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Is NESTROFT better than the hematological indices for screening of beta-thalassemia trait: an answer in rural scenario

Sanjay Piplani[1,*], Manjot Kaur[2], Manas Madan[3], Monika Lalit[4]

[1,3]Associate Professor, [2]Resident, Dept. of Pathology, [4]Assistant Professor, Dept. of Anatomy, Sri Guru Ram Das Institute of Medical Sciences & Research, Amritsar, Punjab

***Corresponding Author:**
Email: sanjaymikki@gmail.com

Abstract

Introduction: Clinical and pathological distinction between beta thalassemia trait **(**β-TT) and iron deficiency anemia (IDA) is of paramount importance. On day to day basis, these two are differentiated by many hematological indices which are derived by mathematical calculations. In this scenario, naked eye single tube red cell osmotic fragility test (NESTROFT) has been slightly relegated. The aim of the present study was to compare by statistical means the standings of these indicators with NESTROFT and also measure efficiency of both by Youden's index.

Material & Methods: The study was conducted on 225 patients diagnosed with mild microcytic hypochromic anemia on complete blood count and PBF. 12 discrimination indices were calculated with simultaneous NESTROFT and HbA2 levels estimation. The results were compiled and analyzed by statistical means.

Results: The Shine & Lal and Ricerca et al indices exhibited the highest sensitivity of 100% each while The England & Fraser index demonstrated the highest specificity of 90.2%.

The highest PPV was found for Mentzer index(77.3%).

The highest NPV was found for Ricerca et al & Shine & Lal indices of 100% each.

The highest Youden's index was shown by Mentzer index(81.3%).

NESTROFT showed a sensitivity, specificity, PPV and NPV of 98.6%, 83.7%, 74.0%, 99.2% respectively. The Youden's index for NESTROFT was 82.3%.

Conclusion: NESTROFT proved to be a better test than all other hematological indices for detection of β-TT. It scores due to its good statistical end results including good efficiency indicator. Additionally, it is observed that it is a test with good cost benefit ratio, easy performability and does not require tedious and hi-tech equipment. Hence NESTROFT should be the primary screening tool for beta thalassemia trait.

Keywords: Iron deficiency anemia(IDA), Beta thalassemia trait (β-TT), NESTROFT, Discrimination Indices, HBA2

Introduction

Iron deficiency anemia (IDA) and beta thalassemia trait (β-TT) are the commonest hematological abnormalities presenting with mild microcytic anemia.[(1)] Deficiency of elemental iron in diet accounts for the commonest hematological disorder in the form of IDA with approximately 30% of the world population (with majority in third world countries) suffering from it.[(2)] In contrast, a common mimicker of microcytic anemia which often confounds a diagnosis of IDA (nutritional case) in third world countries is thalassemia syndrome (trait) which is seen in 3% of the population. The main patho-physiology of these syndromes is absence / lack of either alpha or beta globin chains resulting in alpha or beta thalassemia respectively.[(3)]

In traditional teaching, most of the thalassemia cases are thought to be present mainly in Mediterranean countries, middle east and south east Asia where the prevalence accounts for 8%, 10% and 9% of population respectively.[(4)] Comparing these findings with Indian scenario, it is well established that certain geographical locations and communities (Punjabis, Sindhis, Kutchis, Lohanas Saraswats, Gowdas etc.) are under high risk for genetic transmission of thalassemia syndromes. The prevalence in India also varies from a high of 3-18% in Northern parts of India to only 1.3% in South India.[(5)] The geographical representation of thalassemia syndrome in the form of thal band is quite compromised in today's jet set age where globalization and migration has led to blurring of these lines with spread of thalassemia genes worldwide.[(6)]

Since IDA and β-TT are well represented in North Indian Punjabi population, the absolute diagnosis with patients suffering from microcytic anemia becomes paramount, as in patients with thalassemia trait unnecessary iron administration not only is a source of economic burden to the health services but also can lead to morbidity associated with iron intoxication. A correct diagnosis in addition can promote pre-marital counseling of prospective parents there by eliminating chances of genetic transmission of beta thalassemia gene.[(7)]

Many expensive and practically tedious procedures are available amongst which HBA2 electrophoresis by high performance liquid chromatography is the gold standard[(8)] but these are neither widely available in the resource challenged countries nor adequate trained manpower is available.[(7)]

A number of parameters which utilize mathematical formulae while incorporating at least two RBC parameters such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), RBC count, Red Cell distribution Width (RDW) and Hemoglobin (Hb) in different combinations have been described in literature for a quick and easy preliminary distinction between β-TT and IDA in out- patient and clinical lab settings.[9]

However for mass population screening of β-TT, a screening test which is simple, can be performed on a large number of people, does not require skilled manpower or advanced lab machines, can identify those who have or are likely to develop a specified disease without burdening the health care resources is desired.

Katamis et al in the year 1981, introduced a modified osmotic fragility test 'NESTROFT' (Naked Eye Single Tube Red Cell Osmotic Fragility Test) to be used as a screening test for β-TT.[10] Many researchers have highlighted the advantages of NESTROFT being a good screening test with excellent cost effectiveness.[11]

Not many comparative studies are available from Northern India which is a hub for beta thalassemia trait. The present study was undertaken, to evaluate if NESTROFT is a better screening test for beta thalassemia trait as compared to the various available hematological indices by calculating their sensitivity, specificity and Youden's index[12] values in a population group of North India.

Materials and Methods

The study was conducted in the department of pathology of a tertiary hospital catering mainly to the rural population. A total of 225 patients (167 children and 58 adults) coming for treatment in various specialties and diagnosed with mild microcytic hypochromic anemia on complete blood count and peripheral smear formed the study group.

Family members of beta thalassemia patients coming for blood transfusion of the affected child were also included in the study group because parents are always beta thalassemia trait carriers. Half of the siblings of a known case of beta thalassemia also have a high probability of carrying the gene.

Under aseptic conditions the blood sample was drawn and analyzed on XS-800i Particle counter. Estimation of HBA2 was done by high performance liquid chromatography. The iron profile parameters (Serum Iron, Total iron Binding capacity, Transferrin saturation and Serum Ferritin) were adjudicated in all. The mean values and standard deviation of various hematological and biochemical parameters are shown in Table 1.

Patients with hemoglobin concentration between 8.6-11.5g% and MCV less than 80 fl were included in the study. Those having HB less than 8.6g% were excluded. The cut off value for HBA2 was kept at 3.5%. Patients with HBA2 levels more than 3.5% were labeled as β-TT group and those with value less than 3.5% were labeled as IDA group.

Values of HB, RBC count, MCV, MCH and RDW obtained from the electronic cell counter were used to calculate 12 discrimination indices by various mathematical formulae as given in (Table 2).

In all 225 cases, NESTROFT was done according to the set protocol and the results were recorded.

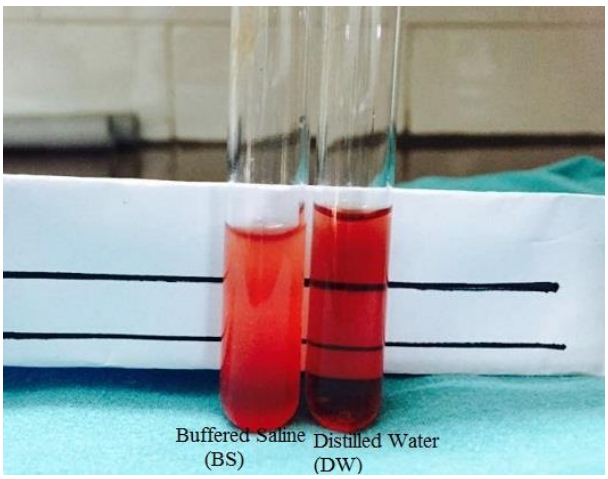

Fig. 1: Showing positive nestroft test

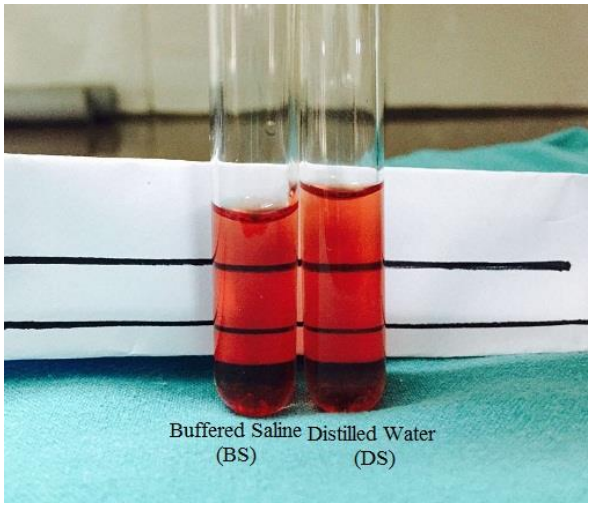

Fig. 2: Showing negative nestroft test

Table 1: Showing the mean values and standard deviation of various hematological and biochemical parameters

S. No.	Parameter	β-TT		IDA	
		Range	Mean+ SD	Range	Mean+ SD
1.	Hb	9-11.5	10.7+0.8	8.6-11.3	9.9+1.43
2.	RBC	4.6-6.4	5.79+0.76	3.7-5.3	4.77+0.53
3.	MCV	56.4-71.1	63.6+5.49	54.2-78.4	69.4+5.16
4.	MCH	16.1-22.4	18.4+2.15	14.2-26.7	20.67+1.34
5.	RDW	14-22.9	15.9+1.62	12.7-27.1	17.2+2.96
6.	SI	26.1-178	78.2+18.3	5.3-27.4	21.1+9.4
7.	SIBC	261-398	312+26.2	279-473	386+38.1
8.	TS	7.1-62	23.7+7.1	1.1-7.9	6.4+2.1
9.	S Ferritin	14.7-87	36.1+18.4	2.3-12.8	6.92+2.9

Table 2: Showing different RBC indices and mathematical formulae used to differentiate between β-TT and IDA

Hematological index	Year	Formula
Mentzer index	1973	MCV/RBC
England and Fraser Index	1973	MCV − (5 × Hb) − RBC − 3.4
Srivastava Index	1973	MCH/RBC
Shine and Lal Index	1977	MCV × MCV × MCH/100
RDW Index	1987	MCV × RDW/RBC
Ricerca et al Index	1987	RDW/RBC
Green and King Index	1989	MCV × MCV × RDW/Hb × 100
MDHL Index (Mean Density of Hb/liter of Blood)	1999	(MCH/MCV) × RBC
MCHD Index (Mean Cell Hb Density)	1999	MCH/MCV
Sirdah Index	2007	MCV − (5 × Hb) − RBC − 3.4
Ehsani et al Index	2009	MCV − (10 × RBC)

Principle of NESTROFT

Normally the lyses of RBC's starts when they are added to a saline solution having a concentration between 0.4-0.5% and they are completely lysed at a saline concentration of 0.32%. In patients of beta thalassemia trait, there are changes in the ratio of volume to surface area of RBC's and these affected RBC's are more resistant to osmosis.,[13] So in β-TT patients, they may not be completely lysed even at a saline concentration of 0.1%. The test is performed in a saline solution having a concentration of 0.36%.

Procedure of the test

Two test tubes were taken. 2 ml of buffered saline (BS) was added to one tube and in the second tube, two ml of distilled water (DS) was added. The tubes were kept untouched at room temperature for 30 min after adding a drop of blood to each tube. Thereafter two black lines drawn on a white paper were visualized through these tubes. The lines could be clearly seen through the contents of DW tube and if the findings were same through the BS tube also, the test was labeled negative otherwise the subject was labeled positive for beta thalassemia trait.

The tubes were revisited again after three hours where the contents in the tube showing a negative test were pink while in a positive test, a sediment was seen settled at the bottom with a clear supernatant at the top.[14]

The observations collected from the NESTROFT test were recorded. The formulae used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index (YI) for the hematological indices and NESTROFT are shown below.

Sensitivity = True Positive / True Positive + False Negative × 100.

Specificity=True Negative/ True Negative + False Positive × 100.

PPV = True Positive / True Positive +False Positive × 100.

NPV = True Negative/ True Negative+ False Negative × 100.

Youden's index = (sensitivity + specificity) – 100.

All the discrimination indices were evaluated according to the differential values proposed by the original researchers.[15-23] The number and proportions of correctly identified patients (True positive) were calculated (Table 3). Sensitivity, specificity, PPV, NPV

and Youden's index for each discrimination index and NESTROFT were calculated (Table 4).

Table 3: Showing number of correctly identified patients by each discrimination index and NESTROFT

S. No.	INDICES	β-TT	IDA	Total No. of Correctly Diagnosed Cases	Percentage
1.	Mentzer β-TT<13 IDA>13	 68 04	 20 133	68+133=201	89.3
2.	RBC β-TT>5 IDA<5	 67 05	 55 98	67+98=165	73.3
3.	England & Frazer β-TT<0 IDA>0	 44 28	 15 138	44+138=182	80.9
4.	Srivastva β-TT<3.8 IDA>3.8	 63 09	 55 38	63+38=101	44.9
5.	Shine & Lal β-TT<1530 IDA>1530	 72 00	 144 09	72+9=81	36.0
6.	RDWI β-TT<220 IDA>220	 58 14	 24 119	58+119=177	78.6
7.	Ricerca et al β-TT<4.4 IDA>4.4	 72 00	 127 26	72+26=98	43.6
8.	Green & King β-TT<65 IDA>65	 57 15	 46 107	57+107=164	72.9
9.	MDHI β-TT>1.63 IDA<1.63	 54 18	 70 83	54+83=137	60.9
10.	MCHD β-TT>0.3045 IDA<0.3045	 53 19	 109 44	53+44=97	43.1
11.	Sirdah β-TT<27 IDA>27	 54 18	 25 128	54+128=182	80.9
12.	Ehsani et al β-TT<15 IDA>15	 67 05	 26 127	67+127=194	86.2
13.	NESTROFT Positive Negative	 71 1	 25 128	71+128=199	88.5%

Table 4: Showing Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Youden's index for all twelve discrimination indices and NESTROFT for β-TT and IDA

S. No.	Indices	Sensitivity	Specificity	PPV	NPV	Youden's Index
1.	Mentzer β-TT IDA	 94.4% 86.9%	 86.9% 94.4%	 73.3% 97.1%	 97.1% 77.3%	81.3%
2.	RBC β-TT IDA	 93.05% 64.05%	 64.05% 93.05%	 54.9% 95.1%	 95.1% 54.9%	57.1%
3.	England & Frazer β-TT	 61.1%	 90.2%	 76.6%	 83.1%	51.3%

	IDA	90.0%	61.1%	83.1%	76.6%	
4.	Srivastva					
	β-TT	87.5%	64.05%	53.4%	90.7%	51.55%
	IDA	64.05%	87.5%	90.7%	53.4%	
5.	Shine & Lal					
	β-TT	100%	6.25%	33.3%	100%	6.25%
	IDA	6.25%	100%	100%	33.3%	
6.	RDWI					
	β-TT	80.5%	77.7%	70.7%	89.47%	58.2%
	IDA	77.7%	80.5%	89.47%	70.7%	
7.	Ricerca et al					
	β-TT	100%	16.9%	39.2%	100%	16.9%
	IDA	16.9%	100%	100%	39.2%	
8.	Green & King					
	β-TT	79.2%	69.9%	55.3%	87.7%	49.1%
	IDA	69.9%	79.2%	87.7%	55.3%	
9.	MDHI					
	β-TT	75.0%	54.24%	43.54%	82.17%	29.4%
	IDA	54.24%	75.0%	82.17%	43.54%	
10.	MCHD					
	β-TT	73.61%	28.75%	32.71%	69.84%	2.36%
	IDA	28.75%	73.61%	69.84%	32.71%	
11.	Sirdah					
	β-TT	75.0%	83.65%	68.4%	87.6%	58.65%
	IDA	85.65%	75.0%	87.6%	68.4%	
12.	Ehsani et al					
	β-TT	93.05%	83.0%	72.04%	96.2%	76.05%
	IDA	83.0%	93.05%	96.2%	72.04%	
13.	NESTROFT					
	β-TT	98.6	83.7	74	99.2	82.3%
	IDA	83.7	98.6	99.2	74	

Results

The present study comprised of 225 patients which mainly belonged to the age group of 5-15 years (134, 59.5%) with age ranging from 1.5-39 years. Most of the patients were asymptomatic (57%) with a significant proportion complaining of weakness (38%) followed by easy fatigability (5%).

On the basis of HBA2 levels, 72 patients having HBA2 more than 3.5% were grouped into β-TT group. Rest 153 patients having HBA2 less than 3.5% were grouped under IDA parameter.

The mean values for HB in β-TT group were 10.7+ 0.8 and those in the IDA group were 9.9+ 1.43.

In β-TT group, the mean MCV and MCH were 63.6+5.49 and 18.4+2.15 respectively which were lower as compared to those found in the IDA group (69.4+5.16 and 20.67+1.34 respectively).

The mean RBC count in β-TT group was definitely higher 5.79+0.76 as compared to the IDA group 4.77+0.53.

The mean RDW values were increased in both the groups, but the IDA group showed the values to be higher (17.2+2.96) as compared to the β-TT group (15.9+1.62).

The mean values for serum iron, transferrin saturation and serum ferritin were much lower in IDA group as compared to those in the β-TT group but TIBC showed an inverse trend. (Table 1).

Hematological Discriminants

The Shine and Lal and Ricerca et al indices exhibited the highest sensitivity of 100% each but the specificity was very low to the tune of 6.25% and 16.9% respectively.

The lowest sensitivity was recorded for the England and Fraser index (61.1%).

The highest specificities of 90.2% and 86.9% were recorded for the England and Fraser and Mentzer indices respectively.

The highest and the lowest PPV were found for Mentzer index (77.3%) and MCHD (32.71%) respectively.

The highest NPV of 100% each was demonstrated by Ricerca et al. and Shine and Lal indices while the lowest NPV was recorded for MCHD (69.8%).

The highest and the lowest values for Youden's index were shown by Mentzer index (81.3%) and MCHD (2.36%) respectively.

Nestroft

In the present study, out of 72 cases of β-TT, NESTROFT was positive in 71 cases and negative in only 1case. In IDA group, 25/153 (16.3%) cases showed a false positivity with remaining 128 cases (83.7%) being true negatives.

From the data thus obtained; various statistical parameters in connection to NESTROFT were calculated which revealed a relatively high sensitivity and specificity of 98.6% and 83.7% respectively. The predictive values were also quite high with a positive predictive value (PPV) calculated as 74.0% and negative predictive value (NPV) of 99.2%.

Discussion

Beta thalassemia is not only a cause of major morbidity and mortality but also a source of economic burden to the community at large.[24]

The best laboratory index as a screening test for β-TT should have a very high sensitivity as well as a reasonably high specificity so that the false positivity can be decreased.

Since 1973, several indices have been introduced in an attempt to distinguish these two conditions in a cheaper and easier way. Initially the authors who described these indicators had claimed almost 100% sensitivity for detection of β-TT.[15,16,20,25-27] However later studies disproved it and showed these to be in an actual range of 61-91%.[28-30]

Increased RBC count as a flag for microcytic anemia of β-TT is not an absolute indicator as a proportion of IDA patients (almost 1/3rd) also had an increased RBC count in the present study. Therefore, though the sensitivity of RBC count for β-TT in the present study was 93.05%, a low specificity and sub-optimal Youden's index of only 65.05% and 57.1% respectively ruled out RBC count as a reliable indicator between the two.

Many studies done worldwide have documented RDW (a measure of the degree of variation in red cell size) to be a good discriminator between β-TT and IDA[31-33] but in our study the values were elevated in both the groups, though they were slightly higher in the IDA group. In the present study, RDW index showed the sensitivity of 80.5%, specificity of 77.7% and Youden's index of 58.2%. Our results concur well with results of[34,35] who found that RDW alone is reasonable but not sufficiently specific or sensitive enough to differentiate between β-TT and IDA.

Of the 12 hematological indicators which were compared in the present study, Mentzer index exhibited a reasonably high sensitivity, specificity and good Youden's index of 94.4%, 86.9% and 81.3% respectively. This was followed by an index proposed by Ehsani and co-workers in which sensitivity specificity and Youden's index of 93.05%, 83.0% and 76.05% respectively were noted. These findings noted in our study were corroborated by Ehsani et al. themselves in their seminal study of 2009.[23] Almost similar results have been documented by Rahim & Keikhaei who recorded a sensitivity and specificity of 85% and 93% respectively for Mentzer index.[36] Almost Similar results were echoed by Ghafouri et al.[37]

Although Al Fadhli et al in 2007 and later Ferrera et al in 2010 recorded not only high efficiency value (Youden's index) but also high specificity for the primordial parameter England and Frazer index.[38,39] but in our study though the specificity was 90.2%, the England and Frazer index showed a Youden's index of just 51.3%.

Same researchers had put a question over claims of high Youden's value by Shine and Lal to discriminate between β-TT and IDA. Our study also proved that it was an inferior discriminant for differentiating β-TT from IDA with a Youden's index of only 6.25%.

In the present study, the Sirdah index demonstrated a sensitivity, specificity and Youden's index of 75.0%, 83.6% and 58.6% respectively which are comparable with the findings of Vehapoglu et al who found these to be 85.7%, 79.4% and 65.0% respectively.[12]

In the present study, the Srivastva index showed a sensitivity, specificity and Youden's index of 87.5%, 64.05% and 51.5% respectively. Alfadhli et al found a Youden's index of 54.9% for Srivastva index.[38]

Ntaios et al. in 2007 tested the efficacy of Green and King index and concluded that it had a reasonably fair sensitivity and Youden's index of 75.06% and 70.86% for detecting β-TT.[40] Urrechaga et al. in 2011 also demonstrated a high Youden's index of 80.9% for the Green and King discriminant for β-TT.[9] However in our study the results were below expectations with the sensitivity, specificity and Youden's index values of 79.2%, 69.9% and 49.1% respectively.

The Ricerca index also exhibited a sensitivity of 100% but the specificity was very low (16.9%) and a Youden's index of 16.9% ruled it out as a reliable indicator. Vehapoglu et al in 2014 found a youden's index of 14.7% for the Ricerca index. [12]

In the present study, the MDHL index showed a sensitivity of 75.0% and a Youden's index of 29.4%. The MCHD index exhibited a very low Youden's index of 2.36% only. These findings are in coherence with the works done by Vehapoglu et al.[12]

NESTROFT was also one of the parameters evaluated for its work efficiency (Youden's index) and it was calculated to be 82.3%.This itself spoke volumes as it was the highest Youden's index obtained of all the 13 parameters under study in the present research conducted by us. Similar results were obtained by Manglani et al and Han KE et al in their studies on beta thalassemia trait.[11,41]

NESTROFT also had good sensitivity and specificity rates of 98.6% and 83.7% respectively. This has been corroborated by many researchers (Table 5).[42-49] NESTROFT also showed a high negative and

positive predictive value which are quite comparable with the findings of other researchers.[42-49]

Thus NESTROFT proved superior to any other hematological index in differentiating β-TT from IDA. The low false positive rates added to its quality as a better indicator.

Table 5: Showing comparative statistics of NESTROFT in present study with some Earlier Research Works

S No.	Authors	Year	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
1.	Mehta et al.[42]	1988	95	82.1	73.1	97
2.	Raghwan et al[43]	1991	95.5	87	70.5	98.3
3.	Thomas et al.[44]	1996	98.7	66.6	87	96.5
4.	Maheshwari et al.[45]	1999	91	95	55	99
5.	Suri & Sidhu[46]	2001	97.7	71.7	51.9	99
6.	Bobhate et al.[47]	2002	97.1	100	100	98
7.	Sirichotivakul et al.[48]	2004	97.6	72.9	33.6	99.5
8.	Chakraborty et al.[49]	2012	94.12	95.23	41.02	99.78
9.	Present study	2016	98.6	83.7	74	99.2

Conclusion

The study assessed twelve commonly used calculated hematological indices and a practical hematological indicator (NESTROFT) as a preventive screening tool to differentiate β-TT and IDA. In comparison, NESTROFT had the highest efficiency in the form of Youdens index making it the screening tool of choice to differentiate between the two. NESTROFT has an additional advantage of being a quick relatively inexpensive test (having a good cost benefit ratio) with easy performability and does not require high end technological equipments. This makes it an ideal contender to be adopted in all the third world developing countries especially in rural settings where such an easy tool can go a long way to decrease the morbidity associated with β-TT and lessen the economic burden of raising a thalassemic child.

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