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Superiority of Five Discriminant Indices to Distinguish Thalassemia Trait from Iron Deficiency Anemia

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Abstract: The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia and thalassemia trait. It is important to distinguish between anemia and thalassemia trait to avoid unnecessary iron therapy. Many formula of the index are helpful in distinguishing the two disorders, but none of the method showed high sensitivity and specificity. Hb analysis became the gold standard and also required a type of thalassemia mutation that causes microcytic diagnostic anemia thus providing strong evidence for diagnosis. This review suggested that 5 discriminant indices to distinguish thalassemia trait from Iron Deficiency Anemia are best performed in adults than in children.

Keywords: Discriminant Indices, Iron Deficiency Anemia, Thalassemia Trait.

INTRODUCTION:

The most common causes of microcytosis are iron deficiency anemia and thalassemia trait. Iron deficiency anemia is a very frequent finding, not only in developing countries due to deficient nutritional status, but also in the western world, where women of childbearing age are often diagnosed with Iron deficiency anemia due to intermittent blood loss in combination with insufficient iron intake[1]. Thalassemia traditionally has a high prevalence in the Mediterranean area, countries in the Middle East, the Arabic peninsula and Southeast Asia, but nowadays population migration has spread thalassemia genes over nearly the entire globe. To differentiate mild or moderate Iron deficiency anemia from thalassemia trait can be a diagnostic dilemma, as both conditions has many characteristics. Obviously a correct diagnosis in patients with microcytic anemia is important: it can provide an indication for supplementing iron to Iron deficiency anemia patients, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes in premarital counseling in high-prevalence areas.

WHY IT IS IMPORTANT TO DO THIS REVIEW

Key diagnostic parameter for Iron deficiency anemia and thalassemia trait is laboratory tests like ferritin, hemoglobin analysis (HbA2 and abnormal Hb) and DNA analysis. However, in thalassemia endemic areas often have low health care resources and facilities. Therefore, several simple screening indices from the basic

complete blood count have been developed for differentiating between thalassemia trait and Iron deficiency anemia. Some of the comparative studies show a superior discriminant indices but in other studies perform less well. This review was focus on sensitivity and specificity of discriminant indices to distinguish thalassemia trait from iron deficiency anemia.

EXPERIMENTAL :

OBJECTIVES

METHODS

Criteria for considering studies for this review

Type of studies

Random or quasi-random controlled trials or cluster-random trials

Types of participant

Participant with anemia or thalassemia trait

Type of outcome measures

Primary outcomes

The primary outcome was using CBC with the performance of different markers in the differential diagnosis of microcytic anemia as quantified in terms of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) values. These values were retrieved from the publications found and entered into a database.

Secondary outcomes

The secondary outcomes were quantification of Hb A2 (for detection of b-thal) and identification of hemoglobinopathies was performed using High Performance Liquid Chromatography (HPLC). The genomic DNA was analyzed by multiplex amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).

The levels of Serum Iron, Total Iron Binding Capacity were measured with the colorimetric procedure, while the serum ferritin was quantified with the Enzyme Linked Immune Sorbent Assay (ELISA) procedure.

Searching of resources

For finding relevant literature we used PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Scopus (www.scopus.com) and Pro Quest (www.proquest.com), which together cover practically all biomedical journals and many other publications in the field. First, we used the combination search “microcytic and iron deficiency and/or thalassemia” and filtered using the terms “distinguish or differentiate or discriminant”. Second, we identified the original publications of all discriminant indices and searched for publications citing them. Finally, we filtered the original publications by the last ten year of publication and select the discriminant indices most frequently used by researches for review.

Heterogeneity of this review

Heterogeneous results of the research of each journal are not analyzed by using meta analyze but high heterogeneity is exposed due to differences in subject, population, research design.

RESULTS:

Description of studies

Included studies

12 reports for this review Niazi (2010), Ferrara, Trivedi[2], Batebi (2012), Schoorl[3], Sirachainan[4], Pomprasert[5], Moghadam[6], Plengsuree[7], Zaghloul (2016), Salim (2016), Ullah (2016)

Population

1. Niazi (2010)[8] : 312 subject included 213 thalassemia trait and 89 IDA
2. Ferrara (2010)[9] : a cohort study of 458 children of both sexes with 215 thalassemia trait and 243 were shown to be affected by IDA
3. Trivedi (2011)[2] : 216 subjects (age<30 years) included 135 thalassemia trait and 81 IDA
4. Batebi (2012)[10] : 901 subject included 444 thalassemia trait and 457 IDA
5. Schoorl (2012) [3]: 34 subjects thalassemia trait and 142 IDA
6. Sirachainan (2014)[4] : 345 subject with 121 thalassemia trait and 174 IDA
7. Pornprasert (2014)[5] : 265 subject with 21 thalassemia trait and 56 IDA
8. Moghadam (2014) [6]: 177 subject with 100 thalassemia trait and 77 IDA
9. Plengsuree (2015)[7] : 166 subject with 102 thalassemia trait and 64 IDA
10. Zaghloul (2016)[11] : 249 subject with 123 thalassemia trait, 91 IDA and 35 control
11. Salim (2016)[12] : 178 subject with 80 thalassemia trait and 98 IDA
12. Ullah (2016)[13] : 800 subject with 230 thalassemia trait and 570 IDA

Outcomes

The secondary outcomes of this review reported 4 original articles: Plengsuree[7], Sirachainan[4], Pornprasert[5] and Plengsuree[7] using gold standard to detect thalassemia mutations using PCR by various methods including ARMS and q-PCR.

The others reported 12 original articles using HbA2 level for detection thalassemia trait and serum ferritin level to detect Iron Deficiency Anemia.

Heterogeneity of this review

Heterogeneous results of the research of each journal are not analyzed by using meta analysis but high heterogeneity is exposed due to differences in research design, participants, including things that will affect the results of research

DISCUSSION:

Differentiating IDA from thalassemia carrier status is a common issue in medical practice, in particular in subjects with mild or moderate Anemia and in regions where thalassemia is common. Generally to distinguish iron deficiency anemia and thalassemia using simple routine blood counts, as they are both

associated with microcytic and hypochromic erythrocytes. However, in thalassemia RBC do tend to be more microcytic, whereas iron deficient RBC are often more hypochromic[14,15]. These differences have been exploited by developing simple mathematical formulas for emphasizing the differences in RBC indices as a tool for distinguishing IDA from thalassemia trait[15–19]. However, the discriminative power of these simple indices never reached maximum diagnostic performance. The large number of discriminant indices described in the literature reflects that researchers were continuously stimulated devising new and supposedly better indices for applying in their local patient population. In the last decade, multiple studies have been published which compared different discriminant indices in the same patient cohort, aimed at identifying the index with the best overall performance. Yet, no single index emerged as the best and it became evident that even the performance ranking of the indices was different across the various investigations. Therefore we review some articles to provide an overview of the advantages and disadvantages of 5 discriminant indices most frequently used in study from 2010 to 2016. For this review we discuss Mentzer index[17], England & Fraser[16], Shine&Lal[18], Bessman[14] and Green&King[19] based on the frequency of use of 12 studies on discriminant indices

Most discriminant indices were designed for distinguishing IDA and thalassemia in subjects with microcytic RBC. These two conditions explain the vast majority of microcytic RBC, but other diseases may be associated with microcytosis, too. For example, patients with

anemia of chronic disease (ACD), although most often normocytic, may occasionally have microcytic anemia. In this review 12 studies classified patients similar to Anemia than to thalassemia carriers using Serum ferritin level, TIBC and level HbA2.

The patient's age did not become one that was measured in the 12 studies reviewed. Review article meta-analiysis[20] reported that some indices performed better in adults than in children. The older indices (England and Fraser, Mentzer, Green and King) evidently performed better in adults. In contrast, some newer discriminant indices (Jayabose, Sirdah and Ehsani) had a much better performance in children. The Srivastava and RBC indices appeared to be equally powerful in adults as in children. In this reviewed we found that England&Fraser and Mentzer performed better in adults. Table 2 showed the lower performed Study of Ferrara with a lower sensitivity Mentzer 59%, RDWI 63.7% and 65.1% of England&Fraser.

The type of thalassemia mutation affecting discriminant indices cannot be explained with certainty because in this review only 4 studies (Batebi[10], Sirachainan[4], Pornprasert[5], Plengsuree[7]) have used PCR techniques to analyze the type of mutation in the patient. But they not explained by the mutation details of the patient and its effect on the sensitivity and effectiveness of discriminant indices. In this review 4 studies analayze the mutation of β^0 thalassemia cause they have a similar RBC, Hb, and red cell indices, thus it is impossible to differentiate the β^0-thalassemia mutation types by using red cell indices or formulas[5].

As many of the discriminant indices are based on these and other basic RBC parameters, one might expect that the indices would perform similarly in different areas of the world. However, based on research there is indications for considerable differences between different geographical regions. Based on 12 article for review 5 from mediterranian region (Pakistan[8,13], Iran[6,10],Saudi Arabia[11]), 5 from South East Asia (Indonesia[12], Thailand[4,5,7], India[2]), 1 from Europe[3] and 1 from Italy[9] showed a similar values of 5 discriminant indices based on geographical regions it means there is no significant effect of geographical regions for performance of 5 discriminant indices.

The sensitivity and specificity of the mentzer index to differentiate thalassemia trait varied in the 12 reviewed articles. The most notable variation was seen in the lowest sensitivity value obtained in Schoorl (2012) study that was 48% and the mean sensitivity value in the Moghadam study was 72%. Both did not test the mutation of thalassemia in the patient so it cannot be known whether the type of mutation affects the sensitivity and specificity of the index of the mentzer. In different studies in adults in Thailand the results were very low on 5 discriminant indices and they created a new discriminant indices formula. The formula of (1.5 Hb - 0.05 MCV >14)[4] had the highest AUC with sensitivity and specificity of 84.6% and 87.5%, respectively.

In the England & Fraser index of 5 studies showed the highest sensitivity 100% and specificity 92,9% with PPV value 84% and NPV value 100% in studies conducted by Pornprasert[5]. The lowest value is earned by Plengsuree[7] showed 61.76% sensitifity and specificity 87.50% with value PPV 87.73% and NPV value 58.95%.

In Index Shine& Lal different value of sensitivity and specificity was showed in studies of Pornprasert[5] with the highest sensitivity 100% and specificity 82.1% and studies Zaghloul[11] showed the lowest sensitivity 65.9% and 61.8% specificity. The lowest value can occur cause Zaghloul did not test the type of mutation while Pornprasert test the type of mutation with ARMS-PCR.

Of the several studies using the Bessman index showed a high 90% -100% sensitivity and specificity. Different results in the Pornprasert[5] study showed high results for sensitivity of 100% but low values in specificity of 12.5%.

Several studies using Green&King index showed variation value in sensitivity and specificity. In Moghaddam[6] studies showed consistent value with 84% of sensitivity and specificity. But in different studies of Pornprasert[5] showed the highest sensitivity 100% and lowest specificity 33.9%.

Cut off values used in their research was using the original cut-off values of discriminant indices, with the original formula (Table 2).

Tables:

Discriminant Indices		Niazi (2010)	Ferrara (2010)	Trivedi (2011)	Batebi (2012)	Schoorl (2012)	Sirachainan (2014)	Pornprasert (2014)	Moghadam (2014)
Mentzer	Sensitivity	89%	59%	82.96%	86.3%	48%	-	100%	72%
	Specificity	81%	97.9%	80.24%	85.4%	95%	-	92.9%	82%
	PPV	93%	96.2%	85.4%	85.1%	30%	-	84%	68%
	NPV	71%	36.2%	46.42%	92.2%	98%	-	100%	67%
	Youden Index	70%	56.9%	63.2%	-	-	-	92.9%	-
	AUC (95% CI)	-	-	-	-	0.854	0.50	-	0.819
England & Fraser	Sensitivity	91%	65.1%	79.25%	87.2%	51%	-	100%	67%
	Specificity	56%	99.1%	90.12%	62.9%	96%	-	92.9%	90%
	PPV	74%	98.5%	91.3%	69.5%	36%	-	84%	94%
	NPV	82%	76.2%	33.9%	73.1%	98%	-	100%	53%
	Youden Index	47%	64.2%	69.37%	-	-	-	92.9%	-
	AUC (95% CI)	-	-	-	-	0.918	0.66	-	0.907
Shine & Lal	Sensitivity	72.5 %	-	99.25%	83.1	-	-	100%	64%
	Specificity	100%	-	29.62%	90.6	-	-	82.1%	57%
	PPV	100%	-	88.88%	89.6	-	-	67.7%	44%
	NPV	3%	-	44.8%	86.4	-	-	100%	75%
	Youden Index	72%	-	28.87%	-	-	-	82.1%	-
	AUC (95% CI)	-	-	-	-	-	0.37	-	0.709
Bessman (RDWI)	Sensitivity	91%	63.7%	71.11%	-	-	-	100%	72%
	Specificity	81%	92.5%	74.07%	-	-	-	12.5%	79%
	PPV	94%	88.3%	88.88%	-	-	-	30%	82%
	NPV	76%	74.2%	48.38%	-	-	-	100%	68%
	Youden Index	72%	56.2%	45.18%	-	-	-	12.5%	-
	AUC (95% CI)	-	-	-	-	-	0.34	-	0.819
Green & King	Sensitivity	86%	73.4%	72.5%	-	64%	-	100%	84%
	Specificity	78%	95.06%	70.3%	-	97%	-	33.9%	84%
	PPV	93%	92.6%	86.95%	-	44%	-	36.2%	84%
	NPV	78%	80.2%	46.6%	-	99%	-	100%	84%
	Youden Index	64%	64%	42.8%	-	-	-	33.9%	-
	AUC (95% CI)	-	-	-	-	0.945	0.68	-	0.909

		Plengsuree (2015)	Zaghloul (2016)	Salim (2016)	Ullah (2016)
Mentzer	Sensitivity	82.35%	87.9%	83.6%	83%
	Specificity	79.69%	74.8%	66.2%	84%
	PPV	86.60%	-	75.2%	93%
	NPV	73.91%	-	76.8%	73%
	Youden Index	62.04%	62.7%	-	
	AUC	-	0.878	-	
England & Fraser	Sensitivity	61.76%	95.6%	-	-
	Specificity	87.50%	74.85%	-	-
	PPV	87.73%	-	-	-
	NPV	58.95%	-	-	-
	Youden Index	49.26%	70.4%	-	-
	AUC	-	0.955	-	-
Shine & Lal	Sensitivity	98.04%	65.9%	-	100%
	Specificity	31.25%	61.8%	-	39%
	PPV	69.44%	-	-	93%
	NPV	90.91%	-	-	61%
	Youden Index	29.29%	27.7%	-	-
	AUC	-	0.657	-	-
Bessman (RDWI)	Sensitivity	91.18%	93.4%	91.8%	100%
	Specificity	76.69%	72.4%	75%	93%
	PPV	87.74%	-	81.8%	83%
	NPV	85%	-	88.2%	100%
	Youden Index	70.86%	65.8%	-	-
	AUC	-	0.939	-	-
Green & King	Sensitivity	83.33%	93.4%	96.9%	82%
	Specificity	85.94%	74%	67.5%	88%
	PPV	90.43%	-	78.5%	94%
	NPV	76.39%	-	94.7%	73%
	Youden Index	69.27%	67.4%	-	-
	AUC	-	0.936	-	-

Table 2. Cut off Value of discriminant indices

Discriminant Index	Formula	Cut-off value	Reference
Mentzer	MCV/RBC	13	[17]
England and Fraser (E&F)	MCV-RBC-(5 Hb)-3.4	0	[16]
Shine and Lal (S&L)	MCV^2xMCH	1.53	[18]
Bessman	RDW	15	[15]
Green and King (G&K)	MCV^2xRDW/100 Hb	65	[19]

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