's Hospital, aimed to compare the volumetric characteristics of platelets, including MPV and PDW, in diabetic children with and without DKA. Based on the results of the study, the mean values of MPV and PDW were significantly higher in the diabetic children with DKA than in those without DKA. As the severity of DKA rose in these patients, the mean MPV and PDW increased significantly. Also, there was a direct and significant correlation between these parameters and the duration of hospitalization. Since the studies on children are limited in this area, the present article cites some studies on adults. According to the findings of the study, the mean MPV and PDW were significantly higher in the diabetic children with DKA than in those without DKA. which is in line with the studies of Mousa et al. (14), Gang Ma et al. (12), Dwivedi et al. (16), and Malachowska et al. (17). In the study by Mousa et al. (14) conducted on 90 Egyptian children and the study by Gang Ma et al. (12) conducted on diabetic adults, the mean MPV and PDW in subjects DKA diabetic with were significantly higher than the mean values in diabetic subjects without DKA and healthy people. In their study conducted in India, Dwivedi's team found the mean MPV and PDW of 210 adult patients with diabetic complications significantly higher than those in diabetic patients without complications (16). This result was confirmed by Malachowska et al. (17) who studied 389 diabetic patients and 389 healthy children. The increase of MPV and PDW in diabetics with DKA is due to the release and activity of cytokines during different inflammations (18). These cytokines increase the volume of produce megakaryocytes and more platelets from megakaryocytes in the bone marrow (19). Also, the lack of proper

control of blood sugar in diabetic patients causes protein glycation and oxidative which ultimately causes stress. the activation of platelets and their production in different forms (20). These factors change the amount of MPV and PDW in diabetes and its complications, such as DKA. The results of this study, however, were not in line with the studies of Putradi et al. (13), Korkmaz et al. (21) and Baghersalimi et al. (22). In the studies by Putradi's team on 71 adult diabetic patients (21 patients with DKA and 50 persons without DKA), Korkmaz's team on 160 children (80 children with type I diabetes and 80 healthy children) and Baghersalimi's team on 166 children (83 diabetic children and 83 healthy children), the two groups had no statistically significant difference in terms of mean MPV and PDW values. The differences between the present study and the other studies are probably because MPV and PDW were investigated in people with diabetes (type I or II). Also, most studies used a control group, including healthy people, for comparison, while the present study was conducted only on children with diabetes mellitus. In the present study, with a rise in the severity of DKA, the **PDW** MPV and increased mean significantly, which was in line with the studies of Baghersalimi et al. (22) and Dwivedi et al. (16). In those two studies, PDW the mean MPV and were significantly higher in the patients with higher HbA1C compared to the patients with lower HbA1C. In contrast, Korkmaz et al. (21) and Malachowska et al. (17) found no significant linear relationship of MPV and PDW with HbA1C. In this study, MPV and PDW were directly and significantly correlated to the duration of hospitalization. So, with the increase of MPV and PDW, the duration of hospitalization also increased. Increased inflammation in the body leads to an

increase in the length of hospitalization. Indeed, due to the rise of inflammation. more cytokines are released and, as a result, the number, size and function of platelets are changed (20). To the best of our knowledge, there is no study on the relationship of MPV and PDW with hospitalization duration. As for the limitations of this study, it is a crosssectional study, and it lacks the examination of factors such as platocrit count and the ratio of platelets to lymphocytes. Therefore, it is suggested for future researchers to examine these factors as well as ABG in patients at the time of discharge and after it. It will also be of benefit to check whether platelet volume parameters (MPV and PDW) improve with the improvement of the patient's general conditions.

Conclusion

As the study showed, the mean MPV and PDW in diabetic children with DKA are higher than in diabetic children without DKA, and their levels rise with an increase in the severity of DKA. Furthermore, MPV and PDW were found directly and significantly correlated to the duration of hospitalization. These findings suggest that MPV and PDW can be used as biomarkers to predict the severity of DKA in diabetic children. potentially enabling earlier intervention and improving patient outcomes. This study was not without limitations. Of them, one may refer to the negligence of examining factors such as platocrit count and the ratio of platelets to lymphocytes. It is, thus, suggested for the future research to check these factors as well as ABG in patients at the time of discharge and after it. It is also advisable to check weather platelets increase with the improvement of the patient's general conditions.

Ethical considerations

This study was approved by the Ethic Committee of Babol University of Medical Sciences (IR.MUBABOL.REC.1401.080).

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Authors' contributions

F.M, M.A, H.M and H.SH Contributed to the design and implementation of the research, H.SH contributed to the analysis of the results and P.A to the writing of the manuscript. M.P supervised the findings of this work and planned the experiments. All the authors provided critical feedbacks and helped to shape the research, the analyses involved, and the manuscript.

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Conflict of interest

There is no conflict of interests to declare.

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