Original Article

Role Of Red Cell Indices In The Differentiation Of Beta-Thalassemia Trait From Iron Deficiency Anemia In Duhok Province-IRAQ

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Abstract

Background and objective: Anemia is considered as one of the most widespread health issues; anemia is also a key clinical indicator of a number of other diseases and conditions. Hypochromia and microcytosis can be caused by many conditions. The most frequent cause of hypochromia and microcytosis is beta-thalassemia trait (β-TT) and iron deficiency anemia (IDA). The purpose of this research is to determine the accuracy of many distinct red blood cell (RBC) indices and formulae that are used to distinguish iron deficiency anemia from beta-thalassemia trait.

Material and Method: A total of 300 blood samples of premarital couples with hypochromia (MCH<27pg) and Microcytosis (MCV<76fl) will be included. These samples will be evaluated using serum iron, complete blood count, and serum ferritin and hemoglobin electrophoresis.

Results: Out of 300 individuals undergoing premarital screening, 154 (51.3%) IDA cases and 84(28%) of β-TT, and 62(20.7) other types of hypochromic microcytic anemia's were included in this research. Depending on the age, the mean ±SD age of IDA is 25.9 ±5.63years (range 20 – 66 years), β-TT cases with aged 24.88 ± 8.11 years (range 17 – 45 years). There was no discernible and significant difference between IDA instances and β-TT cases when it came to the age of the patients. Comparison of IDA and β-TT participants' red blood cell and hemoglobin profiles. Those with β-TT had higher RBC, and Hb counts, on average 5.91(0.75) and 12.3(1.52), compared to patients with IDA, whose counts varied from 154 (51.3%) to 4.93(0.65) and 11.35(9.3). (1.91). statistically, there is a significant difference between two groups ($p<0.05$). The RDW was considerably higher in IDA participants compared to ß-TT subjects. However, the mean± SD of RDWI (209.73±33.83) vs 152.85±23.93 was significantly higher in IDA cases as seen in ß-TT cases. MCV and MCH values for IDA patients (69.99±4.41) were significantly higher than those observed for β-TT patients (63.62±5.86). Whereas HCT level IDA was significantly lower than those observed for β-TT patients.

Conclusion: RDW was considerably higher in IDA participants compared to ß-TT subjects. As well as, (RDW) above 16% is the best index of IDA. Our results show that RDWI, an easily accessible automated cell count-based index, is a good discriminator of IDA and β-TT. When it comes to diagnosing β-TT, criteria and formulae that are based on the cell count, in particular, the MCV and RBC counts and together with their associated indices, have a good capacity to discriminate between patients.

Introduction:

Anemia is a condition that affects populations all around the world, including those in both developing countries and more industrialized nations. Anemia is estimated to affect 1.62 billion individuals, which is equal to approximately

25% of the total population of the globe, as reported by the WHO. It is estimated that fifty percent of all cases of anemia are caused by insufficient amount of iron content in meals. This is particularly true in young kids, vegetarians, and mature women who have a substantial quantity of monthly blood loss or who are pregnant (McLean, Cogswell, et al. 2009). Iron deficiency anemia, often known as IDA, together with beta thalassemia characteristic are the two circumstances that are commonly seen in patients that have moderate microcytic anemia (BTT). There are a few more diagnoses that need to be taken into consideration, including sideroblastic anemia, anemia caused by chronic illness, and lead poisoning.

Thalassemia is a kind of hemoglobinopathy that affects people all over the world, and it is passed down via families in an autosomal recessive pattern (El-Harth et al., 1999). It is particularly prevalent in the countries that are located in the region that includes the South East Asia, Mediterranean and the Far East. Thalassemias are hemoglobinopathies that result from mutations in the genes that produce the protein responsible for making hemoglobin (Hb). Reduced or lack of synthesis of globin chains is the outcome of these mutations (Yang, Chaffin, Easley, Thigpen, & Reddy, 2009). We have two categories of Thalassemia that is; alpha thalassemia, and beta thalassemia, based on the clinical symptoms that are present and the genetic history of the patient (Langlois, Ford, & Chitayat, 2008). The three subtypes of the most common kind of thalassemia include ; beta thalassemia intermediate, beta thalassemia major, and beta thalassemia minor/trait (Safizadeh, Farahmandinia, Nejad, Pourdamghan, & Araste, 2012). According to (EJ Benz, 2007), a normal or increased red blood cell (RBC) count, microcytosis and a raised level of hemoglobin A2 (HbA2) are all common adverse reactions that can occur as a result of obtaining blood from another individual (BTT). There are two chains and two chains in the HbA2 variant, making it a typical type of Hb.HbA2 serves a purpose that is analogous in comparison to HbA. The proportion of HbA2 in the blood can range anywhere from 1.5% to 3.5%, depending on the assay, but it's often somewhere in that range 2. The majority of individuals identified with BTT had an elevated level of HbA2; moreover, some writers have claimed that an HbA2 level of greater than 4% is enough for diagnosis (Safizadeh et al., 2012).

It would be helpful to have diagnostic methods that are both dependable and effective in order to differentiate between thalassemic and iron limited microcytic hypochromic anemia.

The red blood cell (RBC) index has been shown to have a correlation with IDA as well as the beta thalassemia characteristic (B-TT). As a result, it is essential to establish trustworthy methods that are capable of easily diagnosing and differentiating between these two illnesses. Serum transferrin receptor levels, total iron-binding capacity (TIBC), Hemoglobin electrophoresis, transferrin saturation, serum iron, and serum ferritin are all examples of diagnostic methods that can be applied to show a distinct of IDA and B-TT. Other possible diagnostic tests include: (Zypchen LN, 2006). IDA is characterized by a lack of ferritin, a low trans- ferrin saturation, a lack of serum iron, and an elevated TIBC (Zypchen LN, 2006). Carrier status for beta thalassemia is determined by a normal iron profile and hemoglobin electrophoresis in the presence of erythrocytosis with a low MCV, which is independent of level of hemoglobin (Aslan, 2008). IDA and B-TT can be distinguished from one another using a variety of mathematical formulas that many researchers have proposed as an easy, quick, and low-cost method (AlFadhli, Al-Awadhi, & AlKhaldi, 2007). These two are England and Fraser, which together equal MCV-RBC-5XHb-3.4 (England & Fraser, 1973). Mentzer equals MCV/RBC (Mentzer, 1973). MCV multiplied by red cell distribution (RDW) divided by 100 x Hb is the formula developed by Green and King (Green & King, 1989). The formula developed by Strivastava is as follows: mean corpuscular hemoglobin (MCH)/red blood cell (Srivastava, 1973). Ehsani et al. formula's is: MCV- (10XRBC) (Ehsani, et al. 2009). Ricerca =RDW/RBC (Ricerca et al., 1987). RDW index = MCV2XMCHX0.01 (Shine & Lal, 1977), and Shine and Lal's index equals MCV2XMCHX0.01 (Shine & Lal, 1977). (D. A. Rathod et al., 2007). The findings of computation of ferritin, hemoglobin electrophoresis and serum iron levels may be used to arrive at a definitive differential diagnosis of iron deficiency anemia and IDA. This makes it possible to create a treatment plan that is both more accurate and more timely (Goddard, James, et al., 2011).

The goal of this study was to develop red cell formulas to help doctors screen patients for beta-thalassemia trait and iron deficiency anemia and using commonly available indices of red cell and to determine whether or not certain

indices of red cell and formulas can accurately show a distinct between iron deficiency and B-TT in unmarried couples living in the province of Duhok.

Subject and Methods

The research was conducted at Gulan General Hospital and the Directorate of Preventive Health Affairs in Duhok Governorate from September 1st, 2021, to April1st 2022. A cross-sectional design was used to recruit a sample of patients with hypochromic microcytic anemia of both sexes (Female and Male) attending the Gulan general hospital and the Directorate of preventive health affairs in Duhok.

In this study, automated hematology equipment was used to measure a variety of hematological parameters, such as Red Blood Cell count (RBC), Hemoglobin, Red Blood Cell Distribution Width, Mean Corpuscular Volume, and Mean Corpuscular Hemoglobin (MCH). Three hundred premarital couples of both sexes (male and female) provided blood samples, and those samples were selected at random (sweed).

Exclusion criteria:

- Subjects with MCV > 76fl.
- Subjects with MCH > 27pg.
- Subjects under the age of 17.

Each participant in the current study had five milliliters of venous blood drawn, with two-thirds of the blood volume going into EDTA tubes and one-third into gel tubes. An automated hematology analyzer (swelab alfa lyse, M-series Medonic lyse) (Sweed) was used to measure the number of white blood cells (WBC), mean corpuscular volume (MCV), haemoglobin (Hb), red blood cells (RBC), haematocrit (Hct), and mean corpuscular hemoglobin concentration (MCHC) in the blood contained in the EDTA tubes.

The EDTA tube was also utilized to confirm BTT, which was confirmed by HbA2 determination by (Biorad D10) (Germany), with samples with HbA2 > 3.5% identified as βTT.

Serum iron was measured in the current investigation using an automated biochemical analyzer (Biolis 24i) (Japan). An automated biochemical analyzer was used in order to determine the serum ferritin level (Cobas e 411). (Germany). Iron deficiency is indicated by blood iron levels of (< 65 ng/dL) and serum ferritin levels of (< 15 ng/ml).

Result

Out of 300 individuals undergoing premarital screening, this study included 154 IDA cases with a mean ± SD age of 25.9 ± 5.63 years (range 20 – 66 years), 84 (28%) β-TT cases with aged 24.88 ± 8.11 years (range 17 – 45 years). Regarding the age, there was not a discernible difference in outcomes between IDA and β-TT. ((Table 1)).

Table 1: Comparisons of age between β-TT and IDA patients

Hematological factors (n=300)	**Study groups**		**p-value**
	IDA 154 (51.3%) (Mean±SD)	**β-TT 84 (28.0%) (Mean±SD)**	
Age	25.9(5.63)	24.88 (8.11)	0.3905

Table 2 demonstrated that the RBC and Hb features of both groups, namely IDA in addition to β-TT. Participants with β-TT had higher RBC and Hb counts, on average 5.91(0.75) and 12.3(1.52), than the 154 (51.3%) patients with IDA, whose counts averaged 4.93(0.65) and 11.35. (1.91). Statistically there is a significant difference between the two groups ($p<0.05$).

Table 2: Comparisons of RBC and Hb between β-TT and IDA patients

Hematological factors (n=300)	Study groups		p-value
	IDA 154 (51.3%) (Mean±SD)	**β-TT 84 (28.0%) (Mean±SD)**	
RBC	4.93 (0.65)	5.91 (0.76)	**<0.0001**
Hb	11.35 (1.91)	12.3 (1.52)	<0.0001

Table 3 demonstrates that (RDW) was considerably greater in IDA subjects compared to β-TT subjects. However, the mean± SD of RDWI (209.73±33.83) vs 152.85±23.93 were statistically substantially higher in the IDA group compared to the β-TT group as seen in table 4.

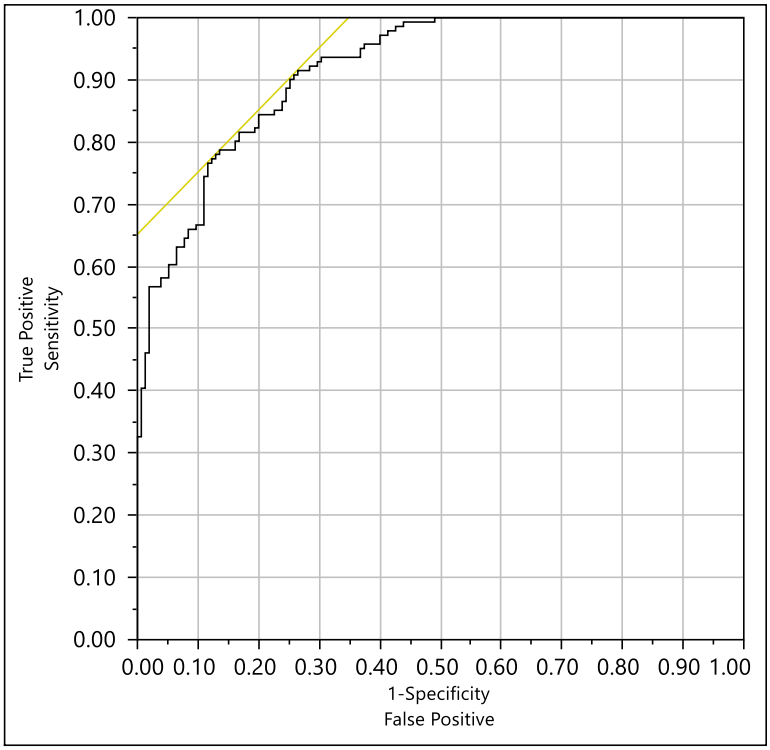
Table 3: Comparisons of RDW between β-TT and IDA patients

Hematological factors (n=300)	Study groups		p-value
	IDA 154 (51.3%) (Mean±SD)	**β-TT 84 (28.0%) (Mean±SD)**	
RDW	(14.46 ±2.18)	(14.11 ±1.77)	<0.0001

Table 4: Comparisons of RDWI and it's in the diagnosis of BTT from IDA

Study groups (n=300)	RDWI		P-value
	Mean	**Std Dev.**	
IDA	209.73	33.83	**<0.0001**
β-TT	152.85	23.93	

Receiver Operating Characteristic



Using Groups='BTT' to be the positive level

AUC
0.91636

MCV and MCH values for IDA patients (69.99±4.41, 23.06 ±2.30) were positively greater than those observed for β-TT patients (63.62±5.86, 20.92 ±1.33), respectively. Whereas HCT level (34.50 ±5.76) in IDA were positively smaller than those observed for β-TT patients (37.65 ±4.98) as shown in table 5. Figure 1 displays the hematological information of the two research categories.

Table 5: Comparisons of HCT, MCV and MCH between β-TT and IDA patient

Hematological factors (n=300)	**Study groups**		**p-value**
	IDA 154 (51.3%) (Mean±SD)	**β-TT 84 (28.0%) (Mean±SD)**	
MCV	(69.99 ±4.61)	(63.62 ±5.86)	**<0.0001**
MCH	(23.06 ±2.30)	(20.92 ±1.33)	<0.0001
HCT	(34.50 ±5.76)	(37.65 ±4.98)	<0.0001

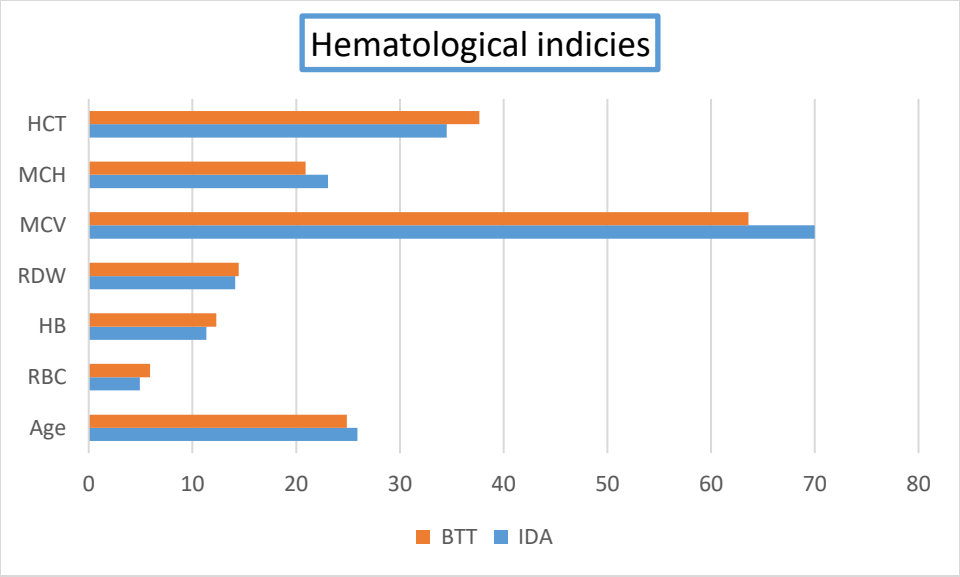


Fig 1: Comparisons of hematological factors of patients with β-TT and IDA

The data displayed a positive increase of England and Fraser Index, Mentzer Index, Srivastav Index, Shine and Lal index, Green and King Index, Ricerca Index, Sehal index, Sirdah Index, and Ehsani Index in the IDA group (14.4 ±2.18, 1152.37±266.13, 11.70 ±9.10, 62.30 ±13.14, 4.75 ±0.74, 2.91 ±0.56, 1016.70 ±200.65, 31.00 ±6.46 and 20.66 ±8.74), respectively with P<0.0001 compared to βTT group, similarly, there was a significant decrease of MDHL in IDA group (1.62 ±0.26) with P<0.001 as compared to βTT group. As well as, there is no significant difference in MCHD between IDA and βTT, as shown in table 6.

Table 6: Comparisons of hematological index between β-TT and IDA patients

Hematological factors (n=300)	**Study groups**		**p-value**
	IDA 154 (51.3%)	**β-TT 84 (28.0%)**	
Mentzer Index (MI)	14.4 (2.18	10.96 (1.85	**<0.0001**

Shine and Lal index (SLI)	1152.37 (266.13	857.41 (168.92	**<0.0001**
England and Fraser Index (EFI)	11.70 (9.10	-0.620 (9.07	**<0.0001**
Green and King Index (GKI)	62.30 (13.14	48.35 (11.68	**<0.0001**
Srivastav Index (SRI)	4.75 (0.74	3.60 (0.59	**<0.0001**
Ricerca Index (RI)	2.91 (0.56	2.48 (0.50	**<0.0001**
Sehal index	1016.70 (200.65	72.87 (158.21	**<0.0001**
Sirdah Index (SI)	31.00 (6.46	20.67 (6.70	**<0.0001**
Ehsani Index (EI)	20.66 (8.74	4.45 (9.84	**<0.0001**
MDHL	1.62 (0.26	1.98 (0.26	**<0.0001**
MCHD	0.330 (0.29	0.32 (0.14	0.6562
MDHL index: mean density of Hb/liter of blood; MCHD index: mean cell Hb density.			

When comparing the two groups, there was a significant gap in terms of the number of instances that were accurately diagnosed. Tables 7, respectively, list the PPV, NPV, sensitivity, and specificity of the techniques that were applied to distinct instances of IDA and cases of β-TT. EFI, Srivasta I and Sehal I indices demonstrated the highest sensitivity (85.71%, 85.06% and 85.06%) but had low specificities (60.71%, 76.1% and 76.19%) for correctly identifying IDA and β-TT. Ricera I had the lowest sensitivity of all of the indices, measuring in at 68.83%; incorrect identification occurred in around 61.90% of all β-TT instances. The MI index and the Ehsani I index both showed the greatest level of specificity, with 82.14% and 80.95%, respectively. In addition, the PPV that was found to be the greatest was for the MI index (89.36%), while the PPV that was found to be the lowest was for the Ricera I (76.81%). Ricera I had the lowest NPV of any of the indices, coming in at 52%, while the Sehal I indices had the greatest NPV of 73.56%. This

was followed by Srivasta I, whose NPV was 72.41%. In addition, the Ehsani I index had the greatest Accuracy value of 82.35%, while the Ricera I index had the lowest Accuracy value of 66.38%.

Table 7: Sensitivity, specificity, and accuracy of hematological index in the diagnosis of BTT compared to IDA patients

			Percentage				
Index	**AUC**	**Cut-off**	**Sensitivity(IDA)**	**Specificity(BTT)**	**PPV**	**NPV**	**Accuracy**
MI	0.887	IDA>12.62682 BTT≤12.62682	81.81	82.14	89.36	70.10	81.9372
SLI	0.815	IDA>955.8881 BTT≤955.8881	75.97	72.61	84.17	62.24	75.21
EFI	0.833	IDA>2.275000 BTT≤2.27500	85.71	60.71	80.00	69.86	76.89
GKI	0.799	IDA>52.06928 BTT≤52.06928	74.02	69.04	81.42	59.18	72.26
Srivasta I	0.880	IDA>3.965046 BTT≤3.965046	85.06	76.1	86.75	72.41	81.93
Ricera I	0.723	IDA>2.556761 BTT≤2.556761	68.83	61.90	76.81	52,00	66,38
Sehal I	0.888	IDA>813.0769 BTT≤813.0769	85.06	76.19	86.75	73.56	81.9327
Sirdah I	0.871	IDA>26.6000 BTT≤26.6000	78.57	79.76	87.68	67.00	78.99
Ehsani I	0.890	IDA>13.25000 BTT≤13.25000	83.11	80.95	88.88	72.34	82.35
MDHL	0.182	IDA≤1.788486 BTT>1.788486	77.27	69.04	82.06	62.36	74.36

DISCUSSION

Generally, sideroblastic anemia, chronic disease-related anemia, ß-TT, and iron deficiency are the four diseases that might result in microcytic anemia. ß-TT and iron deficiency are more prevalent, especially among out-patient community in regions like the basin of the Mediterranean and Southeast Asia (Xiao, Wang et al. 2021) – (Kamil, K. H., & Mohammad, N. S. (2014). Various indices of red cell and formulae have been suggested as simple and low-cost methods for first differentiation of IDA patients from ß-TT patients, these indices and formulas aim to distinguish IDA patients from ß-TT patients. The current study examined the diagnostic validity of a number of different indicies of red cell and formulae in Duhok Province, with the goal of distinguishing between iron deficiency and ß-TT. The current study included 154 IDA cases with a mean±SD age of 25.9 ± 5.63 years (range 20 – 66 years), 84 (28%) β-TT cases with aged 24.88 ± 8.11 years (range 17 – 45 years). There was no obvious differences between IDA and ß-TT. The result agreed with Pornprasert et al., 2014 which revealed that, there were no mathematically significant dismilarities between the iron deficiency group and the ß-TT group based on mean age, platelet levels, gender, white blood cell (WBC) levels. As indicated in Table 2, we discovered that the RBC count and Hb levels in the ß-TT category were higher compared to those in the IDA category. Our findings concurred with (Mondal, Parvez et al. 2021) findings, Results shown that the mean RBC count (4.420.52 vs. 5.350.74) x 1012/L and Hb concentration (7.251.30 vs. 10.491.62 g/dl, p0.001, 95% CI, -3.705,-2.308) were positively greater in the ß-TT group than the IDA group. As well as, (Vehapoglu, Ozgurhan et al. 2014) They found that, out of 290 children with microcytic anemia were diagnosed,

64.1% had a higher RBC count. However, children with IDA had a frequency rate of high RBC counts of 29.4%, indicating that more children with ß-TT have more RBC counts compared to patients having IDA.

On the other hand, (Bhargava, Kumar et al. 2020) revealed that, IDA cases had a mean Hb concentration of 9.6 2 g/dl and the mean Hb value was 12.1 0.4 g/dl. Furthermore (Mondal, Parvez et al. 2021) noted that in individuals, with iron deficiency anaemia, the RBC count was higher at the start of iron therapy and declined in the end period.

Automated analyzers offer red cell distribution width in CBCs, which might be applied in conjunction with a derived value (RDWI) to discriminate IDA and ß-TT (Jameel, Baig et al. 2017). According to our study, patients with IDA have higher RDW levels than those with beta thalassemia trait. (Mondal, Parvez et al. 2021) which expressed that, RDW were positively higher in IDA cases 17.9 (16.6–19.4) as compared with β-TT cases 15.9 (15.3–16.9). Furthermore, they reported that, RDWI were positively greater in the IDA category in comparison to the ß-TT group, and the distict within the two categories was greately significant ($p < 0.001$). Also (Mohammed, Ahmed et al. 2019) showed that, between the examined cases in the ß-TT group (18.06±3.51) and the IDA group (18.76±2.77), there was an insignificant rise in the red cell distribution width of RBCs with a p value of 0.584. The IDA had the highest RDW, which was followed by the ß-TT, indicating that IDA had more anisocytosis than ß-TT. Also (Rahim, Kazemnejad et al. 2021) observed that, RDW was greater in patients with IDA as compared to those with ß-TT. The majority of RBCs in people with the ß-thalassemia trait are microcytic because all RBC precursors display a deficiency in the synthesising globin chains caused by thalassemia mutations. RDW values are hence essentially constant. If the patient has persistent blood loss, IDA progresses rather than stabilizes. Additionally, IDA results in aberrant erythropoiesis, which increases the variability in shape and size (Poikilocytosis and anisocytosis). Distinguishing ß-TT from IDA has significant therapeutic implications, due to the causes, prognoses, and treatments of each disease are completely distinct. In the current research, MCV and MCH values for IDA cases were significantly greater than those expressed for β-TT patients. The result agreed with (Pessar 2019) which showed that, MCV (63.9+4.5) and MCH were significantly lower in β-TT (63.9+4.5, 19±1.8) than IDA (65+6, 19.7+3), respectively. As well as, (Rathod, Kaur et al. 2007) expressed that, comparing MCV and MCH values between the ß-TT group and non ß-TT cases, the ß-TT group showed considerably lower values ($P < 0.001$). Furthermore, (Nesa, Tayab et al. 2009) revealed that, the mean MCV was showed 71.8 fl in IDA patients and 63.9 fl in β-TT patient. As well as, (Rashwan, Ahmed et al. 2022) revealed that, MCHC , MCV and MCH significantly decreased in the analyzed patients in ß-TT in comparison to IDA, which goes hand in hand with (Beyan, Kaptan et al. 2007) and (Yousafzai, Khan et al. 2010), who discovered that the values of MCH and MCV in the ß-TT category were lower compared to those in IDA category. MCV and MCH levels were found to be helpful indicators toc identify ß-TT. This result contrasts with that of (Jameel, Baig et al. 2017), who found that there was no a positive differences in MCV, MCH, and MCHC values within groups. However, according to (Xiao, Wang et al. 2021), MCV, MCH, and MCVm (mean corpuscular volume of mature red blood cells) were considerably greater in the IDA group compared to the ß-TT groups ($P<0.05$). The haemoglobin (HGB), haematocrit HCT), and %MICROr (the proportion of reticulocytes less than 60fl in volume) of the ß-TT and IDA groups did not significantly differ from one another ($P<0.05$). The differentiation between IDA and ß-TT is crucial because if ß-TT is hypochromic, MCV would not normalize as it would in iron deficient conditions. Because of this, MCV and MCH are frequently lower in ß-TT than IDA. However, RBC was misdiagnosed as IDA and was given iron therapy. RBCs tend to be more microcytic and have greater counts in ß-TT than IDA. As a result, the majority of indices use the MCV, MCH, and RBC count to highlight these variations (Ntaios, Chatzinikolaou et al. 2007). Various studies have been conducted to evaluate and compare the efficacy of indicators for the differential diagnosis of IDA and ß-TT (Jahangiri, Rahim et al. 2019). These results of the current study proved to be widely varied. This study examined eleven indices: MI, SLI, EFI, GKI, SRI, RI, and Sehal index, SI, EI, MDHL and MCHD. Table 6 demonstrates significant variations of hematological characteristics between the ß-TT and IDA groups', which could help distinguish between the two anemias, with the exception of MCHD, which does not significantly differ between the ß-TT and IDA groups.

The result agreed with (Rashwan, Ahmed et al. 2022) in Egypt which revealed that, a significant decrease ($P<0.001$) of indices of Green and King, Mentzer, Ehsani, Sirvistava indices Sirdah, and Ricerca in the β-TT group (10.7±1.13, 3.47±5.51, 22.85±4.4, 62.48±18.8, 3.39±0.77, 196.44±53.78, and 3.46±0.67 respectively) in comparison to IDA

group, while statistically, there were a significantly greater in MDHL index in the β-TT cases (1.72±0.2) with P<0.001 compared to IDA cases. According to research done by Pornprasert, Panya, and colleagues (2014), the Srivastava indices and Sirdah have the best differential diagnostic effectiveness in the Thai population. While the Sirdah, G&K, and RDWI tests show the greatest differential diagnostic effectiveness in the Palestinian population.

Additionally, MI, S&L and Srivastava have been identified as the indices in the Indian population that provide the best differential diagnostic efficacy (Rathod, Kaur et al. 2007), and G&F and E&F exhibit the highest differential diagnostic effectiveness in the Chinese population (Shen, Jiang et al. 2010). These variations result from a variety of genetic mutations (Shen, Jiang et al. 2010, Pornprasert, Panya et al. 2014, Hafeez Kandhro, Shoombuatong et al. 2017). Additionally, the perception of the validity of the various indices for various populations may be influenced by the sample size, average age of the study group, and population heterogeneity (Hafeez Kandhro, Shoombuatong et al. 2017). The results may be unreliable and deceptive if the definitive diagnosis is incorrect, as can be the case when choosing subjects from a community of ß-TT patients who also have IDA or when choosing patients under treatment for iron deficiency (Xiao, Wang et al. 2021). To identify the most people with ß-TT, the optimal indicator for IDA should have both a very high sensitivity and a moderately acceptable specificity. According to the findings of numerous research (Hoffmann, Urrechaga et al. 2015, Hafeez Kandhro, Shoombuatong et al. 2017), The results of this investigation showed that the EFI, Srivasta I and Sehal I indices demonstrated the highest sensitivity (85.71%, 85.06% and 85.06%) but had low specificities (60.71%, 76.1% and 76.19%) and AUC (0.833, 0.880 and 0.888) for correctly identifying IDA and β-TT. In addition to this, Rashwan, Ahmed, and colleagues (2022) demonstrated that the high AUC, sensitivity and specificity (AUC, 100%; sensitivity and 100% specificity) for both ß-TT and IDA, the Ehsani and Mentzer were significantly higher, reliable, and very accurate. This was the case for both ß-TT and IDA. In contrast to our results, Demir, Yarali, and colleagues (2002) came to the conclusion that the Mentzer index had only a limited amount of validity when applied to pediatric patients. In addition to this, (Rashwan, Ahmed, and Recierca 2022) showed that the Mentzer, Ehsani, and Matos index has the maximum sensitivity (100%) followed by the Sirdah and MDHL index (95%), the Sirvistava index (90%), and the Green & King and Recierca index (80%).

The biggest amount of PPV was produced by Green & King, Mentzer, Sirvistava, Ehsani, Sirdah and Recierca. This was followed by MDHL, who earned 95%, and then Matos, who made 76.9%. After Green & King and Recierca (with an NPV of 71.4%), the next company with the lowest value is Sirvistava (83.3%), followed by MDHL (90%), Sirdah index (90.9%), and finally Mentzer, Ehsani, and Matos (100%).

Similar to (TELMISSANI, KHALIL et al. 1999, Vehapoglu, Ozgurhan et al. 2014), Our research revealed that the Sirdah, Sirvistava, and MDHL indices all have substantial impact in separation of IDA and β-TT with respectively, 95%,90%,and95% sensitivities; (100%,100%,and90%) specificities; and (0.995,0.968and0.970) AUCs. However (Urrechaga, Borque et al. 2011), In contrast to (Vehapoglu, Ozgurhan et al. 2014), discovered that the Recierca index is a dependable index for separating IDA from ß-TT. The Ricerca index's sensitivity is 100% but its specificity is only 16.9%, making it an unreliable predictor. 74.02 69.04. Our investigation found that the Green and King indices had significant sensitivity, specificity, and AUC values of 74.02%, 69.04%, and 0.779 respectively. Likewise, (Urrechaga, Borque et al. 2011), (Ferrara, Capozzi et al. 2010), and (Sirdah, Tarazi et al. 2008) discovered that the Green and King discriminating index is reliable.

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