Formula in differentiating iron deficiency

ABSTRACT

Background : Microcytic anemia is the most common hematologic disorder encountered in Iran. Easy and early differentiation of iron deficiency anemia (IDA) and beta thalassemia trait (TT) is an important action particularly in areas where advance equipment is inadequate.

Objective : The purpose of the present study is to compare the validity of new index with the various discriminating functions (DF) by calculating statistical tests.

Methodology : 317IDA and 506 TT aged 15 to 35 years old enrolled in this study. The present new formula [Bijan Keikhaei index (BK)] and the other published DFs [(Mentzer Index (M), Green & King Index (G&K), RDW Index (RDWI), England & Fraser Index (E&F), Bessman (RDW), Telmissani (T), Srivastava (S), Shine and Lal (S&L), Ricerca (R), Ehsani (E), M, Sirdah (MS), and Red Blood Cell Count(RBC)] were calculated in all patients.

Results : All thirteen DFs didn't have the sensitivity and specificity of 100%. The BK, RDWI, G&K and E&F showed the most sensitivity and specificity for both IDA and TT; moreover, the lowest reliable indices belonged to RDW, S&L and R.

Conclusion : According to Youden's index (YI), DFs in the order of largest to smallest were BK > G&K > RDWI > E&F > RBC> M > E > T > MS > S > R> S&L > RDW.

KEYWORDS : ß-thalassemia trait, Iron deficiency anemia, Discriminating function, new formula

INTRODUCTION

thalassemia minor and IDA are two common microcytic anemias in the Mediterranean area. 1 The incidence of TT and IDA in Iran are 5-10% and 30-45% respectively. 2, 3 Premarriage genetic counseling program for prevention of thalassemia major in Iran has been started since 1997. 2 With respect to high prevalence of anemia among young couple, simple and easy diagnosis of TT and IDA is crucial. Determination of CBC by electronic cell counter is a first step in the diagnosis of TT and IDA in mass screening strategy. Both anemia are usually present with microcytosis (MCV <80) and hypochromia (MCH (<27). 4 Based on the Red Blood Cell indices, many mathematical formulas for possible diagnosis of TT and IDA have been presented. Since the early 1970, different DFs of CBC parameters have been proposed. England&Fraser5 1973; Mentzer 6 1973; Srivastava & Bevington7 1973; Shine-Lal8 1977; Green&King9 1989; Lafferty et al 10 1996; Jayabose et al. 11 1999; Telmissani et al. 12 1999, Ehsani et al. 13 2009 and M, Sirdah et al. 14 2008. Based on the statistical criteria an ideal diagnostic test should have a high sensitivity, specificity and YI. 15 It is necessary to compare a new formula with the existing formulas before applying in practice. Therefore, this study intended to evaluate the reliability diagnostic of 12 published DFs and the new present formula (BK index) in differentiation of TT and IDA in south west of Iran.

METHODOLOGY

The research was approved by ethics and university review boards committees. Over a period of 6 months, totally 823 young people in the age range of 15 to 35 with microcytic anemia enrolled in this study. After taking

informed consent the participants divided into two groups , one group including 506 TT carriers in the range of 20 to 32 years (mean: 24. 06 ±3. 28) and the other group comprised of 317 with diagnosis of IDA in the range of 15 to 35 years (mean: 23. 94±4. 24) were selected randomly. CBC for all subjects and serum ferritin and Hemoglobin A2 column chromatography (Hb A2) were done for patients with IDA and TT respectively. Hemoglobin electrophoresis and molecular beta chain analysis were conducted for most of TT group. RBC parameters of all IDA patients were corrected with iron therapy.

After taking blood samples, CBC, ferritin, Hb A2, Hb electrophoresis were carried out with a cell counter k 1000 sysmex; Japan – Elisa, kit: Monobind; Elysis Uno; Germany – kit Bio system; Spain and cellulous acetate media, PH: 8. 5 respectively. Golden standard for diagnosis of IDA and TT were as below:

Hb level lower than 12g/dl (in women) and 13g/dl (in men) and serum ferritin less than 12 ng/ml indicated a diagnosis of IDA; in addition, RBC indices and Hb level of these patients were corrected at the end of a full course of iron therapy.

Hb A2 more than > 3. 5% and normal serum ferritin were determined the diagnosis of TT.

In both groups, mean cell volume (MCV) and mean cell hemoglobin (MCH) were lower than 82fl and 27pg respectively.

It should be noticed that the cases with mixed anemia (TT+IDA) and patients with Hb level lower than 9 g/dl were excluded from the study.

It was attempted to compare 13 different DFs in two groups of IDA and TT with respect to nine parameters including sensitivity, specificity, efficacy, positive likelihood ratio (+LR), negative likelihood ratio (-LR), positive predictive value (PPV), negative predictive value (NPV), AUC or ROC (Receiver Operating Characteristic) and Youden's index in south west of Iran.

Sensitivity and specificity were calculated according to standard formulas, namely: Sensitivity = (TP)/ (TP + FN) and specificity = (TN) / (TN + FP), PPV = (TP) / (TP + FP), NPV = (TN)/(TN + FN), Efficacy = (TP+TN)/(TP +TN+FP+FN), +LR = TP rate/FP rate or Sensitivity/1-Specificity, -LR = FN rate/TN rate or 1-Sensitivity/Specificity, YI = (Sensitivity + specificity) – 100, where TP = true positives, FN = false negatives, TN = true negatives and FP = false positives . The ROC curves to calculate the AUC for each formula were performed. 19 The collected data were treated using the SPSS (version 16) and Microsoft Excel 2007.

RESULTS

The proposed cut off value for the new present formula is 21; results that are equal to and/or lower than cut off value are compatible with diagnosis of TT and more than 21 agree with the diagnosis of IDA. The calculation of all DFs' formulas mentioned before are simple and clear. The results of Telmissani formula (MDHL) should be compared with healthy reference value. For this reason, based on our previous study, the cut off value of 1. 5 for women and 1. 7 for men was obtained. The diagnostic method was that patients with values equal to or below the mean of healthy subjects were most likely to have IDA, and patients with values higher than the mean of healthy subjects were most likely to have TT.

Based on the findings of Table 2, the DFs of BK, G&K, RDWI, E&F, RBC count, M, E, T, MS had considerable sensitivity and specificity for diagnosis of IDA and TT. The highest and lowest sensitivity for IDA and TT belonged to MS, R and S&L, RDW-cv respectively. In addition, the highest and lowest specificity for IDA and TT were related to R, RDW-cv and MS, S&L respectively. MS and RDWI had the highest PPV, NPV and +LR for TT and IDA respectively. The best DF for -LR is related to RDWI and MS for TT and IDA. The lowest DF for PPV, NPV, +LR, -LR was RDW-cv for IDA and TT. The best DF for efficacy and YI are attributed to BK index and the worst belongs to RDW-cv. All DFs (except RDW-cv) have an acceptable efficacy, but with considering both efficacy and YI, all except three DFs (R, S&L and RDW-cv) had approximately scores more than 63%. With regard to YI, the DFs with more than 80% in decreasing order were BK, G&K, RDWI and E&F and YI between 70-80% the DFs from top to down were RBC count, M, E and T and only two DFs of MS and S took the position of YI between 60-70%. Three DFs with decreasing order of YI less than 40% were R, S&L and RDW-cv.

The other valuable points that can be found from table 3 are the comparison between cut-off published value and criterion values for every DF that was obtained from the present patients' hematologic data. Based on the new criterion value the best sensitivity and specificity, preferentially more than 80%, was obtained for each DF. RDW-cv could not take a good sensitivity and specificity even by changing the cut-off value.

DISCUSSION

Since 1970, various DFs have been proposed. The aim of all DFs is to magnify the hematological parameter differences between TT and IDA, so as

to reach a cut-off point with high sensitivity and specificity. The discriminative power of each DF is defined as how much it can amplify the hematological parameter differences between IDA and TT. The performance of each DF not only depends on the formula itself, but is also related mostly to hematological parameters. Based on the hematological parameter differences among populations studied, the varieties of formula results may be observed.

Ehsani et al. 13 Rahim et al. 20 George Natios et al. 21 and Suad M et al. 22 The differences of hematological parameters in inter- populations are related to different thalassemia mutations. There is a significant correlation between MCV and beta chain mutations in TT. 23 The other reason to justify the DFs result differences is attributed to severity of IDA in the range of mild anemia. In addition, population selection bias such as incorporation of children, pregnant women, TT plus IDA and or TT plus alpha thalassemia and IDA with infection should be considered in different studies. Therefore, in on my opinion all DFs are efficient in differentiation of TT and IDA , based on this, with changing the cut-off values of DFs we can reach a considerable set of sensitivity and specificity . 14

A diagnostic test which shows the following characteristics is valuable: superior sensitivity, specificity, efficacy, YI, high PPV, NPV, high score for +LR and low score for -LR and high ROC curve score. 24

The present formula agrees with all of these characteristics and therefore it is a good diagnostic test for differentiation of TT and IDA. However, it needs to be verified by other studies . It also requires to pass a trial in children.

One important point in the comparison of efficiency of different DFs is using the selection of statistical index. The YI is used as an acceptable statistical indicator with the least bias is defined as: (specificity + sensitivity) – 100. 24 With regard to YI, the present study shows the highest reliability for DF of BK followed by G&K, RDWI and E&F. This finding somehow agrees with the data of M, Sirdah et al. 14 George Natios et al. 21 Urrechega et al. 25 Demir et al. 26 and Rahim et al. 20 Rahim et al. proved that DF of RDWI was the best discriminative index in differentiation of TT and IDA. This index is placed in the third position in my study. G&K was not a good index in studies done by Rahim et al. and Suad M et al. Suad M et al. 22 found the DF of E&F was the first. This DF came fourth in my thirteen DFs analysis. In studies by Demir et al. and Beyan et al. 26, 27 RBC count was the reliable index, while in the present research RBC count was rated at number five of the thirteen DFs analyzed. 19 Ehsani et al. showed the DFs of M and E were the best (they did not calculate the DFs RDWI and G&K), while these indices were ranked in the sixth and seventh positions in this study. 13 The DFs of T and MS took the places of moderate reliability in the present study. The indices of RDW, S&L and R showed the least reliability in this research which is in line with studies by M, Sirdah, George Natios and Suad M.

CONCLUSION

None of the DFs shows the sensitivity and specificity of 100%. The present study indicates that the DFs of BK, G&K, RDWI and E&F are the highest reliable discriminator indices in differentiating of TT and IDA in south west Iran. Conversely the DFs of RDW, S&L and R appear ineffective in our population. The remainders suggest a moderate reliability in distinguishing

TT and IDA in the population study. It was found that the two DFs of BK and G&K have totally covered all the statistical characteristics of the good diagnostic tests in differentiation TT and IDA, and the DF of RDW-cv was the worst.

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