Physiological Analysis of Haemoglobin Electrophoresis and Complete Blood Picture in Pregnant Women

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ABSTRACT

Background: Different types of Haemoglobin is synthesized as consequence of the differences in oxygen (O2) demands at different stages of growth to mantis optimal O_2 delivery to the tissues. Thus, haemoglobin A1 is the most common form of haemoglobin in the adult people , haemoglobin A1, is a combination of two alpha chains and two beta (α2β2)chains.

Aim: The study aimed to assess the level of adult haemoglobin and fetal haemoglobin in normal pregnancy and correlate the results with complete blood picture.

Methods: Study design: cross sectional study carried from Oct. 2024- Jan. 2025. Blood samples were used to determine complete blood picture by using hematology autoanalyser and haemoglobin electrophoresis by using Gel electrophoresis chamber.

Result: Seventy pregnant women in the third trimester were included in the study. The mean ± SD of their age was 27.55±9.17years.The mean of HbA1 was (92.63±2.47) while for HbA2 was (2.80±0.83) and fetal haemoglobin was (2.15±2.48). Twenty four of them were taking iron supplement (group 1) and 46 pregnant women not taking iron supplement (group 2), HbA1 higher in group 1 and Hb F was lower in group 1 but both results were statically not significant. Positive association between HbA1 with Hb, PCV, RBC and reticulocytes ($P < 0.0001$), ($P<0.0001$), ($P<0.044$) ($P < 0.034$), respectively.There was a negative correlation of Hb A1 with the platelets ($P<0.037$).
Inverse correlation was found between HbA1 and HbA2 ($P<0.017$). A significant negative association between HbA2 and HbA1($p < 0.017$).

Conclusion: Adult hemoglobin positively associated with red blood cell count packed cell volume and reticulocyte count and negatively correlated with platelets count. Significant negative correlation of HbA1 with the HbA2 ,fetal hemoglobin higher in pregnant women and in those not taking iron it has negative association with monocyte.

Keywords: Adult haemoglobin, Complet blood picture, fetal haemoglobin ,haemoglobin electrophoresis, hemoglobin A2.

التحليل الفسلجي للفصل الكهربائي للهيموكلوبين وفحص صورة الدم لدى النساء الحوامل

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الخلاصة

الخلفية: يتم تصنيع أنواع مختلفة من الهيموكلوبين نتيجة للاختلافات في متطلبات الأوكسجين في مراحل مختلفة من النمو من أجل التوصيل الامثل للأوكسجين إلى الأنسجة. وبالتالي، فإن الشكل الأكثر شيوعًا للهيموجلوبين في الإنسان البالغ،هو الهيموكلوبين A١، هو مزيج من سلسلتي ألفا وسلسلتين بيتا (α٢β٢). هدفت الدراسة إلى تقييم مستوى الهيموكلوبين البالغ والهيموكلوبين الجنيني والهيموكلوبين A٢ في الحمل الطبيعي وربط النتائج مع نتائج صورة الدم الكاملة.

طرق البحث: تصميم الدراسة: دراسة مقطعية أجريت في الفترة من أكتوبر ٢٠٢٤ إلى يناير ٢٠٢٥. تم استخدام عينات الدم لتحديد صورة الدم الكاملة باستخدام محلل أمراض الدم الذاتي والرحلان الكهربائي للهيموكلوبين باستخدام غرفة الفصل الكهربائي الهلامية.

النتائج: شملت الدراسة سبعين امرأة حامل في الثلث الثالث من الحمل. كان متوسط ± DS لأعمارهم ٢٧.٥٥ ± ٩.١٧ سنة. وكان متوسط الهيموكلوبينA١ (٩٢.٦٣ ± ٢.٤٧) بينما كان متوسط الهيموكلوبين A٢ (٢.٨٠ ± ٠.٨٣) والهيموجلوبين الجنيني (٢.١٥ ± ٢.٤٨). كانت ٢٤ امرأة منهم يتناولون مكملات الحديد (المجموعة ١) و٤٦ امرأة حامل لا يتناولون مكملات الحديد (المجموعة ٢)، وكان HbA١ أعلى في المجموعة ١ وكان Hb F أقل في المجموعة ١ ولكن كلا النتيجتين لم تكن ذات دلالة إحصائية. ارتباط إيجابي بين HbA١ مع Hb و PCV و RBC والخلايا الشبكية (P<٠.٠٠٠١ ، P<٠.٠٠٠١) (٠.٢٠٢، P <٠.٠٤٤) (، P<٠.٠٣٤) على التوالي. كان هناك ارتباط سلبي لمستوى Hb A١ مع الصفائح الدموية (P<٠.٠٣٧).
كان هناك ارتباط سلبي كبير بين HbA١ وHbA٢ ، P<٠.٠١٧)). وجود علاقة سلبية كبيرة بين HbA١ و HbA٢ (p <٠.٠١٧).
الاستنتاج: الهيموجلوبين البالغ يرتبط إيجابيا مع عدد خلايا الدم الحمراء وحجم الخلايا المعبأة وعدد الخلايا الشبكية ويرتبط سلبا مع عدد الصفائح الدموية. كان الارتباط السلبي الكبير لـ HbA١ مع هيموجلوبين الجنين HbA٢ أعلى عند النساء الحوامل وفي أولئك الذين لا يتناولون الحديد، وله ارتباط سلبي مع الوحيدات.

الكلمات المفتاحية: الفصل الكهربائي للهيموجلوبين، الهيموجلوبين البالغ، الهيموجلوبين الجنيني، الهيموجلوبين A٢، صورة دم كاملة.

INTRODUCTION

The main function of red blood cells (RBC) is to transport oxygen by Haemoglobin which is a protein that carries oxygen in the blood [1,2]. Haemoglobin (Hb) is formed from combination of iron with protoporphyrin IX, to form the heme molecule, and then each heme molecule joins with long polypeptide and forms hemoglobin [1,3]. There are minor variations in the different subunit of haemoglobin chains, depending on the amino acid constitution of the polypeptide chain. The distinct types of chains are named alpha, beta, gamma, and delta chains. Each Hb molecule contains four haemoglobin chains. So four iron atoms in each haemoglobin molecule; bind with one molecule of oxygen, making a total of 4 O_2 molecules that can be carried by each Hb molecule. In the lungs heme molecules pick up oxygen while in the tissue they deliver it [4].

Different types of Haemoglobin is synthesized as consequence of the differences in O_2 demands at different stages of growth to mantis optimal O_2 delivery to the tissues. Thus, haemoglobin A1(HbA1) is the most common form of haemoglobin in the adult people , haemoglobin A1, which is consist of two alpha chains and two beta chains (α2β2). Haemoglobin A2(HbA2) normal variant with two alpha chains and two delta chains [1,3,4].

Fetal haemoglobin is the haemoglobin of the fetus (HbF), that formed before birth . Fetal haemoglobin is made up of 2 alpha chains and 2 gamma chains (α2γ2). Embryonic haemoglobins (HbE) are Gower 1, composed of two zeta and two epsilon chains (ζ2ε2), Portland, composed of two zeta and two gamma chains (ζ2γ2), and Gower 2, composed of two alpha and two epsilon chains (α2ε2) [3,5 -8].

At the low oxygen tension, the fetal haemoglobin can carry 20 - 50 % more oxygen than maternal haemoglobin [1].

Haemoglobin electrophoresis measures the levels of the varied types of this protein in the blood. Haemoglobin A: 95% to 98%, HbA2: 2% to 3%, HbE: Absent, HbF: 0.8% to 2%, HBS: Absent, HbC: Absent [2,8].

Expression of HbF in adults is largely genetically regulated. Few articles reported the elevated level of HbF in pregnancy. Different mechanisms have been recommended, but the explination of HbF manifestation during pregnancy still unclear [9,10].

Fetal haemoglobin ,a minor haemoglobin of normal adults, has major clinical significance for sickle cell disease [11,12].

Hormonal change during pregnancy is the cause of many physiological changes in pregnancy and after labour. Many hematological changes also, occurring during these periods are physiological and are considered as a small problem to the haematologist [13].

In order to compensate for blood at delivers and the increase need of the blood for the new vascular bed during pregnancy , the total blood volume increases by about 1.5 liters [14]. This indicates that, in pregnancy, the increases in plasma volume to compensate for the demand of vascular under filling resulting from systemic vasodilatation and increase in vascular capacitance [15,16] .

Elevated red cell count as result of high maternal erythropoietin production, as general its less than the increase in plasma volume, so result being a decrease in haemoglobin level and dilutional anemia will present. In pregnancy small changes in indices of the RBC like little increase in mean corpuscular volume (MCV).

Increased production of red blood cells to comply with the demands of pregnancy, realistically explains why there is an increased MCV [17].

During pregnancy, an enhanced immune response is necessary to combat infections that could harm mother and baby. This can cause a normal elevation in white blood cells count(WBC) , especially before labour. Physiologic stress during pregnancy produce leukocytosis (increase in white blood cells count) [18].

The aim of this study was to assess the level of adult haemoglobin, and fetal haemoglobin in normal pregnancy and correlate the results with complete blood picture (RBC ,WBC, differential count, Platelets count, and blood indices).

SUBJECTS AND METHODS

The current study sample includes 70 pregnant healthy women in the third trimester, whose gestational age was estimated by taking into account the last menstrual cycle, early ultrasound measurements.

Those pregnant women were attending private clinic for their usual booking visit. Their ages were between 18-38 years with mean of (27.55) years and their weights ranged between 60-84 kg, with a mean of (72) kg. The mean of their blood pressure was 130/80mmHg and the mean of their pulse was 75 beat/minute.

The duration of this study was from October, 2024 to January, 2025.

Specimens

Blood samples from pregnant women in the third trimester were taken by clean antecubital venipuncture. A total of 5 ml of blood was taken from each pregnant woman and collected into a tube containing EDTA as an anticoagulant.These samples were used to determine haemoglobin A1 (HBA1), haemoglobin A2 (HBA2) and fetal haemoglobin (HBF) by using Gel electrophoresis chamber. Haemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell count (WBC), differential white blood cell count (lymphocyte, neutrophils, basophiles, eosinophils), RBC count , mean corpuscular volume (MCV), mean corpuscular haemoglobin(MCH), mean corpuscular haemoglobin concentration (MCHC), platelets count (pl), and reticulocyte count were measured by using hematology autoanalyser.

Statistical Analysis

Statistical analysis was done with SPSS software, version 25.The following statistical methods were used for the analysis of data :

1. Standard statistical methods were used to determine the mean ± SD, minimum and maximum.
2. Z-test was used in the comparison of the haematological parameters of pregnant women during the third trimester.
3. Pearson correlation coefficient (r) was used to determine correlation between haematological parameters.

RESULTS

Seventy pregnant women in the third trimester were included in the study. The mean ± SD of their age was 27.55±9.17years.

The mean of HbA1 was (92.63±2.47) while for HBA2 was (2.80±0.83) and fetal haemoglobin was (2.15±2.48). Twenty four of them were taking iron supplement (group 1) and 46 pregnant women not taking iron supplement (group 2).

Comparison of HbA1, HbA2 and HbF between the two groups, we found that HbA1 higher in group 1 and Hb F was lower in group 1 but both results were statically not significant as shown in table (1) .

Table (1): Comparison between group 1 and group 2 regarding HbA1,HbA2 and HbF.

Parameters	Pregnant Women		P
	Taking Iron (n=24)	Not taking Iron (n=46)	
	mean ± SD	mean ± SD	
HbA1%	92.6±2.79	92.17±2.67	0.55
HbA2%	2.81±0.86	2.81±0.84	0.49
Hb F%	1.29±1.71	2.58±2.68	0.084

Table 2 shows the correlation between HbA1 with other parameters, in the present study the following results were obtained:

Spearman's correlation analysis revealed a considerable positive association between HbA1 with Hb, PCV, RBC and reticulocytes ($r = 0.389$, $P < 0.0001$), ($r=0.393$, $P<0.0001$), ($r=0.202$, $P<0.044$) ($r = 0.212$, $P < 0.034$), respectively.

There was a negative correlation of Hb A1 with the platelets ($r=-0.208$, $P<0.037$).

Inverse correlation was found between HbA1 and HbA2 ($r=-0.238$, $P<0.017$).

Table 2: the correlation between HbA1 with other hematological parameters.

Variables	HbA1	
	r- value	p-value
Hb gm/L	0.389	0.0001**
PCV l/l	0.393	0.0001**
RBC x10^{12}/L	0.202	0.044*
WBC x10^{9}/L	-0.119	0.237
B%	0.077	0.449
N%	-0.055	0.589
L%	0.046	0.650
E%	-0.187	0.063
R%	0.212	0.034*
M%	0.106	0.292
Platelets x10^{9}/L	-0.208	0.037*
MCV fl	0.114	0.259
MCH Pg	0.088	0.384
MCHC g/L	0.046	0.651
HbF %	-0.092	0.361
HbA2 %	-0.238	0.017*
**. Correlation is significant at the 0.01 level (2-tailed).		
*. Correlation is significant at the 0.05 level (2-tailed).		

A significant positive correlation was found between HBF and monocyte (r = 0.202, p < 0.044), as well negative correlations with HbA1 and Hb A2 but it was not significant. Table (3)

Table 3: The correlation between HbF with other hematological parameters.

Variables	HbF	
	r- value	p-value
Hb gm/L	-0.134	0.183
PCV l/l	-0.141	0.161
RBC x10^{12}/L	-0.190	0.059
WBC x10^{9}/L	-0.166	0.099
B%	-0.118	0.243
N%	-0.029	0.775
L%	0.39	0.700
E%	-0.127	0.208
R%	-0.163	0.106
M%	0.202	0.044*
Platelets x10^{9}/L	0.004	0.971
MCV fl	0.081	0.426
MCH Pg	0.102	0.312
MCHC g/L	0.093	0.356
HbA2%	-0.073	0.473
HbA1%	-0.092	0.361
**. Correlation is significant at the 0.01 level (2-tailed).		
*. Correlation is significant at the 0.05 level (2-tailed).		

A significant negative association between HbA2 and HbA1(r = -0.238 , p < 0.017).These correlations can be demonstrated in Table 4

Table 4: the correlation between HBA2 with other hematological parameters.

Variables	HBA2	
	r- value	p-value
Hb gm/L	0.128	0.204
PCV l/l	0.126	0.211
RBC x10^{12}/L	0.126	0.211
WBC x10^{9}/L	0.096	0.341
B%	-0.073	0.472
N%	-0.045	0.658
L%	0.041	0.685
E%	0.049	0.628
R%	0.116	0.251
M%	-0.060	0.554
Platelets x10^{9}/L	0.058	0.564
MCV fl	-0.023	0.821
MCH Pg	-0.014	0.892
MCHC g/L	0.039	0.698
HbF%	-0.073	0.473
HbA1%	-0.238	0.017*
**. Correlation is significant at the 0.01 level (2-tailed).		
*. Correlation is significant at the 0.05 level (2-tailed).		

DISCUSSION

Fetal haemoglobin is the normal haemoglobin that is present in the fetus and usually almost absents in adults in our study the results of fetal haemoglobin was (2.15±2.48%) which is slightly elevated than normal range.

In study of James et al. In normal pregnancies successive maternal serum HbF levels were measured. During the first trimester the maternal HbF level remains within the normal range. In second trimester, HbF in mothers go up in some cases; while in other HbF remains within the normal range. The maternal HbF level may be an indicator of transplacental hemorrhage [19].

Other studies observed a significant increase in the level of maternal HbF compare to the non pregnant control group. Studies the association between the gestational age and level of HbF declaire no significant increase of HbF with the progress of pregnancy. The cause of the rise in HbF is in need to be clarified [20].

High maternal HbF levels (≥ 70%) can lead to problematic pregnancy and increased risk of intra-uterine growth retardation or small for gestational age fetuses. Frequent antenatal care is recommended [21] .

Heightened maternal levels of HbF represent a special issue where both mother and fetus produce haemoglobin with comparable O_2 affinities. Fetal haemoglobin adapted to carry O_2 from maternal blood to fetal tissues, accomplished by the elevated affinity of HbF for O_2 compared with HbA. This is mainly due to the insensitivity of HbF to 2,3 biphosphoglycerate [1,22] .

In the study a significant positive correlation that found between HbF and monocyte is demonstrated .

Studies have demonstrated that raised HbF level is associated with mild disease but there is no proof for the correlation between HbF levels and inflammatory markers. monocyte has an inverse correlation with HbF, chemoattractant protein-1 and platelet count in sickle cell anemia [23].

In the present study HbA2 was (2.80±0.83%) which is near the upper standard limit. Study by Kang L et al. recommended the reference range of HbA2 for pregnant ladies was significantly lower than other groups. Therefore, they suggested limit of HbA2 in pregnant ladies for α-thalassemia is 2.3% [24].

A study by Nijboer et al. declare direct association between Hb and PCV and between Hb and RBC count in general [25].

Also Farid Y et al. found a positive relation between haemoglobin and RBC count, which was slightly lower than the correlation between Hb and PCV [26] .

Haemoglobin levels were positively correlated with PCV in males and females of all ages [27].

CONCLUSIONS

Adult hemoglobin positively associated with red blood cell count packed cell volume and reticulocyte count and negatively correlated with platelets count. Significant negative correlation of HbA1 with the HbA2 fetal hemoglobin higher in pregnant women and in those not taking iron it has negative association with monocyte. Increased expression of HbF in pregnancy. antenatal surveillance is suggested.

Ethical Consideration

Study protocol approved by the Ethics Committee of the University of Mosul No. 60, date 28/10/2024 code:CCMRE-MED-24-23 and informed consent obtained from all.

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Conflicts of Interest

None declared.

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