# Hematological parameters of β-thalassemia Trait in Premarital Screening in Nineveh Province

**Bassma Adnan Yonus* , Professor Muna Abdulbasit Kashmoola****
***Postgraduate Student , Department of Pathology, College of Medicine University of Mosul , * **Department of Pathology, College of Medicine, University of Mosul, Mosul, Iraq**
**Correspondence: bassmaadnan85@gmail.com**

**(Ann Coll Med Mosul 2020; 42 (2):157-161).**
**Received: 13th Oct. 2020; Accepted: 29th Nove. 2020.**

## ABSTRACT

***Background:*** β-Thalassaemia syndromes are inherited group of abnormalities of hemoglobin synthesis. These abnormalities characterized by a defect in β-globin genes which may result either in the reduction or absence of one or more of the β-globin chains of hemoglobin. This genetic defect can be homozygous or heterozygous.

***Aim of this study:*** was to detect the hematological parameters of β-thalassaemia trait in premarital screening individuals in Nineveh province.

***Subjects and Methods:*** This is a cross sectional study that was performed in Nineveh province in three main centers for premarital screening program. In the period between the 15 November 2019 and the 15 May 2020 a total of 1127 subjects who attend to these centers were screened for carrier conditions of β-thalassemia. Blood samples of the couples were obtained during attendance to the marriage office. Complete blood counts and hemoglobin variant analysis were performed with automated counter and high performance liquid chromatography technique.

***Results :*** Out of 1127, β-thalassaemia trait was diagnosed in 47 subjects, 31 were male (66 %) and 16 were female (34 %). The mean of their hemoglobins was 14.4 g/dl in males, and 12.5 g/dl in females. The mean of mean cell volume and mean cell hemoglobin were 68.3fl and 22.4pg respectively. The mean for HbA2 concentration was 4.9 % and for HbF was 1.2%.

***Conclusion:*** β-thalassaemia trait is usually presented with low MCV and/or low MCH but the accurate and dependable technique in detecting carriers is the use of High Performance Liquid Chromatography (HPLC).

***Keywords:*** β-thalassaemia trait, HPLC, MCH, MCV.

# مؤشرات الدم لصفة البيتا ثلاسيميا في فحص ماقبل الزواج في محافظة نينوى

**بسمة عدنان يونس* ، الاستاذة منى عبد الباسط كشمولة****
***طالب دراسات عليا ، فرع علم الامراض ، كلية الطب ، **فرع علم الامراض ، كلية الطب ، جامعة الموصل ، الموصل ، العراق**

## الخلاصة

**الخلفية:** متلازمات بيتا-الثلاسيميا هي مجموعة وراثية من تشوهات تخليق الهيموجلوبين ، وتتميز هذه التشوهات بخلل في جينات البيتا-غلوبين والتي قد تؤدي إما إلى تقليل أو عدم وجود واحد أو أكثر من سلاسل بيتا-غلوبين من الهيموجلوبين. يمكن أن يكون هذا الخلل الجيني (متماثل الزيجوت) أو (متغاير الزيجوت).

**الهدف من هذه الدراسة:** هو تحديد المتغيرات الدموية لخاصية الثلاسيميا بيتا في فحص الأفراد قبل الزواج في محافظة نينوى.

**طريقة البحث:** في الفترة ما بين ١٥ تشرين الثاني ٢٠١٩ و ١٥ اب ٢٠٢٠ ، تم فحص مجموعه ١١٢٧ فردا ممن حضروا إلى مراكز فحوصات ما قبل الزواج في محافظة نينوى لإجراء فحوصات روتينية بحثاً عن حالات حاملي بيتا ثلاسيميا. تم الحصول على عينات دم من الزوجين. تم إجراء تعداد الدم الكامل وتحليل متغير الهيموجلوبين باستخدام العداد الآلي وتقنية كروماتوجرافيا السائل عالية الأداء.

**النتائج:** شملت الحالات التي تم تشخيصها على أنها صفة ثلاسيميا بيتا ٣١ ذكر (٦٦٪) و ١٦ أنثى (٣٤٪). كان متوسط الهيموجلوبين (Hb) لديهم ١٤.٤ جم / ديسيلتر عند الذكور ، و ١٢.٥ جم / ديسيلتر عند الإناث. كان متوسط حجم الخلية (MCV) هو (٦٨,٣) فل ومتوسط الهيموجلوبين الخلوي (MCH) هو ٢٢.٤ بيكوغرام ، وكان متوسط تركيز HbA2 هو ٤,٩٪ و متوسط تركيز HbF كان ١.٢٪ .

**الاستنتاج:** عادةً ما ترتبط سمة ثلاسيميا بيتا مع MCV منخفض و / أو MCH منخفض ولكن الأسلوب الدقيق والموثوق في الكشف عن الحاملات هو استخدام الكروماتوجرافيا السائلة عالية الأداء (HPLC).

**الكلمات المفتاحية:** حاملي بيتا ثلاسيميا, تقنية كروماتوجرافيا السائل عالية الأداء, متوسط حجم الخلية (MCV) , متوسط الهيموجلوبين الخلوي (MCH).

## INTRODUCTION

β-Thalassaemia syndromes are inherited group of abnormalities of hemoglobin synthesis which is autosomal recessive. These abnormalities characterized by a defect in β-globin genes which may result either in the reduction or absence of one or more of the β-globin chains of hemoglobin. This genetic defect can be homozygous in which both genes are affected or heterozygous in which only one gene is affected. [1,2]. The β_thalassaemia alleles may be classified functionally as $\beta^0$ or β+ resulting in: $\beta^0$_ thalassaemia phenotype in which there is a total absence of β-globin synthesis and the most severe form, and β+ thalassaemia in which there is some, decreased β globin formation. Mild β-thalassaemia, occasionally referred to as β++, there is production of a moderate amount of β globin, where as in the 'silent' β thalassaemia, there is minimal reduction in β chain formation, carriers have normal or minimally reduced red cell parameters and normal HbA2 levels. The main molecular defects in β- thalassaemia are point mutations result from single base substitutions, minor insertions or deletions of a few bases inside the gene.[3]. β-thalassaemia trait, usually show mild or no anemia in their complete blood count (CBC) , and their mean cell volume (MCV) and mean cell hemoglobin (MCH) are frequently low, the red blood cell (RBC) count is usually high. Decreased synthesis of β-globin leads to an inability to produce normal amount of HbA (α2β2), this is compensated by increasing synthesis of other β-like chains, like δ and γ, these increase the levels of the minor hemoglobins HbA2 (α2δ2) and HbF (α2γ2). This is routinely diagnosed by Hb electrophoresis or Hb HPLC: these will show elevation of HbA2 (i.e., > 3.5% of total hemoglobin) and usually HbF (i.e., >1%).[4]. The aim of this study was to detect the hematological parameters of β-thalassaemia trait in premarital screening individuals in Nineveh province.

## SUBJECTS AND METHODS

The subjects were couples who attend the primary health care centers in Nineveh governorate for routine premarital investigations. A questionnaire was used for each individual and each person's name, age, sex, were recorded, RBC indices (HB, PCV, MCV, MCH, MCHC, RDW, RBC count), HBA0, HBA2 and HBF were done. 1127 subjects were included. All were of Iraqi nationality and living in different parts of Nineveh governorate.

This was a cross sectional study and permissions for this study were taken.

The study was performed in Nineveh Province. The premarital screening program in Nineveh province is controlled by the Ministry of Health and Nineveh Health Directorate. It is performed in three main centers in Nineveh, these are:

-Ibn Al-Atheer teaching hospital in Mosul city.

-Talafar general hospital in Talafar district.

-Al-Hamdania primary health care center in Al-Hamdania district.

Blood Sampling and Sample handling:

A seven mL of blood was aspirated from each subject by venipuncture, 3 ml of which were placed in an EDTA anticoagulant sterile vacutainer tube for manual blood grouping , hematological and red cell indices estimation by an automated cell counter (Swelab/Coulter counter-From Sweden) and also to perform Hb variant analysis by using high performance liquid chromatography technique (the used instrument was the variant Π Beta Thalassemia Short Program, from Bio-Rad laboratories-USA) for measuring and detection of HbA2, HbA0, HbF and other abnormal hemoglobin, while remaining 4 ml of blood were collected in a plain tube for viral screen .

The individual was diagnosed to have β-thalassemia trait when HbA2 >3.5.

## RESULTS

Out of 1127, β-thalassaemia trait was diagnosed in 47 subjects, 31 were male (66 %) with mean age of 25 years and 16 were female (34 %) with mean age of 19 years, their main hematological parameters are shown in table (1). In male, the range of their hemoglobins were 11.3-17.1 g/dl with a mean of 14.4 g/dl and a range of 8.8-15 g/dl with a mean of 12.5 g/dl in female. The mean MCV and MCH were 68.3 fl and 22.4pg respectively, and the mean of HbA2 concentration was 4.9% with a range of 3.6-6.9% figure (1). The mean and range of HbF was 1.2% and 0.2% -10.3% respectively. The mean RBC count was 6.3 × $10^{12}$/L. The MCV and MCH were >76 fl and >25 pg

in 9 cases, and 2 cases their MCV were >76 fl but their MCH <25 pg and only one case had MCH >25 pg but low MCV <76 fl. This signifies that not all cases with β-thalassemia trait should have low MCV and low MCH. Mean of (RDW %) was 14.1 % and only in four cases it was elevated.

Table (1): The main hematological parameters in cases with β-thalassemia trait.

| SEX | | Hb g/dl | Pcv % | MCV fl | MCH pg | MCHC g/dl | RDW % | HbA0* % | HbA2 % | HbF % | RBC×$10^{12}$/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M | N=31 (66.0%) | | | | | | | | | | |
| | Mean | 14.4 | 44.1 | 67.7 | 22 | 32.4 | 13.9 | 83.5 | 4. 9 | 1 | 6.7 |
| | Std. Deviation | 1.5 | 3.9 | 9.6 | 4 | 2. 5 | 1.3 | 2.3 | 0.9 | 1.4 | 0. 9 |
| | Minimum | 11.3 | 34.8 | 56.3 | 16 | 25 | 11.4 | 79 | 3.6 | 0.2 | 4.9 |
| | Maximum | 17.1 | 50 | 90.9 | 31.5 | 36.4 | 17 | 87.5 | 6.9 | 7.9 | 8.8 |
| F | N=16 (34.0%) | | | | | | | | | | |
| | Mean | 12.5 | 37.4 | 69.5 | 23.2 | 33.3 | 14.5 | 79.5 | 4.8 | 1.5 | 5.5 |
| | Std. Deviation | 1.9 | 5. 4 | 9.4 | 3.7 | 1.6 | 2.7 | 20. 2 | 0.9 | 2.5 | 0.8 |
| | Minimum | 8.8 | 25.7 | 57 | 18 | 30.7 | 11.4 | 4 | 3.6 | 0.2 | 3.3 |
| | Maximum | 15 | 45 | 84.4 | 30.5 | 36.2 | 21.5 | 87.3 | 6.4 | 10.3 | 6.9 |
| Total | N=47 | | | | | | | | | | |
| | Mean | 13.7 | 41.9 | 68.3 | 22.4 | 32.7 | 14.1 | 82.1 | 4.9 | 1.2 | 6.3 |
| | Std. Deviation | 1.9 | 5.5 | 9.5 | 3.9 | 2.2 | 1.9 | 11.8 | 0.9 | 1.8 | 1 |
| | Minimum | 8.8 | 25.7 | 56.3 | 16 | 25 | 11.4 | 4 | 3.6 | 0.2 | 3.3 |
| | Maximum | 17.1 | 50 | 90.9 | 31.5 | 36.4 | 21.5 | 87.5 | 6.9 | 10.3 | 8.9 |

*HbA0 is the pure non glycated hemoglobin. [5].

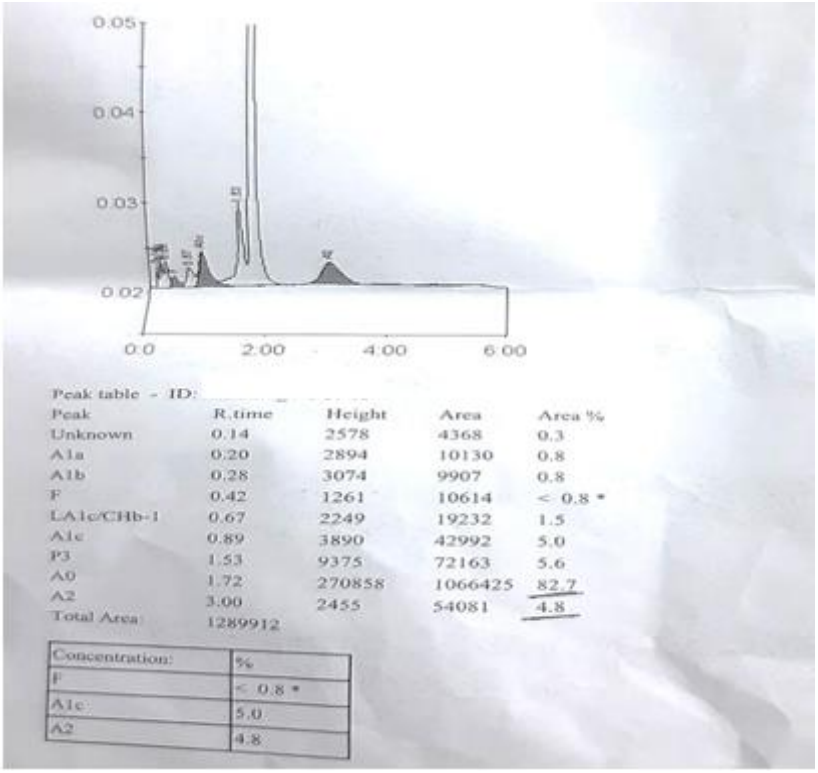


Figure (1): Chromatogram of a case with β-thalassaemia trait

## DISCUSSION

Nineveh is the second largest Iraqi province regarding population size. It lies at the northwest of Iraq. An important finding in this study is the mean of red cell indices in cases with β_thalassemia trait which include relatively normal hemoglobin with elevated RBC count and reduced MCV, MCH which is disproportional to the level of hemoglobin, and this finding is consistent with other studies as.[6,7,8]. Al-Suliman also mentioned low MCV & MCH in β-thalassemia trait in his study [9].

An important issue was that the majority of cases in this group their MCV and MCH were <76 fl and <25 pg (as these were the cut off points of the kits used), but still there were some cases with higher levels of MCV and MCH (The MCV and MCH were >76 fl and >25 pg respectively in 9 cases, and 2 cases their MCV were >76 fl but their MCH <25 pg and only one case had MCH >25 pg but low MCV <76). This signifies that not all cases with β-thalassaemia trait should have low MCV and low MCH. This also signifies that we should depend on both MCV and MCH not only one of them (at least one of them is lower than normal) and this is similar to other studies like.[4,9,10] . While other depended on MCV only as in.[11], Karimi & Pranpanus had considered the MCH as the only parameter to depend on in the screening for thalassemia.[12,13] . Findings also signify the importance of using HPLC in detecting β-thalassemia trait and not to depend on MCV, MCH only to diagnose β_thalassemia trait.

The RBC count was elevated in nearly all cases (43 cases & 93.5%) and this is consistent with other study. [14,15] This increase is due to pathophysiology of the disease as globin chain excess precipitate in erythroid precursors and RBC leads to a discrete ineffective erythropoiesis that causes increased production of RBC in order to compensate for anemia.[16].

Red cell distribution width (RDW), which is one of the red blood cell indices in (CBC), reveals the degree of anisocytosis of red blood cells. This is considered as a significant value in differentiation between patients with IDA and β-thalassemia trait.[17,18] . RDW tends to be higher in iron deficiency anemia than in thalassemia minor because thalassemia minor show homogeneous microcytosis in comparison with that of iron deficiency anemia patients.[19] . In microcytic MCV ranges, RDW-CV had the best sensitivity (86.8%) and efficiency (86.8%) in detecting anisocytosis. Whereas, RDW-SD was more sensitive and efficient in normocytic (82.9% and 83.3%) and macrocytic (90.2% and 90.2%) MCV ranges.[20] . The mean of RDW in this group of β-thalassemia trait was within normal range, and it was found normal in β- in many studies as in.[18, 21], while Yousafzai found that RDW in β_thalassemia trait was higher than normal. [7]. This is because reduction of β-globin synthesis in thalassemia minor lead to homogeneous microcytosis.[19] .

The cut-off point of HbA2 used for diagnosing the β-thalassemia trait is more than 3.5%, which is consistent with other studies such as.[8, 22, 23]. The mean value of HbA2 in this group was 4.9%. The mean of HbF in β-thalassemia trait was 1.2% which was considered significantly different from that of normal individuals (0.5%) and this is expected in β-thalassaemia trait as a compensatory mechanism for the reduction in the synthesis of β_globin chain.

If the individual has low MCV, low MCH, but normal HbA2, this may be due to iron deficiency anemia or may be a silent B-thalassemia carrier; this is differentiated by measuring serum ferritin which is low in iron deficiency anemia.

When a subject had a low Hb, low MCV, low MCH, borderline HbA2, measure serum ferritin to detect any evidence of iron deficiency anemia which affect HbA2 level.[24, 25, 26] .

## CONCLUSION

β-thalassaemia trait is usually presented with low MCV and/or low MCH but the accurate and dependable technique in detecting carriers is the High Performance Liquid Chromatography (HPLC) where HbA2 is usually elevated to >3.5%.

## REFERENCES

1. Weatherall DJ, Clegg JB. The Thalassaemia syndromes. (4th ed.). Blackwell Science 2001.
2. Savongsy O, Fucharoen S, Fucharoen G,Sanchaisuriya K,Sae-Ung N et al. Thalassemia and hemoglobinopathies in pregnant Lao women: carrier screening, prevalence and molecular basis. Ann Hematology. 2008; 87(8): 647–54. doi: 10.1007/s00277-008-0490-z
3. Thein SL. Beta thalassaemia. Bailliere's Clin Haematol . 1998; 11(1): 91–126. doi:10.1016/S0950-3536(98)80071-1
4. Leung TN, Lau TK , Chung T. Thalassaemia screening in pregnancy. Curr Opin Obstet Gynecol. 1998; 17(2): 129–34. doi: 10.1097/01.gco.0000162180.22984.a3
5. Muñoz-Piñeiro A, Schimmel H, Klein C. Certification of the amount-of-substance fraction of HbA0 versus the sum of all Hb isoforms forming the glycated or non-glycated N-terminal hexapeptide of the β -chain in haemoglobin isolated from whole blood (internet) Publications Office of the EU (JRC Scientific and Technical Reports). 2007; Doi: 978-92-79-06043-4

6. Faraj S, Mahdi LS, Ghali H. Significance of Red Blood Cell Indices in Beta-Thalassaemia Trait. Mustansiriya Medical Journal. 2015; 14(2): 28-32. doi: 10.13140/RG.2.1.1866.6000
7. Yousafzai YM, Khan S, Raziq F. Beta-thalassaemia trait: haematological parameters. JAMC. 2010; 22(4): 84–86.
8. Denic S, Agarwal M M, Al Dabbagh B,El-Essa A, Takala M, Showqi S,Yassin J et al. Hemoglobin A2 Lowered by Iron Deficiency and α -Thalassemia: Should Screening Recommendation for β -Thalassemia Change?. ISRN Hematology. 2013;2013:1-5 858294. doi: 10.1155/2013/858294
9. Al-Suliman A. Prevalence of beta-thalassemia trait in premarital screening in Al-Hassa, Saudi Arabia. *Ann Saudi med. 2006; 26*(1): 14–16. doi: 10.5144/0256-4947.2006.14.
10. Ma ES, Chan AY, Ha SY,Lau YL, Chan LC. Thalassemia screening based on red cell indices in the Chinese. Haematologica. 2001; 86(12): 1310–11.
11. Lafferty J D, Crowther MA, Ali MA,Levine M. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. *AJCP.* 1996;*106*(2): 201–05. doi: 10.1093/ajcp/106.2.201.
12. Karimi M, Rasekhi AR. Efficiency of premarital screening of beta-thalassemia trait using MCH rather than MCV in the population of Fars Province, Iran. Haematologia. 2002; 32(2): 129–133. doi: 10.1163/156855902320387961.
13. Pranpanus S, Sirichotiyakul S, Srisupundit K, Tongsong T. Sensitivity and specificity of mean corpuscular hemoglobin (MCH): for screening alpha-thalassemia-1 trait and beta-thalassemia trait. J Med Assoc Thai. 2009; 92(6): 739–43.
14. Hesham M, Besher M , Khalifa N. Screening for B-Tthalassemia carrier among students in secondary school in Diarb Negm, Sharkia. Z.U.M.J. 2018; 24(1): 72-79. doi: 10.21608/zumj.2018.13007.
15. Jameel T, Baig M, Ahmed I,Hussain MB, Alkhamaly MBD . Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. Pak J Med Sci. 2017;33 (3):665-69. https://doi.org/10.12669/pjms.333.12098
16. Hoffbrand A V, Catovsky D, Tuddenham E G, et al. Postgraduate hematology. (6th ed.). Willey-Blackwell 2011; 39-40, 88, 91.
17. Aslan D, Gumruk F, Gurgey A, Altay C. Importance of RDW value in differential diagnosis of hypochrome anemias. Am J Hematol. 2002; 69(1):31-3.
18. Bessman J D, Gilmer P R Jr , Gardner F H. Improved classification of anemias by MCV and RDW. AJCP. 1983; 80(3): 322–26. doi: 10.1093/ajcp/80.3.322
19. Trent RJA. Diagnosis of the haemoglobinopathies. Clin Biochem Rev. 2006; 27(1): 27-38.
20. Caporal F , Comar S. Evaluation of RDW-CV, RDW-SD, and MATH-1SD for the detection of erythrocyte anisocytosis observed by optical microscopy. J Bras Patol Med Lab. 2013; 49(5): 324-31.Doi:10.1590/S16762444201300050005.
21. Romero A J, Carbia CD, Ceballo MF,Diaz NB. Red cell distribution width (RDW): its use in the characterization of microcytic and hypochromic anemias. Medicina. 1999; 59(1): 17–22.
22. Firkin F, Chesterman C, Penington D,Rush B. de Gruchy's Clinical Hametology in Medical practice. (5th ed). Blackwell 1996; 137-71.
23. Ou Z, Li Q, Liu W,Sun X. Elevated hemoglobin A2 as a marker for β-thalassemia trait in pregnant women. Tohoku J Exp Med. 2011; 223(3): 223–26. doi: 10.1620/tjem.223.223
24. Old J, Harteveld CL, Traeger-Synodinos J,Petrou M, Angastiniotis M, Galanello R et al. *Prevention of Thalassaemias and Other Haemoglobin Disorders: Volume 2: Laboratory Protocols*. (2nd ed.). Thalassaemia International Federation 2012.
25. Paglietti ME, Satta S, Sollaino MC,Barella S, Ventrella A, Desogus MF et al. The Problem of Borderline Hemoglobin A2 Levels in the Screening for β-Thalassemia Carriers in Sardinia. Acta Haematol. 2016; 135(4): 193–99. doi: 10.1159/000442194.
26. Viprakasit V, Limwongse C, Sukpanichnant S, Ruangvutilert P, Kanjanakorn C, Gomglao W et al. Problems in determining thalassemia carrier status in a program for prevention and control of severe thalassemia syndromes: a lesson from Thailand. Clin Chem Lab Med. 2013; 51(8): 1605–14. doi: 10.1515/cclm-2013-0098.