Estimation of Complete Blood Count and Platelets Indices in Sudanese Patients with Malaria

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Abstract

Background: Malaria remains today one of the major health problem in the tropical and subtropical countries particularly Africa and Asia, with increased morbidity and mortality. Hematological changes, which are the most common complication, play a significant role in these serious complications. The hematological abnormalities that have been reported to consistently companion which comprise are anemia and thrombocytopenia.

Objectives: This study was conducted to determine complete blood count and platelets indices among Sudanese patients with malaria, to determine haemoglobin level, RBCs count in Sudanese patients with malaria, to determine WBCs count in Sudanese patients with malaria and to determine platelets count, mean platelets volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR) in Sudanese patients with malaria.

Methods: A cross-sectional descriptive study was conducted in Sudan among 95 individuals as patients and 50 as controls. Blood samples were collected from each individual into K_2EDTA containers.

Results: The results showed statistically significant difference in the mean hemoglobin and hematocrit level between malaria patient (Hb 13.2g/dl ± 2.1, HCT 39.4 % ± 5.6) and control (Hb14.2±1.8, HCT±41.3±4.6), *p*.value of Hb=0.005) (*p*. value of HCT=0.041), and the malaria patients are associated with normocytic normochromic anemia. It showed there was

significant differences in platelets count and platelets indices between malaria patient (platelets count 190.7 x 10^{9}/L ± 102, PDW13.5fl ± 2.4, P.LCR 26.8% ± 6.6) and control (platelets count 269.1 ± 63.7, PDW11.9 ± 1.7, P.LCR 23.7 ± 6.6), (p.value of platelets count=0.000, *p*.value of PDW=0.000, *p*.value of P.LCR=0.012). This study also showed difference in mean platelets count and platelets indices according to severity of infection was highly statistically significant according to ANOVA test (platelets count 190.7 x 10^{9}/L ±102, *p*.value=0.000) (PDW 13.5 fl ± 2.4, *p*.value=0.00) (P.LCR 26.8 %± 6.6, *p*.value= 0.012) but there was no statistical different in MPV (10.0 fl ± 1.2, *p*.value=0.296). Also there was no statistical different in WBCS count between malaria patients (6.3 x 10^{9}/L ± 2.1) and control (6.79±1.9), (*p*.value=0.163) and RBCS count between malaria patients (4.9x 10^{12}/L ± 0.7) and control (9.8 ± 0.6), (*p*.value=0.498). The study showed no statistical different in all parameter in comparison with age groups and gender.

Conclusion: This study concluded that all this hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complication.

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Introduction

Malaria remains the most serious and widespread protozoan infection of humans. Over 40% of the world's population is at risk of contracting malaria, which is endemic in 91 countries, mostly developing. Malaria was defined as a "typical blood disease" characterized by fever, anemia and splenomegaly [1].

It is currently considered a typical example of a hemolytic anemia in more recent hematology textbooks, due to an acquired extra-corpuscular cause. As parasites of the blood for the majority of their complex life cycle, they expectedly induce hematological alterations. The

hematological abnormalities that have been reported to invariably accompany infection with malaria include anemia, thrombocytopenia, splenomegaly, and mild-to-moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (DIC) [2].

There have also been reports of leucopenia and leucocytosis. Other hematological reactions to malaria that have been reported include neutropenia, eosinophilia, neutrophilia and monocytosis [3].

Some contraversies appear to exist; however, many of the studies on the hematological abnormalities have been conducted in endemic countries, some only in children and some only in severe malaria patients [4].

Relatively few studies have been done among non-immune or semi-immune travelers returning from endemic areas or patients returning from their endemic countries [3,5].

This work was carried out to determine complete blood count and platelet indices among Sudanese patients with malaria.

Materials and methods

Study design

Cross-sectional descriptive study aim to determine CBC and platelets indices among Sudanese patients with malaria from July 2014 to December 2014.

Study population

Individual infected with malaria parasite in Khartoum and Senga state.

Study area

Sample collected from several areas in Khartoum and Senga state.

Sample collection

2.5ml of venous blood collected using sterile disposable plastic needle with EDTA vacationer and aseptic standard non traumatic vein puncture technique and using immediately.

The blood films

Preparation of thin blood film

clean slide wiped immediately before use small drop of fresh anti coagulant blood placed in the center line of slide about 1-2 cm at an angle of 45c to slide and move back to make contact with the drop, the drop was spread quickly along the line of contact of spreader with slide . The film lifted to air dry [6].

Staining blood film

The slide filled with Lieshman's stain on the staining rack, after 3 minutes double volume of buffer added for 7 minutes and then washed with tape water and left to air dry [6].

Examination of blood film

Blood film is examined for cell size, shape, haemoglobin, distributed, leukocyte deferential, abnormality in staining properties, and inclusion bodies. The films were examined by X10 of eye lens for staining quality and X40 and X100 for blood cells differentiation [6].

Complete blood count (CBC)

CBC and blood film examination usually medicate wherever there is any abnormalities in blood cells, sysmex KX2IN (automated haemoglobin analyzer) is use.

Principle of sysmex

Principle of automated haematological analyzer system (sysmex):

The counting of cellular elements in blood is done with impedancemetery technique. Thus technique based on the modification of the impedance of a calibrate aperture soaked in an electrolyte and going through a constant course deliver by two small aperture on the wall is immersed into a breaker that contains particle suspend in a low concentration electrolyte two electrodes, one inside the aperture tube and one outside the aperture tube but inside the breaker, are place and a current path is provide by the electrolyte when an electrodes is then measure. The aperture creates what is called (a sensing zone) particles in low concentration. Suspended in the electrolyte can be count by passing them through the aperture. As a particle passes through the aperture a volume of electrolyte equivalent to the immersed volume of the particle is displaced from the sensing zone. This causes a short term change in the impedance across the aperture. This change can be measure as a voltage pulse or current pulse. The pulse height is proportional to the volume of sense particle. If constant particle density is assume, the pulse height is also proportional to the participle mass. This technology thus also called aperture technology. Using count and pulse height analyzer orcuits, the number of particle and volume oh each particle passing through sensing zone can be measure. If the volume of liquid passing through the aperture can be precisely control and measure, the concentration of the sample can be determined [6].

Procedure

The reagent needed was checked for expiry date before use. The power switch was turned and background check will be automatically performed and the vend (vend for analysis) will

appear. Sample number inputted by pressing simple number then number of sample was entered. The enter key was pressed. Sample was mixed sufficiently. The tube was sited to the sample probe, and in that condition the start switch was pressed. When the LCD screen display analyzing the tube was removed. After that the unit executes automatic analysis and the result was display in the LCD screen. The result was point out.

WBCs, RBCs and Platelets count

The counting of the cellular elements in blood sample was done with impedancemetery technique. This technique was based on the modification of the impedance of calibrated aperture soaked in an electrolyte and going through constant course delivered by two electrodes located on both side of the aperture. Avacuam applied on aside of aperture allows the cells passage, they oppose their physical volume to the coarse passage voltage impulse was registered at the electrode terminal. The height of the impulse is proportional to the cell volume.

Hemoglobin

Hemoglobin is intensely colored and this property has been utilized in the methods for estimation its concentration in the blood. Erythrocytes contain a mixture of hemoglobin, oxyhemoglobin, carboxyhemoglobin, methemoglobin and minor amount of other form of hemoglobin. To determine hemoglobin concentration in the peripheral blood, red cells were lysed and hemoglobin variants were converted to stable component cyan methemoglobin for quartation by absorption at 450 nm. Hemoglobin measurement was directly done in the WBCs chamber, by spectrophotometer by formation of chromogen cyano methemoglobin for lytic solution without cyanide measurement of the blank Hb was done for each analytical cycle and during the startup rising steps.

Platelets (PLTS) analysis

Platelets analysis was made by empedancemetery in the RBCs count chamber at the same time with the red blood cells.

Statistical analysis

SPSS soft ware program, to obtain mean, standard deviation and *p.value* by T. Test and One Way ANOVA. Data were presented in form of tables.

Ethical consideration:

The consent of the selected individuals to the study was taken after being informed with all detailed objectives of the study and its health benefit in future.

Results

Ninety five malaria patient's blood samples tested against fifty control blood sample to evaluate the hematological parameters, and platelet indices of them.

Table (1): The mean value of WBCs, RBCs, hemoglobin and hematocrit of patients with malaria parasite and control

- Showed significant differences in hemoglobin and hematocrite in comparison with control.

Parameters	Sample	N	Mean	*p* value
WBCs x 10^9/L	Case	95	6.3 ± 2.1	0.163
	Control	50	6.8 ± 1.9	
RBCs x 10^{12}/L	Case	95	4.9 ± 0.7	0.498
	Control	50	4.8 ± 0.6	
Hb g/dl	Case	95	13.2 ± 2	0.005
	Control	50	14.2 ± 1.8	
Hct (%)	Case	95	39.4 ± 5.6	0.041
	Control	50	41.3 ± 4.6	

Table (2): The mean value of platelet count and platelet indices of of patients with malaria parasite and control

- Showed significant differences in platelets count and platelet indices except (MPV) in comparison with control.

Parameters	Sample	N	Mean	*p* value
Platelet x 10^9 /L	Case	95	190.1 ±102	0.000
	Control	50	269.1 ± 63.7	
PDW (fl)	Case	95	13.5 ± 2.4	0.000
	Control	50	11.9 ± 1.7	
MPV (fl)	Case	95	10.0 ± 1.2	0.296
	Control	50	9.8 ± 0.9	
P. LCR (%)	Case	95	26.9 ± 7.6	0.012
	Control	50	23.7 ± 6.6	

Table (3): The mean value of hematological parameters of patients with malaria parasite and age groups

- Showed no significant differences in comparison with age groups.

Parameters	Age group	N	Mean	P value
WBCs x 10^9 /L	<40	42	6.3 ±2.2	0.91
	>40	53	6.3 ± 1.9	
RBCs x10^{12} /L	<40	42	4.8 ± 0.87	0.59
	>40	53	4.9 ± 0.60	
Hb (g/dl)	<40	42	13.0 ± 2.4	0.47
	>40	53	13.3 ± 1.7	
HCT (%)	<40	42	38.6 ± 6.6	0.27
	>40	53	39.9 ± 4.8	
Platelets x10^9 /L	<40	42	191.1 ± 109.7	0.93
	>40	53	189.2 ± 96.6	
PDW (fl)	<40	42	13.2 ± 2.7	0.29
	>40	53	13.7 ± 2.1	
MPV (fl)	<40	42	9.9 ± 1.2	0.25
	>40	53	10.1 ± 1.3	
P.LCR (%)	<40	42	25.7 ± 7.6	0.19
	>40	53	27.7 ± 7.5	

Table (4): The mean value of hematological parameters of patients with malaria parasite and gender

-Showed no significant differences in comparison with gender.

Parameters	Sex	N	Mean	*p* value
WBCs x 10^{9} /L	Male	49	6.3 ± 2.2	0.98
	Female	46	6.3 ± 1.9	
RBCs x 10^{12}/L	Male	49	4.9 ±0.85	0.42
	Female	46	4.8 ± 0.58	
Hb (g/dl)	Male	49	13.5 ± 2.3	0.12
	Female	46	12.9 ±1.6	
HCT (%)	Male	49	40.0 ± 6.4	0.22
	Female	46	38.6 ± 4.6	
Platelets x 10^{9}/L	Male	49	182.1 ± 97	0.43
	Female	46	198.5 ±107.5	
PDW (fl)	Male	49	13.3 ±2.3	0.34
	Female	46	13.7 ± 2.2	
MPV (fl)	Male	49	9.9 ± 1.2	0.44
	Female	46	10.1 ±1.3	
P.LCR (%)	Male	49	26.3 ± 7.9	0.43
	Female	46	27.5 ± 7.2	

Discussion

Malaria is a major cause of morbidity and mortality in tropical countries. There is a strong association between change in hematological variables and outcome in malaria [7].

In this study 95 malaria patient's blood samples tested against 50 control blood sample to evaluate the hematological variables and platelets indices of them.

This study showed that there was significant differences in hemoglobin and hematocrit between malaria patient (Hb 13.2g/dl ± 2.1, HCT 39.4 % ± 5.6) and control (Hb14.2 ± 1.8, HCT 41.3 ± 4.6), (*p* value of Hb = 0.005) (*p* value of HCT= 0.041) which agreed with the study done in India by Pradhan [7], showed (Hb level and HCT low in 62.5%). Also agreed

with study done in India by Shamim *et al* [8], showed Hb level low in 84.6% of patients with malaria.

Also the study showed there was no statistical significant differences in RBCs count between malaria patients (4.9x 10^{12}/L ± 0.7) and control(9.8±0.6), (*p* value=0.498).

The present study showed there was no statistical significant differences in WBCs count between malaria patients (6.3 x 10^{9}/L ± 2.1) and control (6.79 ± 1.9), (*p* value= 0.163). This agreed with the study done in Saudi Arabia by Bashawri *et al* [9], showed normal WBCs count in 78.3% in malaria patient. Also agreed with study done in India by Shamim *et al* [8], showed normal WBCs count in 81.1%.

The study showed there was significant differences in platelets count and platelet indices between malaria patients (platelets count 190.7 x 10^{9}/L ± 102, PDW 13.5fl ± 2.4, P.LCR 26.8% ± 6.6) and control (platelets count 269.1 ± 63.7, PDW 11.9 ± 1.7, P.LCR 23.7 ± 6.6), (*p* value of platelets count=0.000, *p* value of PDW=0.000, *p* value of P.LCR=0.012,). This agreed with study done by Shamim *et al* [8] in which showed platelets low in 71.6% and also agreed with Bashawri *et al* [9], showed platelet low in 85% and MPV high in 25%.

This study show no significant differences in MPV between malaria patients (10.0 fl ± 1.2) and control (9.8 ± 0.9), (P value= 0.296).

The study showed no significant different in all parameter in comparison with age groups and gender, it also showed no significant different in severity of infection except in platelets count and platelets indices it was highly statistically significant.

Finally the study showed the main type of anemia in patients with malaria was normocytic normochromic anemia. This result agreed with Bashawri *et al* [9] showed normocytic normochromic anemia in 59.2% and microcytic hypochromic anemia in 17.7 %.

Conclusions

This study was concluded that, some hematological variables affected with malaria parasite. There was decreased in hemoglobin and hematocrit confirmed with peripheral blood film indicated to normocytic normochromic anemia, the red blood cells and white blood cells showed a normal value, compared to control and normal range. This study showed a thrombocytopenia and increased in platelet distribution width (PDW) and platelet large cell ratio (P-LCR).

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