Evaluation of M/H Ratio for Screening of B Thalassaemia Trait

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Abstract

This study was done to evaluate the efficiency of M/H ratio between the percentages of microcytic and hypochromic cells as a screening procedure for thalassaemia trait in Pakistani population. A total of 150 subjects, were included in this study. The analysis of results revealed that M/H ratio is a very sensitive index for B thalassaemia trait. The sensitivity, predictive value and diagnostic accuracy of the M/ll ratio for thalassaemia trait was 100%, 90.4% and 90.4% respectively. It also picked up all cases of B thalassaemia trait with co-existent iron deficiency. It is therefore concluded that M/H ratio is an easy, reliable and sensitive index which can be used for mass screening of B thalassaemia trait, particularly in a population where iron deficiency is also prevalent (JPMA 45: 84, 1995).

Introduction

Thalassaemia is the commonest inherited disorder present world wide. Pakistan also lies in the thalassaemia belt and B thalassaemia is common here[1]. If an average of 5.4% is taken as the National Carrier Rate[2,3], there would be approximately 5-6 million carriers in Pakistan.

The average cost of treating one thalassaemic patient is Rs. 10,000 per annum in addition to blood requirements which is unaffordable with our limited resources. Under these circumstances the best approach will be to prevent the birth of children with thalassaemia major. The single most effective method of prevention is pre-marital screening and genetic counselling[4]. This requires population screening for 13 thalassaemia trait. Several screening procedures have been devised but these are either not cost effective or not sensitive enough[5-10].

A simple procedure, introduced recently primarily for discrimination of B thalassaemia trait from iron deficiency anaemia, is the ratio between percentage of microcytic and of hypochromic cells (M/H ratio). The Technicon H I auto-analyzer research screen in addition to other information, also provides percent value of hypochromic and microcytic cells. M/H ratio iscalculated by dividing percent value of microcytic cells by percentvalue of hypochromic cells. It is high in (>0.9) in 13 thalassaernia trait and low (<0.9) in iron deficiency anaemia. This ratio has yielded sensitivity of upto 100% for beta thalassaemia trait in some studies[11]. In this study we have evaluated the M/H ratio as a screening procedure for B thalassaernia trait, in Pakistani population where iron deficiency is also very common.

Subjects and Methods

The material comprised of 2903 subjects referred to the department of haematology, Armed Forces Institute of Pathology, Rawalpindi, for completeblood counts (CBC)forvarious reasons. They comprised of both sexes, all ages and belonged to personnel of Armed Forces and their families as well as civilian population. M/H ratio was calculated on all the blood samples ran on TechniconTM H 1 auto-analyzer. Individuals having MJH ratio of >0.9 were suspected of having B thalassaemia trait and were called back for confirmatory investigations. The number of such individuals was 100 and they comprised the study group. The control group comprised of 50 subjects who were diagnosed as carriers

of B thalassaemia trait by Hb A2 estimation and family studies, where required. They were calledback forfreshblood samples for estimation of M/H iatio and repeat estimation of Hb A2 and serum ferritin levels. All individuals were subjected to detailed interview and thorough clinical examination. Seven ml of venous blood was collected from each of the subjects from both study and control groups through a clean venepuncture and distributed in the following manner:

1. Two ml blood was mixed with potassium EDTA to a final concentration of 2.0 mg/ml and used forblood count and calculation of MJH ratio.
2. Two ml blood was mixed with potassium EDTA to a final concentration of 2.0 mg/mI and used for preparation of haemolysate for Hb electrophoresis and estimation of Hb A2.
3. Three ml blood was transferred into a sterile glass test tube and allowed to clot. Clear serum was separated into a sterile cryotube and preserved at -20°C forestimation of serum ferritin.

All complete blood count(CBC) and percentage of microcytic (M) and hypocliromic (H) cells were measured on TechniconTM H1 haematology auto-analyzer within two hours of sample collection (percentages of microcytic and hypochromic cells were obtained by using research screen programme). The M/H ratio was then calculated by the following fonnula:

MJH Ratio = % Microcytosis % Hypochromia

The equipment was calibrated daily by using low, normal and high commercial controls obtained from TechniconTM. Quality control was performed by running same normal specimen after every 19 test specimens. Estimation of Hb A2 was done spectrophotornetrically after elution of the haemoglobin bands separated on cellulose acetate strips in TRIS/EDTA/Borate buffer at PH 8.6[12]. Serum ferritin estimation was performed by Radioimmunoassay (RIA) technique utilizing kit manufactured by kodakTM (UK). Calibration was performed by using standards of 0-1000 ng/ml. Quality control was performed within assay and between assay running of controls. The sensitivity, predictive value and diagnostic accuracy of M/H ratio for B thalassaemia trait was calculated according to the method of Galen and Gambino[13].

Results

The results of various haematological parameters and diagnostic tests of control and study groups are presented in Table 1.

Table I. Haematological parameters and diagnostic tests of control and study group.

Parameters and Diagnostic Tests	Control group (n=50)	Study Group (n=100)
Hb (g/l)	126±17.14	117±21.2
TRBC (10^{12}/1)	6.3±0.8	5.3±0.7
MCV (fl)	65.1±4.5	67.0±7.2
MCH (pg)	20±1.5	21.2±3.1
Microcytosis (%)	30.7±16.9	27.8±15.9
Hypochromia (%)	17.4±11.8	17.5±10.2
Hb A_2 (%)	5.0±0.6	3.9±0.8
Serum ferritin levels (ng/ml)	98.3	85.9

The age of controls ranged from 18 to 55 years (mean 30.5 years) and male to female ratio was 1.4:1.

Twenty_five (50%) were offsprings from first cousins, 8(16%) had related parents while parents of 17(34%) had no relationship before marriage. Of these 48 subjects had M/H ratio of >0.9. The sensitivity of MJH ratio for B thalassaemia trait was calculated to be 96%. The serum ferritin was nonnal in all of them.

The age of subjects ranged from 1 to 70 years (mean 22.9 years) and male to female ratio of 0.9:1. The parents of 56 (56%) were first cousins and of 11(11%) were related but not first cousins. The parents of 33 (33%) had no previous relationship. On the basis of Hb A2 and serum ferritin levels, they were divided into 4 groups

Table II. Haematological parameters and diagnostic tests of study group.

Parameters and DiagnosticTests	βTT (n=67)	βTTID (n=18)	IDA (n=9)	Misc. (n=6)
Hb (g/l)	122±16.7	106±27.2	88.0±11.6	147±23.0
TRBC (10^{12}/l)	5.4±0.8	5.0±0.3	4.6±0.4	5.0±0.6
MCV (fl)	66.5±4.9	63.2±8.3	66.8±5.6	83.6±7.4
MCH (pg)	20.8±2.1	19.7±3.2	21.0±2.2	29.3±2.7
Microcytosis (%)	28.6±16.8	29.8±14.8	28.1±6.6	8.3±5.9
Hypochromia (%)	17.5±9.8	19.2±12.5	20.3±7.1	7.8±5.9
Hb A_2 (%)	4.2±0.7	4.0±0.3	2.5±0.6	2.8±0.3
Serum ferritin (ng/ml)	118.6	5.35±2.9	4.3±3.3	86.1

(Table II) f3 thalassaemia trait (BTT) (Hb A2 >3.5% and serum ferritin >16 ng/ml) was seen in 67 subjects (67%), B thalassaemia trait with irondeficiency (BTTID) (Hb A2 >3.5% and serum ferritin<16 ng/ml) seen in 18 subjects (18%), Iron deficiency anaemia (IDA)(Hb A2 <3.5% and serum ferritin <16 ng/ml) in 9 subjects (9%) and miscellaneous/unclassified (Hb A2 <3.5% and serum ferritin >16 ng/ml) in 6 subjects (6%).

The M/H ratio correctly identified all the cases of BTT and BTTID. There were 9 false positives which actually belonged to IDA category. The sensitivity and predictive value of M/H ratio for BTT was 100% and 90.4% respectively. The diagnostic accuracy was 90.4%. Six cases belonged to the miscellaneous (unclassifiable) group with normal Hb A2 and serum ferritin levels. These cases could be of 13 thalassaemia trait with normal Hb A2 levels, A thalassaemia trait or sB thalassaemia but the exact cause could not be elucidated.

Discussion

There are only two studies reported previously on the M/H ratio[11,14]. The results of these studies along with the present regarding sensitivity of MJH ratio for 13 thalassaemia trait are presented in Table III

Table III. Efficiency of M/H ratio for βTT- Previous studies and present study.

Studies	Sensitivity (%)	Predictive value (%)	Diagnostic accuracy (%)
d'Onofrio et al., 1992	100	92.3	93.3
Khattak et al., 1992	94.2	84.4	89.5
Present study	100	90.4	90.4

The present study was undertaken with main object of evaluating efficiency of MJH ratio in screening for B thalassaemia trait only. The sensitivity, predictive value and diagnostic accuracy of this index for B thalassaemia trait was found to be 100%, 90.4% and 90.4% respectively which almost match the results of study carried out by others[11] ". M/H ratio also picked up all cases of thalassaemia trait with in deficiency (BTTID) successfully. Findings of this study indicate that M/H ratio is specific and sensitive enough to be utilized as a population screening procedure. It is superior to all so far described indices. The only drawback of this index is that it is only available on a specific haematology auto-analyzer (Technicon TM H1) which is an expensive equipment and not available in small laboratories. However, since the test is performed on EDTA anticoagulated blood samples, it may still be feasible to collect and transport specimen to a central laboratory and carry out the test there. This may even be more cost effective.

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