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Original Article

Role of Green & King Index in the Diagnosis of Iron Deficiency with Microcytic Anemia

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ABSTRACT

Objective: This study was carried out to determine the diagnostic accuracy of Green and King Index which based upon simple hematological parameters is thus cheap and easily available for the diagnosis of iron deficiency anemia.

Methods: It was a cross-sectional study carried out in Pediatrics ward of PAEC general hospital between 1stJanuary 2014 to 30thJune 2014. Using Nonprobability consecutive sampling, 70 children with microcytic (<70fl) anemia (<12 mg/dl) were selected and serum Ferritin measured using electrochemiluminescence immunoassay (ECLIA). The automated hematology analyzer was used to calculate G & K Index. A cut off value of below 80 percent of G & K Index was considered as indicative of iron deficiency anemia. Statistical analysis was performed using SPSS 20.0.

Results: In this study, the mean age of the patients was 2.99± 2.69 years. There were 36(51.4%) male and 34 (48.6%) female patients. Among the 70 patients showing microcytic anemia serum, Ferritin was < 15ng/L in 40 of the patients and hence were positive for iron deficiency anemia, whereas 30 patients were negative for IDA.31 patients were detected positive by both Ferritin and G & K index. A total of 5 patients were diagnosed as positive on G & K index but were negative on serum Ferritin and there were 9 patients who were diagnosed as negative on G & K index but were positive on Serum Ferritin. The sensitivity and specificity of Green & King index were 77.5 and 83.33% respectively. Diagnostic accuracy was 51.4%.

Conclusion: G & K index is a useful test for correctly identifying the majority of children with iron deficiency anemia.

Keywords: Anemia, Iron deficiency, Ferritin

Introduction

According to the World Health Organization, iron deficiency is defined as a condition in which there are no mobile iron stores and in which signs of compromised supply of iron to tissues are noted.[1] The more severe stages of iron deficiency are also seen associated with anemia. Anemia resulting from lack of sufficient iron for the synthesis of hemoglobin is the most common hematological disease of infancy & childhood.[2,3] Iron deficiency anemia is believed to affect nearly 700 to 800 million people worldwide.

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South Asia and Africa are the most vulnerable regions with the highest degree of prevalence.[4] Iron deficiency anemia has serious consequences including impaired cognitive development, reduced work capacity, an unfavorable outcome during pregnancy, economic implications and increase in morbidity and mortality.[1] Therefore timely diagnosis is necessary so as to combat the serious effects which may alter the quality of life of an individual later on.

Morphologically, anemia can be classified according to red cell size into microcytic, normocytic and macrocytic. Microcytic anemia is defined as a condition in which the size of red blood cells is smaller than normal. Iron deficiency anemia is microcytic as is beta-thalassemia trait thereby creating confusion on peripheral blood smear examination.[5] Beta-thalassemia is the most common inherited hemoglobinopathy in which there is a defective synthesis of beta globin chains and often is

presumptively diagnosed as iron deficiency anemia when based only on red blood cell (RBC) indices and morphologic features.[6] Iron deficiency anemia (IDA) and beta thalassemia trait (TT) are the most common causes of microcytic anemia.[7,8]The differentiation between thalassemic and iron deficiency microcytosis has important clinical implications, particularly in a multiethnic Pakistani population because each has an entirely different cause, pathogenesis, prognosis,and treatment. IDA is usually short-term and can be cured by various oral and intravenous iron supplements whereas this is not the case with Beta thalassemia minor. Prevalence of IDA in Pakistan is 45%.[9]

In an era of rising consciousness, efficient diagnostic approaches, which can rule in or rule out the disease with sufficient accuracy so that invasive and expensive testing is minimized, are particularly welcome. The gold standard for identifying iron deficiency is a direct test; bone marrow biopsy with Prussian blue staining.[10] However, bone marrow aspiration is too invasive for routine use, and is also not available in small set-ups, so indirect assays generally are used. Serum Ferritin at a very low level (<14 ng/ml) gives very high likelihood ratio (LR+42) and is the most powerful test with an area under the ROC curve of 0.95.[11] Hence, serum Ferritin is used as a gold standard for the diagnosis of IDA in this study.

Among laboratory parameters, a low serum Ferritin (<14ng/ml) is virtually diagnostic of iron deficiency, but is relatively expensive while Green and King (G&K) index is a part of routine blood counts in laboratories using automated hematology analyzers thus Green & King Index (MCV x MCV x RDW/100 x Hb) can help diagnose iron deficiency anemia.[12,13] The sensitivity of Green & King index is 91% and specificity 83% in diagnosing iron deficiency anemia.[14]In iron deficiency anemia the ratio of G & K index is greater than 80 percent. If this easily available test could be used to screen IDA, the cost of anemia work-up would drop considerably because patients with a G & K index suggestive of iron deficiency would not have to undergo further evaluation. As G & K index is based upon simple hematological parameters which are derived from complete blood count, therefore, therefore this test is easily accessible in all settings whether urban or rural thus further simplifying the diagnosis for IDA vs Beta-thalassemia and hence patient would not have to undergo complicated tests like bone marrow aspiration and high- performance liquid chromatography. This study was undertaken to determine the diagnostic accuracy of Green and King (G&K) Index in the diagnosis of iIron deficiency in children using serum ferritin levels as a gold standard.

Methods

It was a prospective, cross- sectional validation study carried out in Children Ward, Department of Pediatrics, PAEC General Hospital, Islamabad over a duration of 6 months from 1st January 2014 to 30th June 2014. Using WHO sample size calculator, keeping Specificity of G & K Index: 83%.[12]Prevalence of IDA in Pakistan: 45%**Error! Bookmark not defined.**and Precision 10%. The sample size was found to be 70.

Data was collected after approval from the Biomedical Ethics committee of the hospital and informed consent from the caretakers of patients. Non-probability consecutive sampling technique was used. All patients between age groups 6 months to 12 years and diagnosed with microcytic (MCV < 70fl) anemia (Hb < 12 mg/dL) were included. Patients who had received iron therapy over the past two months or blood transfusion over the past three months were excluded from the study.

Serum Ferritin was measured using electrochemiluminescence immunoassay ECLIA in the laboratory of Pakistan Atomic Energy Commission (PAEC) General Hospital. All children with serum Ferritin less than 15ng/L were considered having iron deficiency anemia. The automated hematology analyzer in the laboratory of PAEC was used to calculate various hematology parameters including red cell distribution width (RDW), Hemoglobin (Hb) and mean corpuscular volume (MCV) required for G & K Index. A cut off value of above 80 percent of G & K Index was considered as indicative of iron deficiency anemia.

Data was entered and analyzed in SPSS version 20.0. Frequency and percentages were calculated for the gender of the patient, RDW, Serum Ferritin, G&K index. Mean and the standard deviation was calculated for the age of the patients.

For validation of the study, the 2x2 table was plotted to calculate sensitivity, specificity, positive predictive value, negative predictive value, and Diagnostic accuracy.

Green & King Index of Iron Deficiency

True Positive (A)	False Positive (B)
False Negative (C)	True Negative (D)

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA) was calculated as follows:

Sensitivity: A/(A+C) × 100
Specificity: D/(D+B) × 100
Positive Predictive Value: A/(A+B) × 100
Negative Predictive Value: D/(D+C) × 100
Diagnostic Accuracy: A+B/ A+B+C+D x 100

Results

In this study, the mean age of the subjects under study was 2.99± 2.69years with a minimum age of 1 year and a maximum age of 12 years. There were 36 males (51.4%) and 34 females (48.6%) . Serum Ferritin level less than 15ng/l was taken as diagnostic for IDA. Results showed that serum Ferritin levels were less than 15ng/L in 40 of the patients corresponding to a percentage of 57.1% whereas 30 of the patients had serum Ferritin greater than 15ng/L which corresponds to a percentage of 42.9%. This is shown in Table-1.

Table-1: Illustrating frequency and percentages of patients with various hematological parameters

Hematology Parameter	Numeric Value	Frequency (n)	Percentage (%)
RDW value	< 21	61	87.1
	>21	9	12.9
Serum Ferritin	<15ng/L	40	57.1
	>15ng/L	30	42.9
Green & King Index	<80	34	48.6
	>80	36	51.4

G & K index > 80% was taken as diagnostic for IDA. It was found to be greater than 80 in 36 of the cases corresponding to a percentage of 51.4%, whereas it was less than 80% in 34 of the cases thus corresponding to a percentage of 48.6 %.

RDW greater than 21 signifies IDA. In our study, RDW was found to be greater than 21 in 9 of the subjects corresponding to a percentage of 12.9% while it was found to be less than 21 in 61 of the subjects corresponding to a percentage of 87.1%.

Out of a total of 70 cases there were 36 cases that were diagnosed as positive for iron deficiency anemia by serum Ferritin as well as G & K index where as 34 cases were diagnosed as negative for IDA by both serum Ferritin and G & K index. There were 9 cases which were positive on serum Ferritin however were falsely labeled as negative by G & K index. Similarly, there were 5 cases thatwhich were negative on serum Ferritin but were falsely labeled as positive by G & K index.

Table-2: A 2x2 contingency table for evaluating diagnostic accuracy of Green & King index

Green & King Index	Serum Ferritin		Total
	Patients with IDA	Patients without IDA	
Positive cases	31	5	36
Negative cases	9	25	34
Total	40	30	70

The sensitivity of G & K index was found to be 77.5%, specificity was 83.3%, positive predictive value was 86.11%, Negative predictive value was 73.5% and Diagnostic Accuracy was 51.4%.

Discussion

The most common causes of microcytic anemia encountered in a pediatric set-up are IDA and TT. Distinguishing these two entities has important clinical implications as they both have entirely different pathogenesis, treatment, and prognosis.[16]Currently, the routine approach of pediatricians here in Pakistan is further testing of microcytic anemia by expensive tests such as Ferritin and Hb electrophoresis. Since healthcare in Pakistan is not free for all so we explored the utility of Green and King's index which is based upon simple hematological parameters in accurately diagnosing IDA. This test would not only be cost- effective for all patients but also widely available even in rural settings. We obtained somewhat mixed results from our study and though G & K index shows a sensitivity & specificity of < 90%, it has every potential to be utilized in specific clinical settings.

To date, various hematological indices have been utilized by researchers worldwide based on only complete blood count. Although bone marrow aspiration and biopsy is the gold standard for iron deficiency anemia, it is highly invasive and not suitable in all patients.[15] Therefore, previous studies have incorporated alternative hematological parameters as reference methods for defining iron deficiency anemia without developing consensus on a single test. In the study by Carmen et al carried out in Brazil 60 patients having mild microcytic anaemia were evaluated. Of these 30 were suffering from IDA while 30 had beta- thalassemia trait. He utilized serum iron, TIBC and serum Ferritin in combination for defining iron deficiency anemia and sensitivity and specificity of Green and Kings Index was measured against it.[15] Another research carried out in the Indian

population used a combination of Hb levels, MCV, serum iron levels, transferrin saturation and serum Ferritin level to define the population with iron deficiency anemia in their study.[16]Likewise, a study by Matos et al utilized Hb, MCV and Ferritin to define IDA.[17]

In our study we have also utilized Hb and MCV to identify hypochromic, microcytic anemia and serum Ferritin is used as a gold standard to identify iron deficiency. It has also been used as a gold standard by some other studies[18] since it is considered a very powerful test with area under curve (ROC) of 0.95.**Error! Bookmark not defined.**When considering the fact that most of the studies depend on a wide variety of hematological parameters for reliably identifying iron deficiency anemia, the need for such a parameter or an index which can correctly identify IDA and is applicable in majority of patients is further highlighted. A great deal of work has been carried out in this regard.

Microcytosis is a feature of both iron deficiency anemia and thalassemia minor however it is usually much marked in beta thalassemia trait as compared to iron deficiency.[15]A number of studies have intended to utilize MCV singly which denotes microcytosis .A study by Sultana et al showed that sensitivity of MCV was 29.2% and specificity was 98.7% for iron deficiency anemia.[19]Another study amongst pregnant Saudi females showed a sensitivity of MCV to be 73.6% and specificity of 69%.[18]

Since in our population, both iron deficiency and thalassemia traits are endemic therefore MCV alone cannot be used to distinguish between these two major entities. RDW has also been widely tested for differentiating between IDA and thalassemia trait in various populations. RDW indicates anisocytosis within a peripheral blood smear. In IDA there is a usually greater amount of anisocytosis as new red cells that which are produced in bone marrow with progressive degrees of iron deficiency are intermixed with microcytic cells thus giving a mixed picture of normocytic and microcytic cells. In contrast as regards thalassemia trait, there are no such fluctuations noted in the disorder, so bone marrow produces a continuum of microcytic cells. RDW is one of the earliest indicators of IDA.[15]

The continent of Africa has the highest prevalence of anemia. A study was carried out at Khartoum teaching hospital, Sudan in 2012 amongst pregnant Sudanese women in which sensitivity and specificity of RDW was measured against serum Ferritin. The results showed very low sensitivity (43.8%) and moderate specificity (73.7%) of the RDW and thus the study concluded a poor performance of RDW in diagnosing IDA.[18]

Another study was carried out by Sunil et al amongst children aged 1-3 years. In this study, Hb and Ferritin were used in combination as the gold standard. The results showed a moderate sensitivity and specificity for RDW that is sensitivity was 76.5% and specificity 73.1%. However, the sensitivity was increased to 99% and specificity to 90% when a combination of low hemoglobin and RDW was used.[20]RDW and MCV when considered alone are not reliable indicators of IDA therefore many hematological indices have been proposed based upon combination of different hematological parameters so that the sensitivity and specificity of results could be improved. These include:

1. Ehsani et al
2. England et al
3. Green and King
4. Shine and Lal

Ricerca et al.[6] In this study we have measured the sensitivity and specificity of Green and Kings Index which has turned out to be 77.5% and 83.3% respectively.

Green & kings index above 80.0% correctly identified 86.11% of the patients as having IDA and correctly identified 73.5% of the patients as being in the non-IDA category.

The results of some other studies show variable figures. In a cross- sectional study by V. Okann (2009) carried out amongst patients with the mean age of 32 years and age range between (20-84)years showed G & K index to be 96% sensitive and 83% specific for detecting IDA.PPV and NPV were 84.9% and 95.4% respectively.[14] In another study by L. Carmen which was carried out in the University of Campinas, Brazil a mixed population was taken into the study group and results showed that sensitivity, as well as specificity of G & K, as well as specificity of G & K was 97.0 % for IDA. The results of this study thus concluded that G & K index is very useful for initial screening of patients with IDA from those having thalassemia trait.[15]

A recent study carried out in 2011 among adult patients showed that the sensitivity & specificity of G & K index was 91% and 99% respectively in differential diagnosis of microcytic anaemia.[6]The results of these studies show increased sensitivity and specificity contrasting with ours demonstrating a slightly lower value. However, another study carried out in 2010 amongst children aged 6months to 12 years showed a sensitivity and specificity of 66.7% and

77.6% respectively which is even lower than that obtained in our study.[21]

There could be a multitude of reasons for increased sensitivity & specificity shown by some other researches. Foremost, is the difference in population characteristics. In other studies, the subjects were mostly adults whereas in our study the pediatric population was taken into account. In one study which was carried out amongst pediatric patients lower levels of sensitivity were seen.[6] In addition, the difference is in the gold standard or reference method used for diagnosing IDA. L Carmen used a combination of serum iron, TIBC and Ferritin as a reference method.[15] V Okan used serum Ferritin value of < 10 ng/ml as gold standard for IDA,[14] whereas H. Narchi confirmed the diagnosis of IDA by combination of serum Ferritin< 10 ng/mL, serum iron < 50 µg/dL, TIBC < 250 µg/dL or correction by iron therapy.[21]We used a serum Ferritin value of < 15ng/L to diagnose IDA.

Moreover, another difference could be due to the cut off value taken for diagnosing IDA by G & K index. Though it is above 80 in our study as well as in some other studies, there are studies that have utilized a cut off value of greater than 65 for diagnosing IDA.[6,14,15]This difference in cut off values may prove to be significant and perhaps there is still need to define a cut off value which has increased sensitivity without compromising on specificity. More studies in this regard can prove to be beneficial.

Moreover, there is difference in analyzer which has been used. In our study we used automated hematology analyzer CELL- DYN Ruby where as other studies have used Cell dyn Model 1600 CS[15] Sysmex XT-2000i[14] and Sysmex KX21N.[18]The different instruments used could lead to different results obtained especially regarding the value of RDW due to different principles of detection, the selection of events used for analysis and calibration factors.

Considering all the facts mentioned above we can say that since iron deficiency is prevalent in this age group in our society and children with anemia are routinely given iron replacement, therefore, therefore a very high sensitivity is not desirable however the test should be specific enough so that patients detected as negative could be evaluated for further testing which may include HPLC. Green & King's index is thus a very useful and cheap test thatwhich serves this function.

Conclusion

In the present study, we found sensitivity and specificity of Green and King's index to be 77.5% and 83.3% respectively with a positive predictive value of 86.11%. Thus, Green and Kings Index is a useful test for correctly identifying the majority of children with iron deficiency anemia and to rule out those with anemia due to other causes. This test is especially recommended in rural settings where the facilities of more invasive and expensive tests are unavailable.

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- Sara Najeeb and Shabana ShumailExperimentation, Study ConductionAnalysis, Interpretation and Discussion