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Diagnostic significance of MCV, MCH AND NESTROFT in thalassemia minor individuals

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ABSTRACT

Thalassemia is a blood disorder passed down through families (inherited) in which the body makes an abnormal form of hemoglobin, the protein in red blood cells that carries oxygen. The disorder results in excessive destruction of red blood cells, which leads to anemia. Thalassemia minor occurs if a person receives the defective gene from only one parent. If a person inherit defective gene from both parents then it will result in thalassemia major. Therefore carrier identification is necessary in order to prevent the major form of thalassemia. Although sophisticated methods of screening have become available, a hunt for a cheap, rapid, objective screening method still remains elusive. So in microbiology department at Jinnah University for women we design a study project to evaluate the significance of cell counter–based parameters that are MCV and MCH and to appraise the usefulness of Naked-Eye-Single-Tube-Osmotic-Fragility-Test (NESTROFT) in detection of thalassemia minor. For this purpose 100 thalassemia minor individuals were selected for the study along with control (normal) sample. Their blood samples were subjected to NESTROFT and CBC samples were analyzed on cell counter Sysmex K21 for the estimation of MCV and MCH. All individuals give positive NESTROFT. MCV and MCH values were significantly low. MCV values were between 56-75fl with a mean of 64.552fl and MCH values were between15-24pg with a mean of 19.603pg. This indicates MCV <80fl and MCH <27pg are the significant diagnostic parameters of thalassemia minor.

Key Words: Thalassemia, MCV, MCH, NESTROFT.

INTRODUCTION

The term thalassemia is derived from the Greek, thalassa (sea) and haima (blood). These are a group of inherited autosomal recessive hematologic disorders that cause hemolytic anemia because of the decreased or absent synthesis of a globin chain. Imbalances of globin chains cause hemolysis and impair erythropoiesis. Originally diagnosed in Greece, the disease was named thalassemia (the sea) because of the geographical location of Greece along the Mediterranean Sea since its original discovery; the disease has been found to be prevalent in the Mediterranean countries, in the Middle East, the Indo-Pak subcontinent and the South-East Asian countries. This stretch of land is collectively called the thalassemia belt. With rapid migration of the population across the globe and the world transforming into a global village, the concept of 'thalassemia belt' is giving way to globalization of thalassemias (Moin, 2010). Thalassemia trait affects 5 to 30 percent of persons in these ethnic groups. Scientists and public health officials predict that thalassemia will become a worldwide issue in the next century (Elena, 2010). Only forty years ago Thalassaemia was considered to be non-entity (Moin, 2010). The gene frequency of â-thalassemia, however, is high and varies considerably from area to area,

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having its highest rate of more than 10% around the Caspian sea, and Persian Gulf. The prevalence of the disease in other areas is between 4% and 8% (Kasimi, et al. 2011).

Hemoglobin is made of two proteins: Alpha globin and beta globin. Thalassemia occurs when there is a defect in a gene that helps control production of one of these proteins. There are two main types of thalassemia: Alpha thalassemia occurs when a gene or genes related to the alpha globin protein are missing or changed (mutated).Beta thalassemia occurs when similar gene defects affect production of the beta globin protein. Alpha thalassemias occur most commonly in persons from Southeast Asia, the Middle East, China, and in those of African descent. Beta thalassemias occur in persons of Mediterranean origin, and to a lesser extent, Chinese, other Asians, and African Americans. There are many forms of thalassemia. Each type has many different subtypes. Both alpha and beta thalassemia include the three forms: Thalassemia major, variably referred to as "Cooley's Anemia" and "Mediterranean Anemia", Thalassemia minor also called "beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia" and the Thalassemia intermedia which is a case between the minor and major types of this disease. One must inherit the defective gene from both parents to develop thalassemia major. Thalassemia minor occurs if you receive the defective gene from only one parent. Persons with this form of the disorder are carriers of the disease and usually do not have symptoms. Thalassemia intermedia is much severe than the thalassemia minor but less severe than the thalassemia major, and children with thalassemia intermedia may develop some of same complications conferred by thalassemia major. All forms of thalassemia cannot be caught from another individual who has it, and transmitted only through heredity, so the disease is passed on through parents who carry the globin gene disorder. . When two carriers become parents, there is a 25% chance of producing an affected child (thalassemia major), 50% chance of producing a carrier child, and 25% chance of producing a healthy child Galanello and Origa, 2010; Wikipedia).

MATERIALS AND METHODS

100 individuals with thalassemia minor were enrolled in this study. Blood sample were collected from all individuals in tubes containing, EDTA anticoagulant for Complete Blood Count (CBC) to analyze MCV and MCH and for Naked Eye Single-Tube Red Cell Osmotic Fragility (NESTROF) Test. All samples were collected from patients under standard conditions. CBC was analyzed by cell counter (Kx-21 sysmex) within 2 hours of sampling. This instrument was calibrated with reference methods and had a regular quality control program.

1. Naked Eye Single-Tube Red Cell Osmotic Fragility (NESTROF) Test:

SAMPLE:
Blood in EDTA.

REAGENTS:
0.36% buffered saline

PROCEDURE:
Fifty ìl of whole blood collected in EDTA is pipetted into a glass test tube (100 x 10 mm) containing 4 ml of 0.36% buffered saline solution.
Tubes were shaken and left at room temperature for 30 minutes.
After 30 minutes tubes were shaken again and reading was taken on a NESTROFT stand on which a thin black line is marked.

2. COMPLETE BLOOD COUNT (for estimation of MCV and MCH):

CBC was analyzed by cell counter (Kx-21 sysmex)

SAMPLE:
Blood in EDTA

REAGENTS:
· Cellpack (diluent)
· Stromatolyser-WH (WBC/HGB lyse reagent)

DETERGENT:
Cellclean

PROCEDURE:
With this instrument, sample mixing, cap removal, and tube setting are performed manually.
· Power switch was turn on.
· Self-check, auto rinse, and background check was automatically performed, and the "Ready" (ready for analysis) appeared on the screen.
· "PD" was indicated in the system status area on the LCD screen, the pre-diluted (PD) mode is in use for analysis, it was changed to the whole blood (WB) mode.
· Sample number was inputted in the system.
· Collected sample with EDTA was mixed sufficiently in the tube.
· Tube was set to the sample probe, and in that condition, start switch was pressed.
· When the LCD screen displayed "Analyzing," tube was removed. After that, the unit executed automatic analysis and displayed the result on the LCD screen. Then the unit turned to the Ready status, becoming ready for analysis of the next samples.
· Next samples were prepared and above procedure were repeated.

Table 1: Values of MCV in thalassemia minor individuals

Number of patients	Mean corpuscular volume (fL)4 mm
48	61-65
29	66-70
14	56-60
9	71-75
Total:100	**Mean: 64.552**

Table 2: Values of MCH in thalassemia minor individuals

Number of patients	Mean corpuscular hemoglobin (pg)
52	15-19
42	20-24
Total:100	**Mean: 19.603**

RESULTS

Naked Eye Single-Tube Red Cell Osmotic Fragility (NESTROF) Test:
The line was not clearly visible behind all tubes that indicate positive test (thalassaemia trait) due to the turbidity of the solution.

Complete blood count (estimation of MCV and MCH):
The results are given in table 1 and 2. All individuals had significantly low MCV values with a mean of 64.552fL and low MCH values with a mean of 19.603pg. Of 100 individuals, 14 had MCV value ranges from 56-60fL, 48 had MCV value ranges from 61-65fl, 29 had 66-70fL MCV and the remaining 9 had MCV value ranges from 71-75fL. MCH values are also much reduced of these individuals. 58 individuals had MCH value between 15-19pg and 42 individuals had MCH value between 20-24pg. Hence MCV <80fL and MCH <27pg are the significant diagnostic parameters of thalassemia minor.

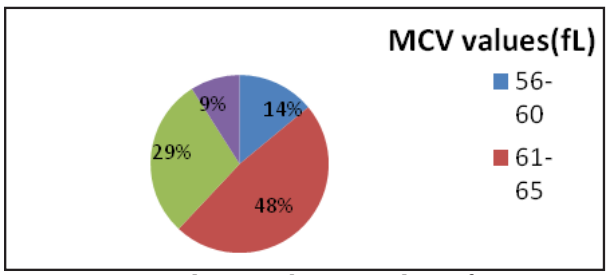

Figure 1: Pie diagram showing values of MCV in thalassemia minor individuals (most of the individuals have MCV value between 61-65fL {<70fL})

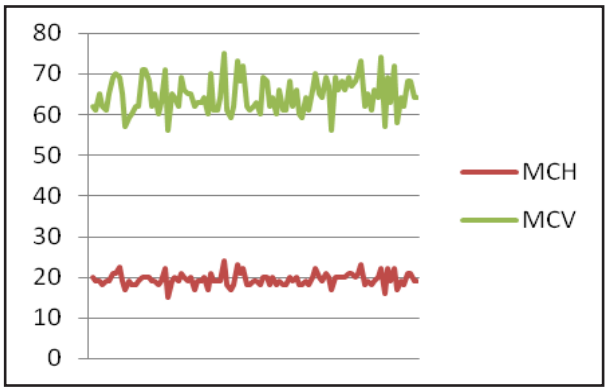

Figure 2: Lower MCV values (<80fL) with lower MCH values (<27fL) in thalassemia minor individuals

DISCUSSION

Thalassemia minor is the most common form of thalassemia. In the present study we check the significance of MCV and MCH values in diagnosing thalassemia minor patients and we also check the efficacy of NESTROF test in determining thalassemia minor patients. We determine MCV and MCH values by CBC cell counter (Kx-21 sysmex). Facility for Hb electrophoresis is not available at many places in Pakistan. Red cell indices given by electronic counters can be reliably used in diagnosing thalassemia. Complete blood counts provided by a routine automated blood counter are major contributors for extensive screening and appropriate detection of carriers. They are inexpensive and easily accessible by individuals who are not professional in hematology.

In present study, we investigated MCV, MCH values and NESTROF test results of 100 thalassemia minor patients. All of the 100 patients give positive NESTROF test indicating its significance in diagnosing thalassemia carriers. The present study shows that the MCV and MCH levels were significantly lower in cases of thalassemia minor. All patients had MCV values between 56-75fl with a mean of 64.552fl and MCH values between15-24pg with a mean of 19.603pg. The normal range for MCV is 80-99 fL (28) and normal value for MCH is 27 to 31picograms/cell (29). Hence MCV < 80fl and MCH <27pg are the best diagnostic parameters along with positive NESTROF test in detecting thalassemia carriers.

From the present study, we concluded that automated cell counter–based parameters are technically good, rapid, cheaper, easily available, and reliable methods for thalassemia minor detection and NESTROFT is a sensitive, cost effective, rapid and reliable screening test for detection of thalassemia trait. An MCV value together with MCH value is very helpful in estimating the risk of thalassemia minor.

CONCLUSION

According to the results of this study, NESTROFT, MCV and MCH are very helpful, sensitive, cost effective, rapid and reliable screening tests for the detection of thalassemia trait. They are inexpensive and easily accessible by individuals who are not professional in hematology.

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REFERENCES

Elena FC. 2010. Beta- thalassemia trait - epidemiological and clinical aspects in children in Constanta County. PhD thesis. University Of Medicine And Pharmacy Craiova.

Moin. 2010. Introduction to Thalassemia. Pak Haematology. Available from: http://www.pakhematology.com/enter/introduction-to-thalassemia/.

Karimi M, Emadmarvasti V, Hoseini J and Shoja L. 2011. Major Causes of Hospital Admission in Beta Thalassemia Major Patients in Southern Iran. Iran J. Pediatr., 21 (4): 509-513.

Galanello R and Origa R. 2010. Beta-thalassemia: Galanello and Origa Orphanet. J. Rare Dis., 5(11):1-15.

Wikipedia. Thalassemia. Available from: http://en.wikipedia.org/w/index.php?oldid=395435875.