ISSN: 2536-9474 (Print) Original article / FYMJ
ISSN: 2536-9482 (Online) **Fayoum University Medical Journal** Mahmoud et al., 2022,10(2), 47-56

Evaluation Of Hematological Indices In Pediatric Patients With Type 1 Diabetes Mellitus

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Abstract

BackGround and Objective: This study was done to evaluate different hematological indices alterations as an inflammatory marker in T1DM children and children with DKA, potentially predisposing patients with diabetes to complications, and to assess the effect of duration of disease and glycemic control on different hematological indices.

Patients and methods: The study was conducted in outpatient clinic and outpatient Endocrinology Clinic of pediatric department jointly with PICU at Fayoum University Hospital, it included 120 children who were classified into three groups: **Study Group:** was divided in 2 groups **Group I:** 40 children with DKA, **Group II:** 40 children with T1DM. **Control group (Group III):**40 apparently healthy children, age and sex matched to the study group.

Results: Mean platelet volume **(MPV)**, platelet distribution width **(PDW)** and platelet-large cell ratio **(P-LCR)** were significantly higher among diabetic and DKA group in comparison with the control group. White blood cells count **(WBCs)** and neutrophil **(NEUT)** were statistically significantly higher in DKA group compared to T1D and control. Red blood cells **(RBCs)**, hemoglobin **(HGB)** and hematocrit **(HCT)** were statistically significantly higher in DKA and T1D group as compared to the control group.

Conclusion: The study revealed that hematological parameters changed as a result of DM and DKA. Platelets of pediatric patients with T1DM and DKA show morphological evidence of hyperreactivity (higher MPV, PDW and P-LCR). High levels of PDW, and WBCs count increased the risk of DKA incidence.

Keywords
Children, haematological parameters, type-1 diabetes, diabetic ketoacidosis.

Introduction:

Type 1 diabetes (T1D) is one of the most common endocrine and metabolic conditions occurring in childhood (1). Diabetic ketoacidosis (DKA) is an acute, major, life-threatening complication of diabetes that

mainly occurs in T1D (2). Higher risk of cardiovascular complications and recurrent ischemic events are reported in diabetic patients (3). The majority of these ischemic events occur due to thrombosis and its related complications (4). There are various mechanisms for increasing atherothrombotic risk, including platelet hyperactivity, endothelial dysfunction, fibrinolysis, and abnormalities in coagulation (3). There is PLT hyperreactivity in patients with diabetes. Because of PLT prothrombinase activity, this hyperreactivity increases PLT size or PLT hyperfunctionality (5). Also Leukocyte count is an inflammatory marker and is communicated with endothelial dysfunction, insulin resistance, incomplete glucose metabolism, and loss of function of beta cells in diabetic patients. Leukocytes are hyperactive in T1DM and its interaction with PLTs may lead to PLT hyperactivity and microvascular complications (5, 6)**.** Similarly it has been shown that hyperglycemia has multiple effects on the RBC (7). The effects of hyperglycemia include glycation of hemoglobin, reduced deformability of RBCs (8, 9), and reduced RBCs lifespan (10).Patients with diabetes mellitus show a significant derangement in various hematological parameters.

Patients and methods:

A case control study was conducted in outpatient clinic and outpatient Endocrinology Clinic of pediatric department jointly with PICU at Fayoum University Hospital.The study included 120 children who were classified into three groups:

Study Group: was divided in 2 groups

Group I: 40 children who present with diabetic ketoacidosis (DKA) and admitted at PICU.

Group II: 40 children with type 1 diabetes mellitus (DM) who follow up in the pediatric endocrinology outpatient clinic.

Control group (Group III):

40 apparently healthy children, age and sex matched to the study group who came for routine health visit in the outpatient clinic of pediatric department.

Inclusion criteria:

1- Children with T1DM treated with insulin and children with DKA.

2- Both sexes.

3- Age lower than 18 years.

4- Duration of diabetes longer than 6 months.

Exclusion criteria:

Subjects who had any of the following conditions were excluded from the study:

1- Children with PLTs count >450000 or <150000.

2- Inherited or acquired diseases which affect PLT count and function.

3- Children received nonsteroidal anti-inflammatory drugs, lipid reducing drugs, aspirin, heparin, chemotherapy, received immunosuppressive therapy.

4- Ongoing infectious disease, C-reactive protein >5mg/L.

5- Abnormal white blood cell (WBC) for age.patient with a known neuromuscular disorder.

Methods:

All patients will be subjected to the following:

1) Full History taking and general examination including :

- Personal history (age, gender).
- Onset and duration of DM.

2) Laboratory investigations, including:

- Complete blood picture using an automated hematology system (Sysmex -XS 800 i; Sysmex Corporation, Japan).
- C-reactive protein (CRP) using latex agglutination (CRP-Latex Cromatest, Mexico).
- HbA1c.

- **Ethical consideration:**

  This study was reviewed by the Faculty of Medicine Research Ethical Committee. The researcher was informed the participants about the objectives of the study, the examination, investigation that will be done. Also the confidentiality of their information and their right not to participate in the study.

- **Statistical methods:**

  Data management was performed using the Statistical Package for Social Sciences (version 15.0; SPSS Inc., Chicago, IL, USA). Compute standard descriptive statistics (e.g., mean, standard deviation) were be used to summarize the data. Nominal data was be analyzed using simple X2 test, while independent sample T-test procedure was be used to compare means for two groups of cases; for more than two groups, data was be evaluated with one-way analysis of variance (ANOVA). A probability value (P value) less than 0.05 was considered significant.

Results:

Platelet parameters:

As shown in table (1):

MPV was statistically significantly higher in DKA group (10.63 ± 1.03, p < 0.0001) and T1D (10.01 ± 0.68, p=0.014) as compared to the control (9.48 ± 0.72). Also, it was higher in DKA group than in T1D (10.63 ± 1.03 vs. 10.01 ± 0.68), with statistically significant difference (p = 0.003).

PDW was statistically significantly higher in DKA group (12.48 ± 2.45, p < 0.0001) and T1D (10.87 ± 1.2, p=0.006) as compared to the control (9.66 ± 1.22). Also, it was higher in DKA group than in T1D (12.48 ± 2.45 vs. 10.87 ± 1.2), with statistically significant difference (p < 0.0001). P-LCR was statistically significantly higher in DKA group (29.99 ± 8.5, p < 0.0001) and T1D (24.35 ± 4.82, p=0.007) as compared to the control (19.8 ± 5.76). Also, it was higher in DKA group than in T1D (29.99 ± 8.5 vs. 24.35 ± 4.82), with statistically significant difference (p = 0.001).

PCT was statistically significantly higher in DKA group (0.37 ± 0.1) compared to T1D (0.33 ± 0.07, p=0.036) and control (0.33 ± 0.07, p=0.036). However, there was no statistically significant difference between T1D and control (p = 0.949). There was no statistically significant difference between the groups in platelet count (p = 0.661).

Table (1):

	DKA group (N=40)		T1D (N=40)		Control (N=40)		P-value#
	Mean	SD	Mean	Mean	SD	Mean	
PLT	344.3	86.62	329.75	68.75	340.58	65.4	0.661
MPV	10.63	1.03	10.01	0.68	9.48	0.72	0.003a* 0.014b* <0.0001c*
PDW	12.48	2.45	10.87	1.2	9.66	1.22	<0.0001a* 0.006b* <0.0001c*
P-LCR	29.99	8.5	24.35	4.82	19.8	5.76	0.001a* 0.007b* <0.0001c*
PCT	0.37	0.1	0.33	0.07	0.33	0.07	0.036a* 0.949b 0.036c*

#One-Way ANOVA *Significant

a DKA vs. T1D b T1D vs. Control c DKA vs. Control

(**PLT:** platelet count; **MPV:** Mean Platelet Volume; **PDW:** Platelet Distribution Width; **P-LCR:** Platelet Large Cell Ratio; **PCT:** Plateletcrit).

WBCs parameters:

As shown in table (2):

WBCs count was statistically significantly higher in DKA group (13.66 ± 7.9) compared to T1D (6.37 ± 1.75, p<0.0001) and control (7.05 ± 1.58, p<0.0001).

NEUT was statistically significantly higher in DKA group (9.29 ± 6.69) compared to T1D (2.84 ± 1.36, p<0.0001) and control (2.83 ± 1.24, p<0.0001).

NLR was statistically significantly higher in DKA group (3.54 ± 2.82) compared to T1D (1.13 ± 0.76, p<0.0001) and control (1.07 ± 0.87, p<0.0001). However, there was no statistically significant difference between T1D and control (p = 0.985).

Table (2):

	DKA group (N=40)		T1D (N=40)		Control (N=40)		P-value#
	Mean	SD	Mean	Mean	SD	Mean	
WBCs	13.66	7.9	6.37	1.75	7.05	1.58	<0.0001a* 0.799b <0.0001c*
LYMPH	3.23	1.73	2.78	0.88	3.23	1.02	0.189
NEUT	9.29	6.69	2.84	1.36	2.83	1.24	<0.0001a* 1.000b <0.0001c*
NLR	3.54	2.82	1.13	0.76	1.07	0.87	<0.0001a* 0.985b* <0.0001c*
PLR	130.51	65.06	125.59	31.39	119.48	54.29	0.639

#One-Way ANOVA *Significant

a DKA vs. T1D b T1D vs. Control c DKA vs. Control

(**WBCs**, white blood cells; **LYMPH**, lymphocyte; **NEUT**, neutrophil; **NLR**, neutrophil to lymphocyte ratio; **PLR**, platelet to lymphocyte ratio).

RBCs parameters:

As showen in table (3):

RBCs count was statistically significantly higher in DKA group (5 ± 0.56, $p < 0.0001$) and T1D (4.94 ± 0.41, p=0.001) as compared to the control (4.54 ± 0.46).

HGB was statistically significantly higher in DKA group (12.36 ± 1.52, $p < 0.012$) and T1D (12.54 ± 1.03, p=0.002) as compared to the control (11.56 ± 1.09).

HCT was statistically significantly higher in DKA group (35.48 ± 3.79, $p = 0.002$) and T1D (35.97 ± 2.41, p<0.0001) as compared to the control (4.54 ± 0.46).

There was no statistically significant difference between the groups in terms of the MCV, MCH, MCHC, RDW%, RDW/MCV (p = 0.224, p =0.395, p = 0.985, p =0.107, p = 0.152 respectively).

Table (3):

	DKA group (N=40)		T1D (N=40)		Control (N=40)		P-value#
	Mean	SD	Mean	Mean	SD	Mean	
RBCs	5	0.56	4.94	0.41	4.54	0.46	0.824a 0.001b* <0.0001c*
HGB	12.36	1.52	12.54	1.03	11.56	1.09	0.796a 0.002b* 0.012c*
HCT	35.48	3.79	35.97	2.41	33.17	2.61	0.741a <0.0001b* 0.002c*
MCV	71.27	6.75	73.11	5.18	73.53	7.05	0.244
MCH	24.83	2.86	25.45	2.29	25.59	2.74	0.395
MCHC	34.81	1.83	34.84	1.4	34.78	1.1	0.985
RDW%	14.51	2.46	13.96	1.78	13.6	1.4	0.107
RDW /MCV	0.002	0.0005	0.0019	0.0004	0.0018	0.0004	0.152

#One-Way ANOVA *Significant

a DKA vs. T1D b T1D vs. Control c DKA vs. Control

(**RBCs**, red blood cells; **HGB,** hemoglobin; **HCT,** hematocrit; **MCV**, mean corpuscular volume; **MCHC**, mean corpuscular hemoglobin concentration; **RDW**, red cell distribution width; **RDW/MCV**, red blood cell distribution width/mean corpuscular volume).

Discussion:

In this study, we selected a group of patients with diabetes, a group of DKA and a group of nondiabetic healthy persons to compare hematological parameters between them. This study was designed to evaluate different hematological indices alterations as an inflammatory marker in diabetic children and children with DKA, potentially predisposing patients with diabetes to complications, and to assess the effect of duration of disease and glycemic control on different hematological indices.

DM is considered as a prothrombotic state characterized by increased platelet activation and coagulation and decreased fibrinolytic activity. In diabetes, there is an alteration in all the systems that maintain the integrity and patency of the blood vessels, and a vicious circle of events is initiated in the vascular wall which includes platelet hyperactivity and dysfunction, increased inflammation, altered coagulation, and endothelial dysfunction (11).The alteration of platelets in diabetes is an increase in its size and hyperactivity, These altered platelet function and activity cause thrombus formation (12).

Regarding platelets parameters alteration in the current study, we found that MPV, PDW, and P-LCR were elevated in patients with diabetes than nondiabetic individuals which are in accordance with Jindal et al (13), Malachowska et al (14), Venkatesh et al (15), Khudhur and Al-Ani (11).

In addition, in the current study, we found that MPV, PDW, PCT and P-LCR were higher in DKA group than in T1D with a statistically significant difference. This is in agreement with Ma et al (16), Erdoğan et al (17), Mousa et al (18), who observed that MPV, PDW were significantly higher in the DKA group than the diabetic and control group. This can be attributed to the cytokines released during inflammatory conditions, like DKA. These cytokines influence the increase in megakaryocyte volume; that allows megakaryocyte to produce several thousand platelets per cell. Platelets produced are activated and are larger and more reactive, with wide variations in their size (18). In the present study, we reported that platelets of pediatric patients with T1DM and DKA show morphological evidence of hyperreactivity (higher MPV, PDW and P-LCR).

Hyperglycemia has effects on erythrocytes. It decreases deformability and changes the mechanical characteristics of erythrocytes while increasing osmotic fragility and adhesion, causing changes in the structure and hemodynamic characteristics of erythrocytes, and effectively decreasing lifespan (17).

Regarding erythrocyte parameters alteration in the current study, we found that RBCs count, HGB and HCT were statistically significantly higher in DKA group and T1D as compared to the control. Elevation these parameters in DKA group can be attributed due to dehydration in children with DKA.

In the current study, we reported that there was no a statistically significant difference between the groups in terms of the MCV,

MCH, MCHC, RDW%, RDW/MCV (p = 0.224, p =0.395, p = 0.985, p =0.107, p = 0.152 respectively).But there was a statistically positive correlation between disease duration and MCV (r= 0.409, p=0.009). This is in agreement with Erdoğan et al (17), who reported that there was no statistically significant difference between the groups in terms of RDW, MCV, and RDW/MCV levels (p>0.05) .

Leukocyte count is an inflammatory marker and is communicated with endothelial dysfunction, insulin resistance, incomplete glucose metabolism, and loss of function of beta cells in diabetic patients. Leukocytes are hyperactive in T1DM and its interaction with PLTs may lead to PLT hyperactivity and microvascular complications (5).

Regarding leucocytes parameters alteration in the current study, we found that WBCs count was a statistically significantly higher in DKA group when compared to T1D and control. This finding is consistent with the results mentioned by Karavanaky et al (19), Xu et al (20), Małachowska et al (21).

Also, the results of studies showed a strong direct relationship between blood pH and level of WBC; i.e. the severity of blood acidity increases with the amount of leukocytes (22). However, in the current study there was no a statistically significant difference between T1D and control (p = 0.799) in term of WBCs count.

In the present study we reported that NEUT was a statistically significantly higher in DKA group when compared to T1D and control which is in accordance with Xu et al (20). In the current study, we found that NLR was a statistically significantly higher in DKA group when compared to T1D and control.

In the present study, we analyzed the effects of different hematological parameters for predicting DKA by using multiple stepwise logistic regression analysis, we found that there was statistically positive correlation just between WBCs and PDW for predicting DKA. We reported that each one percent increase in PDW increased the odds of DKA by 2.453 (95% CI 1.514, 3.976) which is in accordance with Ma et al (16), who reported that a high PDW could pose a risk factor for the presence of DKA. Also this is in agreement with Erdoğan et al (17), who determined that the risk of DKA incidence increased with a high level of PDW. They found that the risk of DKA incidence increased by 3.238 with a high level of PDW.

In addition, in the present study we reported that each one percent increase in WBCs increased the odds of DKA by 2.297. Wasif et al, examined predictors of infection in DKA patients and found leukocytosis is a more accurate predicator for DKA severity rather than infection (23). Also Alamri et al (24), reported that the WBC differential (namely, monocytes, immature granulocytes, basophils, & lymphocytes) is associated with the severity of DKA.

Conclusion:

We concluded that hematological parameters changed as a result of DM and DKA. Especially MPV, PDW, P-LCR were highly elevated and HGB , HCT and RBCs count were slightly elevated in the DKA and diabetic groups as compared with nondiabetic individuals. We found

that platelets of pediatric patients with T1DM and DKA show morphological evidence of hyperreactivity (higher MPV, PDW and P-LCR). We reported that high levels of PDW, and WBCs count increased the risk of DKA incidence.

References:

1) Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark A.(2017). Type 1 diabetes mellitus. Nat. Rev. Dis. Primers 3, 17016.

2) Andrade-Castellanos, C. A., Colunga-Lozano, L. E., Delgado-Figueroa, N., & Gonzalez-Padilla, D. A. (2016). Subcutaneous rapid-acting insulin analogues for diabetic ketoacidosis. Cochrane Database of Systematic Reviews, (1).

3) Szablewski L, Sulima A. (2017). The structural and functional changes of blood cells and molecular components in diabetes mellitus.Biol Chem; 398:411–423.

4) Kakouros N, Rade JJ, Kourliouros A, Resar JR. (2011). Platelet function in patients with diabetes mellitus: from a theoretical to a practical perspective. Int J Endocrinol; 2011, e742719.

5) Baghersalimi A, Koohmanaee S, Darbandi B, Farzamfard V, Hassanzadeh Rad A, Zare R, Tabrizi M, Dalili S. (2019). Platelet Indices Alterations in Children With Type 1 Diabetes Mellitus. J Pediatr Hematol Oncol; 41(4): 227-232. Doi: 10.1097/MPH.0000000000001454.

6) Hu H, Li N, Yngen M, et al. (2004). Enhanced leukocyte-platelet crosstalk in type 1 diabetes mellitus: relationship to microangiopathy. J Thromb Haemost; 2:58–64.

7) Simmons D. (2010). Increased red cell count in diabetes and pre-diabetes. Diabetes Res Clin Pract; 90: e50–e53.

8) Singh DK, Winocour P, Farrington K. (2009). Erythropoietic stress and anemia in diabetes mellitus. Nat Rev Endocrinol; 5: 204–210.

9) Singh M, Shin S. (2009). Changes in erythrocyte aggregation and deformability in diabetes mellitus: a brief review. Indian J Exp Biol; 47: 7–15.

10) Virtue MA, Furne JK, Nuttall FQ, Levitt MD. (2004). Relationship between GHb concentration and erythrocyte survival determined from breath carbon monoxide concentration. Diabetes Care; 27: 931–935.

11) Khudhur KH, Al-Ani MH. (2019). Hematological parameters in children with type-1 diabetes. Med J Babylon; 16:184-7.

12) Gaiz, A., Mosawy, S., Colson, N., & Singh, I. (2017). Thrombotic and cardiovascular risks in type two diabetes; Role of platelet hyperactivity. Biomedicine & pharmacotherapy, 94, 679-686.

13) Jindal S, Gupta S, Gupta R, et al. (2011). Platelet indices in diabetes mellitus: indicators of diabetic microvascular complications. Hematology; 16:86–89.

14) Malachowska, B., Tomasik, B., Szadkowska, A., BaranowskaJazwiecka, A., Wegner, O., Mlynarski, W., & Fendler, W. (2015). Altered Platelets' morphological parameters in children with type 1 diabetes – a case-control study. BMC Endocrine Disorders, 15(1). doi:10.1186/s12902-015-0011-8

15) Venkatesh, V., Kumar, R., Varma, D. K., Bhatia, P., Yadav, J., & Dayal, D. (2018). Changes in platelet morphology indices in relation to duration of disease and glycemic control in children with type 1 diabetes mellitus. Journal of Diabetes and Its Complications, 32(9), 833– 838. doi:10.1016/j.jdiacomp.2018.06.008

16) Ma SG, Yang LX, Qiu XQ. (2013). Assesment of the platelet parameters and serum butyrylcholinesterase activity in type

1 diabetes patients with ketoacidosis. Platelets; 24:544-8.

17) Erdoğan S, Dursun F, Kırmızıbekmez H, Güven Ş, Yıldırım ÜM. (2017). Evaluation of Erythrocyte and Thrombocyte Parameters in Pediatric Patients with Diabetes Mellitus. J Clin Anal Med; 8(2): 98-101.

18) Mousa SO, Sayed SZ, Moussa MM, et al. (2017). Assessment of platelets morphological changes and serum butyrylcholinesterase activity in children with diabetic ketoacidosis: a case control study. BMC Endocr Disord; 17:23.

19) Karavanaki K, Kakleas K, Georga S, Bartzeliotou A, Mavropoulos G, Tsouvalas M, et al. (2012). Plasma high sensitivity C-reactive protein and its relationship with cytokine levels in children with newly diagnosed type 1 diabetes and ketoacidosis. Clin Biochem; 45(16-17):1383-8.

20) Xu W, Wu HF, Ma SG, Bai F, Hu W, Jin Y, et al. (2013). Correlation between peripheral white blood cell counts and hyperglycemic emergencies. Int J Med Sci; 10(6):758-65.

21) Małachowska B, Michałek D, Koptas M, Pietras W, Młynarski W, Szadkowska A, Fendler W. (2020). Changes in hematological parameters during first days of diabetic ketoacidosis treatment in children with type 1 diabetes mellitus. Clinical Diabetology. 2020 Feb 10.

22) Firooz M, Moradi B, Hosseini SJ. (2019). Leukocytosis in Diabetic Ketoacidosis; whether the Time Has Come to Start Antibiotics? A Short Literature Review. Journal of Patient Safety and Quality Improvement; 7(1): 27-28. Doi:10.22038/PSJ.2019.35823.1195.

23) Hosseini, S. J., Firooz, M., & Moradi, B. (2019). Leukocytosis in Diabetic Ketoacidosis; Whether the Time Has Come to Start Antibiotics? A Short Literature Review. Journal of Patient Safety & Quality Improvement, 7(1), 27-28.

24) Alamri, B. N., Ferris, J., Matheson, K., & De Tugwell, B. (2020). MON-638 The WBC Differential in Relation to DKA Severity. Journal of the Endocrine Society, 4(Supplement_1). doi:10.1210/jendso/bvaa046.540.