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Morphological Classification of Anemia in Sudanese Patients with Different Chronic Disease

Amged Husssien Abdelrhman[1]*and Abdelgadir Ahmed Abdelgadir[2]

[1]Department of Hematology and Immunohematology, Omdurman Islamic university / Sudan

[2]Head Department of Hematology and Immunohematology, Omdurman Islamic university / Sudan

ABSTRACT

Background: The morphologic approach to anemia begins with review of the CBC, particularly the mean corpuscular volume (MCV), and the peripheral blood smear. The initial distinction is based on the red cell size: anemias are classified as microcytic, normocytic, or macrocytic. The presence of abnormally shaped erythrocytes (poikilocytes) may suggest a specific disease or cause. A problem with the morphologic approach is that the morphologic changes in early anemia may be subtle and easy to miss. A second problem is that one morphologic abnormality may have several possible causes.

Methods: A sample of 100 participants were included in the study. Informed consent was obtained. Venous blood samples were obtained and serum prepared, the hematology analyzer (Sysmex 3000) used to measure the hematology parameters, Cobas e114 used to estimate the serum ferritin.

Results: The results showed significant differences between the mean level of male and female in the following parameters RBC's count, and RBC's indices MCV, MCH and PLTs count p. value(<0.05) and significant statistical deferens in RBC count , MCV and MCH among study group p .value (0.001 , 0.002 , 0.02) respectively. Platelets count show statistical significant differences among study group p. value (0.03).

The correlation studies showed there are significant correlation between age and Hb concentration p. value (0.01), and positive correlation between weight and Hb concentration p. value (0.01). In current study there were Significant association between Age , RBCs, Hemoglobin , HCT, MCH ,MCHC and Platelets Count among patients with liver disease (P. value = 0.00, 0.001, 0.013, 0.000, 0.00,0.000, 0.000) respectively .The mean of hematological parameters, WBCs mean was 8.3 ±3.3 ($\times 10^9$/l), RBCs 4.5 ± 0.5 ($\times 10^{12}$/l) and the mean of Hb was 9.7±1.4 g/l which was low than normal, PLTs Count mean was 284.5 ±98.9 ($\times 10^9$/l) while the mean was serum ferritin 13.4 ±7.1 mg/l in pregnant women with anemia.

Table (7). In this study there was significant statistical difference between RBCs count and age (p. value 0.040) and statistical insignificant different between (WBCs, Hb, PCV, MCV, MCH, MCHC, PLTs count and Serum ferritin among pregnant women (p. value: 0.14, 0.68, 0.52, 0.18, 0.33, 0.7, 0.23 and 0.97) respectively. Positive statistical correlation was observed between serum ferritin and Hb, also positive correlation was observed between serum ferritin and PCV. In the current study significant positive correlation was observed between first and second trimester in Hb concentration P.value (0.000) and between second and third trimester P.value (0.000), Negative correlation between serum ferritin and different stage of trimester P.value ≥ 0.05. and significant statistical difference in Hb and serum ferritin level among patients use iron supplement P.value (0.009 and 0.000) respectively.

Conclusion: Majority of the study participants had, followed by Normocytic, and macrocytic anemia. This might have negative health and educational implications. In this study result were represented significant when Comparison of age, counts, red count, hemoglobin, Hematocrit, red cells indices, and insignificant with white cell count among liver disease patients and control.

Comparison of age, white cells counts, red cells count, hemoglobin, red cells indices and Platelets Count among gender of liver disease patients the result were represented insignificant. Comparison of age, , red cells count, hemoglobin, red cell ,the result represent significant and white cells counts Platelets Count ,MCH,MCV, represent insignificant among duration of disease. Significantly Decrease in Hb% and S. ferritin was observed in Sudanese pregnant women.

***Corresponding author**
Amged Husssien Abdelrhman, Department of Hematology and Immunohematology, Omdurman Islamic university / Sudan. E-mail: Amgedhussen66@gmail.com

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List of Abbreviation
WHO= Word health organization
MCV= Men corpuscular volume
MCH = Men corpuscular hemoglobin
MCHC= Men corpuscular hemoglobin concentration
Hb= Hemoglobin
DPG = Diphosphoglycerate
CBC= Complete blood count
IDA = Iron deficiency anemia
TIBC = Total iron Binding Cabsti
DNA = Deoxyribonucleci acid
HbF = Hemoglobin Fetal

RBC = Red blood cells
HSCs = Haemopieitic stem cells
HTC = Hematocrit
EDTA = Ethylene DiamineTetraacetic Acid
PBP = Peripheral Blood Picture
PH = Power Hydrogen
SPSS = Statistical Package Social Science
WBC = White blood cells
PLt = Platelet

Introduction

According to the world health organization (WHO) report, anemia considered indicator of both poor nutrition and health. It is recognized as a major public health problem globally, mostly affecting children; it has a negative impact on mental physical development, coordination, language development, and scholastic achievement. It reduce the immunity, which leads to susceptibility to infectious disease, and cause premature death [1]. according to the estimation of international organization, about 1.62 billion people in the world suffer from anemia, whichconstitus a global public health problem in both developing and industrialized countries [2]. Anemia is a public health problem that affects populations in both rich and poor countries. Its primary cause is iron deficiency [3].

Anemia affects 45.7 to 49.1 of school- age children in the world and prevalence of anemia among school- age children in Africa ranged from 64.3 to 71%. Consequences of anemia on school-age children are poor psychomotor development, negative last-longing effects on central nervous system [4]. Anemia can be cause by iron, folate, vitamin B12 and vitamin A deficiency, chronic inflammation, parasitic infection and inherited disorders. In developing countries, anemia can also be resulted from a number of cases but a nutritional deficiency particularly iron deficiency is the most common case.

According to the world health organization (WHO) report, anemia considered indicator of both poor nutrition and health. It is recognized as a major public health problem globally, mostly affecting children; it has a negative impact on mental physical development, coordination, language development, and scholastic achievement. It reduce the immunity, which leads to susceptibility to infectious disease, and cause premature death Anemia is a reduction in the hemoglobin concentration of the blood [5].

Although normal values can vary between laboratories, typical values would be less than 13.5 g/dL in adult males and less than 11.5 g/dL in adult females. From the age of 2 years to puberty, less than 11.0 g/dL indicates anemia. As newborn infants have a high hemoglobin level, 14.0 g/dL is taken as the lower limit at birth. Reduction of hemoglobin is usually accompanied by a fall in red cell count and packed cell volume (PCV). Anemia is considered to be present if the hemoglobin concentration of the red blood cells (RBCs) or the packed cell volume of RBCs (hematocrit) is below the lower limit of the 95% reference interval for the individual's age, gender, and geographical location [6].

The causes of anemia fall into three major pathophysiological categories: Blood loss /Impaired red cell production/Accelerated red cell destruction (hemolysis in excess of the ability of the marrow to replace these losses) Anemia may be a sign of an underlying disorder. Dilutional anemia with normal or increased total red cell mass may occur with pregnancy, macroglobulinemia, and splenomegaly. Some anemias have more than one pathogenetic mechanism and go through more than one morphological state, such as blood loss anemia. In the case of accelerated red cell destruction, hemolysis in excess of the ability of the marrow to replace these losses occurs [7].

ACD is a hypoproliferative anemia resulting from underproduction of red cells. Although the life span of erythrocytes is mildly shortened in this disorder (in some patients, the average erythrocyte survival time is 90 days), decreased erythrocyte survival is not an isolated or major factor in the development of anemi Multiple mechanisms contribute to anemia associated with inflammation or malignancy The principal pathogenesis of ACD is believed to be related to a 2 recently described molecule, hepcidin [8]. Body iron metabolism is regulated by several molecules.

Hepcidin, a small 25-amino acid polypeptide hormone, is a key molecule in controlling iron absorption and recycling. In the liver, hepcidin gene expression is regulated by at least two pathways pathway dependent on iron availability and involves signaling from the surface of the hepatocyte through the BMP receptor complex. pathway that regulates hepcidin gene expression by the IL-6–mediated infl ammatory signaling pathwa [9]. Hepcidin is released by the liver and circulates to interact withitscellularreceptor ,the iron export channel ferroportin, to block release of iron from cells,Inadequate production of erythropoietin (Epo) in response to the anemia [10]. Inadequate response of the erythroid marrow to endogenous Epo Impaired release of iron due to increased hepcidin production producing a functional iron deficiency. Alterations in production of several proinfl ammatory cytokines.

Decreased erythrocyte production the pathogenesis of this anaemia appears to be related to the decreased release of iron from macrophages to plasma and so to erythroblasts caused by hepcidin, reduced red cell lifespan and an inadequate erythropoietin response to anaemia the plasma levels of various cytokines, especially interleukin-l (IL-l), IL-6 and tumour necrosis factor (TNF) are raised and reduce erythropoietin secretion. the anaemia is corrected by the successful Treatment of the underlying disease. It does not respond to iron therapy despite the low serum iron. Responses to recombinant erythropoietin therapy maybe obtained [11].

Anemia is one of the most frequent complications related to pregnancy. The word implies a decrease in the oxygen-carrying capacity of the blood and is best characterized by a reduction in hemoglobin concentration. This may be either relative or absolute. It is known that there is a larger increase in plasma volume relative to red cell mass in almost all pregnancies, and it accounts for ―physiologic anemia. These alterations have been known for centuries, and the term ―plethora gravidarum‖ from medieval ages indicates this condition. However, it is still an open question to what extent this ―hydremia‖ is physiologic or pathologic [1]. There are two contrasting medical philosophies covering this problem.

According to the first, it is preferable to prevent pregnant women from developing too low hemoglobin concentrations. According to another point of view the ―physiologic anemia‖ is of great importance for normal fetal growth and should be passively observed. Moreover, the relationship between a successful outcome of pregnancy and this normal expansion in maternal plasma volume has been noted [12]. This controversy is reflected in the recommendations from the World Health Organization on the optimal hemoglobin (Hb) concentrations or hematocrit (Hct) level. Thus, in 1965 a WHO expert committee suggested that 10 gm/dl should be accepted as the lower limit of the physiologic adjustments made during pregnancy [13].

Materials and Methods

Blood Picture (PBP): A blood film—or peripheral blood smear—is a thin layer of blood smeared on a glass microscope slide and then stained in such a way as to allow the various blood cells to be examined microscopically .

Automated sysmex BC-3000plus technique

The Coulter principle is based on the following: particles suspended in an isotonic diluent, when drawn through an aperture which has an electric current flowing through it was caused a measurable drop in voltage which is proportional to the size of the particle passing through the aperture is constant the particle can be quantified per unit volume. This is also called electrical impedance.

Ferritin Test

The elecsys ferritin assay uses two monoclonal mouse antibodies to form the sandwich complex in the assay. Total duration of assay 18minutes. 1st incubation: 10ml of sample, a biotinylated monoclonal ferritin-specific antibody, and a monoclonal ferritin –specific antibody labeled with a ruthenium complex react to form a sandwich complex.

Results

The results showed significant differences between the mean level of male and female in the following parameters RBC's count, and RBC's indices MCV, MCH and PLTs count p. value(<0.05). Table (1), and no significant differences in WBC count p. value (0.4) Table (2) . The result show significant statistical deferens in RBC count, MCV and MCH among study group p value (0.001 , 0.002 , 0.02) respectively. Platelets count show statistical significant differences among study group p. value (0.03). Table (3). The correlation studies showed there are significant correlation between age and Hb concentration p. value (0.01). Table (4), and positive correlation between weight and Hb concentration p. value (0.01). Table (5). In current study there were Significant association between Age, RBCs, Hemoglobin , HCT, MCH ,MCHC and Platelets Count among patients with liver disease (P. value = 0.00, 0.001, 0.013, 0.000, 0.00,0.000, 0.000) respectively . Table (6). The mean of hematological parameters, WBCs mean was 8.3 ±3.3 ($\times 10^9$/l), RBCs 4.5 ± 0.5 ($\times 101^2$/l) and the mean of Hb was 9.7±1.4 g/l which was low than normal, PLTs Count mean was 284.5 ±98.9 ($\times 10^9$/l) while the mean was serum ferritin 13.4 ±7.1 mg/l in pregnant women with anemia .Table (7). In this study there was significant statistical difference between RBCs count and age (p. value 0.040) and statistical insignificant different between (WBCs, Hb, PCV, MCV, MCH, MCHC, PLTs count and Serum ferritin among pregnant women (p. value: 0.14 ,0.68 ,0.52 ,0.18 ,0.33, 0.7, 0.23 and 0.97) respectively. Table (8). Positive statistical correlation was observed between serum ferritin and Hb. figure (1) also positive correlation was observed between serum ferritin and PCV. figure (2). In the current study significant positive correlation was observed between first and second trimester in Hb concentration P.value (0.000) and between second and third trimester P.value (0.000), Negative correlation between serum ferritin and different stage of trimester P.value ≥ 0.05 show that in Table (9). In the present study there is significant statistical difference in Hb and serum ferritin level among patients use iron supplement P.value (0.009 and 0.000) respectively. Table (10).

Table 1: Descriptive Statistics of study variables

	N	Minimum	Maximum	Mean	Std. Deviation
Age (years)	100	6	12	8.8	2.1
Weight (kg)	100	11	20	14.0	1.6
WBCs ($\times 10^9$/l)	100	3.3	12.0	6.8	1.9
RBCs ($\times 10^{12}$/l)	100	2.85	5.80	4.5	0.4
Hemoglobin (g/dl)	100	6.9	12.0	9.9	1.1
HCT (%)	100	21.9	44.0	30.6	3.8
MCV (fl)	100	54.0	99.9	77.1	11.4
MCH (pg)	100	15.0	38.0	21.9	2.8
MCHC (g/l)	100	22.6	229.0	30.1	20.4
Platelets Count (x10^9/l)	100	108	550	290.1	88.8

Table 2: Comparison between study variables and gender

Parameters	Gender (Mean ± SD)		P. value
	Male (n=50)	Female (n=50)	
Age (years)	8.8 ± 2.2	8.7 ± 2.0	0.812
Weight (kg)	13.9 ± 1.2	14.2 ± 1.8	0.407
WBCs ($\times 10^9$/l)	6.9 ± 1.8	6.6 ± 1.9	0.409
RBCs ($\times 10^{12}$/l)	4.7 ± 0.4	4.3 ± 0.4	0.001
Hemoglobin (g/dl)	9.8 ± 1.4	9.9 ± 0.7	0.927
HCT (%)	31.1 ± 4.8	30.1 ± 2.1	0.183
MCV (fl)	74.9 ± 11.1	79.3 ± 11.4	0.053
MCH (pg)	21.3 ± 2.9	22.6 ± 2.6	0.021
MCHC (g/l)	27.8 ± 3.2	32.4 ± 28.6	0.260
Platelets Count (x109/l)	208.4 ± 92.9	271.8 ± 81.4	0.039

Table 3: Correlations between age and hemoglobin

Correlations			
		Age	Hemoglobin
Agea	Pearson Correlation	1	.596**
	Sig. (2-tailed)		.000
	N	100	100
Hemoglobin	Pearson Correlation	.596**	1
	Sig. (2-tailed)	.000	
	N	100	100
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 4: Correlations between weight and hemoglobin

Correlations			
		Age	Hemoglobin
Hemoglobin	Pearson Correlation	1	.495**
	Sig. (2-tailed)		.000
	N	100	100
Weight	Pearson Correlation	.495**	1
	Sig. (2-tailed)	.000	
	N	100	100
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 6: Comparison of age, total white cells counts, red cells count, haemoglobin, red cells indices and Platelets Count among liver disease patients and control

Parameters	Population study (Mean ± SD)		P. value
	Patients (n=55)	Control (n=56)	
Age (years)	45.9 ± 17.6	25.9 ± 10.2	0.000
WBCs ($\times 10^9$/l)	6.9 ± 4.2	6.2 ± 2.3	0.251
RBCs ($\times 10^{12}$/l)	4.1 ± 1.9	4.9 ± 0.5	0.001
Hemoglobin (g/dl)	12.0 ± 3.9	13.4 ± 1.7	0.013
Hemoglobin (%)	80.1 ± 25.9	89.5 ± 11.1	0.015
HCT (%)	36.4 ± 8.8	41.8 ± 4.2	0.000
MCV (fl)	84.8 ± 7.2	84.3 ± 4.6	0.622
MCH (pg)	29.4 ± 3.2	26.7 ± 1.8	0.000
MCHC (g/l)	34.0 ± 3.1	31.4 ± 1.2	0.000
Platelets Count (x10^9/l)	172.8 ± 95.9	287.9 ± 65.9	0.000

Table 7: mean and STD of Total Red Blood Cells count, White Blood count, Hb, HCT and RBCs indices of pregnant Platelets serum Ferritin in study population

Variables	Mean	Std. Deviation
Age (years)	31.6	4.6
TWBCs ($\times 10^9$/l)	8.3	3.3
RBCs ($\times 10^{12}$/l)	4.5	0.5
Haemoglobin (g/l)	9.7	1.4
PCV (%)	30.7	4.1
MCV (fl)	68.0	5.6
MCH (pg)	21.5	2.3
MCHC (g/l)	30.6	1.0
Platelet count ($\times 10^9$/l)	284.5	98.9
Serum ferritin (mg/l)	13.4	7.1

Table 8: Comparison of white cells counts, red cells count, hemoglobin, red cells indices, Platelet count and Serum ferritin among age group

Parameters	Age group (Mean ± SD)		
	Less than 30 years (n=24)	More than 30 years (n=26)	P. value
TWBCs (×10⁹/l)	7.6 ± 3.2	8.9 ± 3.3	0.141
RBCs (×10¹²/l)	4.4 ± 0.4	4.6 ± 0.5	0.040
Haemoglobin (g/l)	9.6 ± 1.2	9.7 ± 1.5	0.687
PCV (%)	30.3 ± 3.5	31.1 ± 4.6	0.527
MCV (fl)	69.1 ± 4.7	66.9 ± 6.3	0.180
MCH (pg)	21.8 ± 2.1	21. ± 2.4	0.335
MCHC (g/l)	30.5 ± 1.1	30.7 ± 0.9	0.721
Platelet count (×10⁹/l)	267.0 ± 90.1	300.7 ± 104.9	0.233
Serum ferritin (mg/l)	13.3 ± 6.1	13.4 ± 8.5	0.973

Table 9: Multiple Comparisons Hemoglobin and Serum ferritin level according to risk factor of other diseases

Parameters	Sample (I)	Sample (II)	Mean of (I)	Mean of (II)	P value
Hemoglobin (g/dl)	First trimester	Second trimester	10.6 ± 0.6	8.7 ± 0.9	0.000
	First trimester	Third trimester	10.6 ± 0.6	11.1 ± 0.7	0.155
	Second trimester	Third trimester	8.7 ± 0.9	11.1 ± 0.7	0.000

Table 10: Comparison of hemoglobin and serum ferritin according to iron supplement

Parameters	Iron supplement (Mean ± SD)		P. value
	Not use (n=17)	Use (n=8)	
Haemoglobin (g/dl)	9.2 ± 1.3	10.8 ± 1.1	0.009
Serum ferritin (mg/l)	8.9 ± 3.1	22.9 ± 3.7	0.000

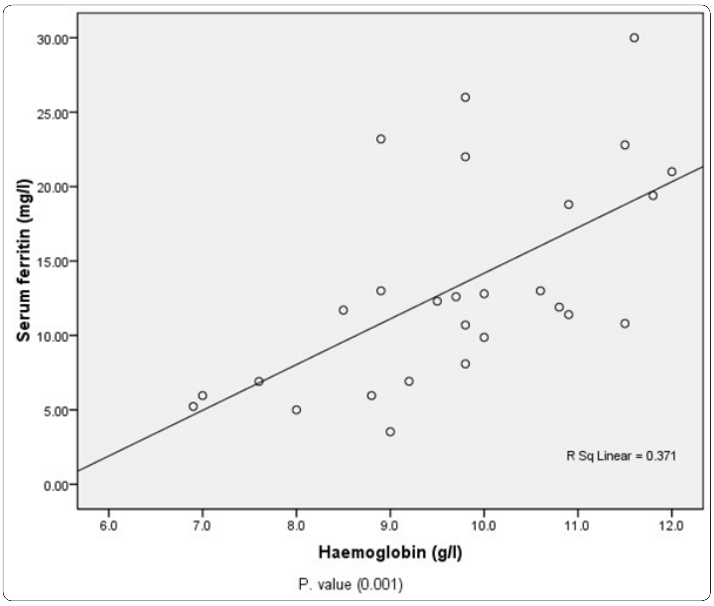

Figure 1: positive Correlation between Serum ferritin and hemoglobin

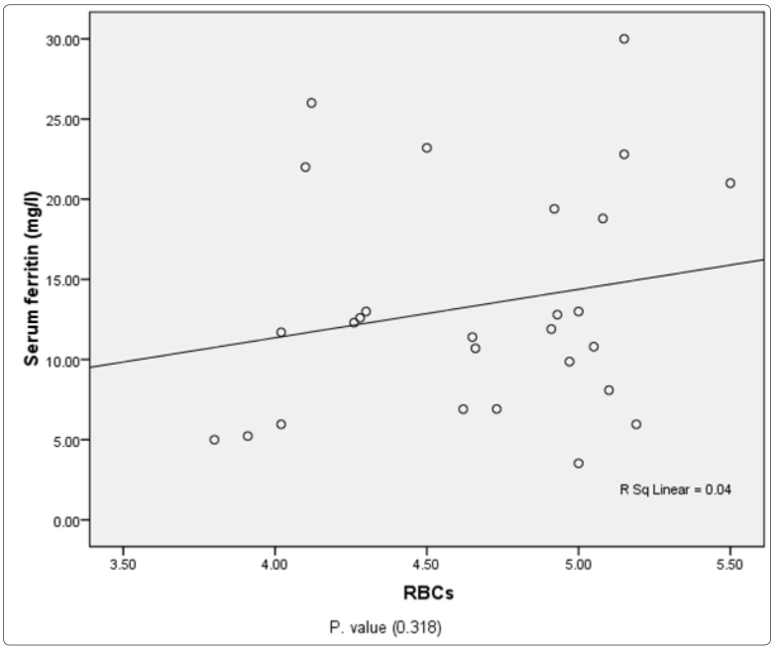

Figure 2: positive Correlation between Serum ferritin and packed cell volume

Discussion

The results showed significant differences between the mean level of male and female in the following parameters RBC's count, and RBC's indices MCV, MCH and PLTs count (p.value<0.05). The results showed there are no differences between hemoglobin, hematocrit, Mean Cell Hemoglobin and Mean Cell Hemoglobin Concentration, WBCs, weight and Age in male and female among study group. (p.value> 0.05). The prevalence of anemia was estimated, by measuring haemoglobin concentration, the results showed that mean of Hb in male of the students was 9.8g/dl and mean of haemoglobin estimations in female 9.9 g/dl(Hb. less than 13.5 g/dl). There was significant relation with the mean Hb value (P value 0,01).

Microcytic anemias was found to be the common type of anemia in the present study and this agreed with another study conducted in Delhi, India, about the Prevalence and etiology of nutritional anemia among schoolchildren of urban slums) [13] . The mean of white blood count is 6.9 x 10^9 µ/L in male and 6.6 x 10^9 µ/L Significant differences showed in platelet counts , the mean less than 208 x 10^9 µ/L occurred in male and 271 x 10^9 µ/L in female (P.value 0.03). In this study, male school-aged childrenhad higher prevalence of anemia (28%)compared with female counterparts (25%).Though, there was no significant difference between the gender subgroups (P=0.42). (14) , this also agreement with our finding.

Also agreed with the finding of study done to detect Prevalence of Anemia in Children from Latin America and the Caribbean and Effectiveness of Nutritional Interventions evaluated between1997 and 2018, disagreed Although in other study; Prevalence of anemia among school-age children in Ethiopia at 2012-2016, and Frequency of Nutritional Anemia among Schoolchildren of Kosti Town – White Nile State [15]. The result were represented insignificant when Comparison of age, white cells counts, hemoglobin, Hematocrit, red cells coun and Mchc represents significant when compared with p value, among. duration of disease. This agree with [16], the result show 86 percent had anaemia helping in early diagnosis of anaemia in decompensated chronic liver disease patients. This agree with [17]. In this study results show Liver diseases are frequently associated with hematological abnormalities. This agree with [18].

In this study the result show Chronic liver disease is accompanied by multiple hematological abnormalities. Iron deficiency anemia is a frequent complication of advanced liver disease. This agree with the result show in this study Thrombocytopenia is a marked feature of chronic liver disease and cirrhosis. This disagree with, result show in this study Liver disease is frequently missed as the cause for a patients thrombocytopenia [19,20]. these results agreed partly with the results of a study in China which showed that during pregnancy Hb concentration, TRBC count, HCT were decreased Hb concentration value was 8.7 ± 0.9 g/dL in third trimester, this similar to study done by Akinbami in Lagos showed that Hb concentration value was 10.38 ±1.27 g/dL in Nigeran women [21,22]. In Sudan there were significant decrease in RBCs count, Hb and HCT of pregnant women compared to non-pregnant women Akingbola et al. in South Nigeria showed that pregnancy is characterized by decreased values of haemoglobin, hematocrit. Women often become anemic during pregnancy because of the increase in demand for iron and other vitamins in the body [23]. It is estimated that the blood volume increases approximately 50 per cent during pregnancy, although the plasma amount is disproportionately greater. This causes dilution of the blood, making the hemoglobin concentration fall, with hemoglobin concentration at its lowest between weeks 25 and 30 [23]. The Platelets count and lymphocytes significantly decreased (P. value 0.00). in pregnant women when compared with non-pregnant women These results agreed with the results of the study in China, which showed PLT count was lower [22]. Also, Akinbami, et al. showed that PLT was lower. Platelets count significantly lower than the normal control [23]

Ethical approval and consent to participant

Approval of This study was obtained from hematology department of medical laboratory science (MLS) , Alwatania University ,and ministry of health issued by the local ethical committee , Khartoum State, Sudan . Written consent was taken from each member of the study.

Consent for publication
Not applicable.

Availability of data and materials
The datasets generated during and / or analyzed in this study are not publicly available due to Bahri hospital center ethical policy in order to protect participant confidentiality.

Competing interest
The authors declare that they have no competing interests.

Funding
No funding was obtained for this study

Authors Contributions
AH and AA contributed in literature search and manuscript writing. AH had the main idea of the study and contributed in manuscript writing, A contributed to clinic work, AH contributed in statistical analysis . AA supervised the study and critically reviewed the manuscript. All authors read and approved the final draft of the manuscript.

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