International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# A Systematic Review on: Diagnostic Accuracy of MCV/RBC Count Ratio (Mentzer Index) for Screening of Beta-Thalassemia Trait

**Ms. Sudeshna Bakshi**

Research Associate, Medical Administration, Tata Memorial Centre

**Abstract**

**Introduction:** Thalassemia is an inherited blood disorder characterized by abnormal hemoglobin production. Beta thalassemia, one of its primary forms, affects populations across regions from Africa to Southeast Asia. Differentiating beta-thalassemia from iron deficiency anemia (IDA) is essential, with the Mentzer index (MCV/RBC count ratio) being a simple and cost-effective screening tool.

**Objective:** To assess the diagnostic accuracy of the Mentzer index for screening beta-thalassemia through a systematic review and meta-analysis.

**Methods:** A systematic search was conducted using relevant keywords and MESH terms in Cochrane Library and MEDLINE. Studies published in English up to March 2017 were included. Quality was assessed using QUADAS-2. Pooled sensitivity, specificity, and diagnostic odds ratios were calculated. Meta-regression analyzed factors affecting diagnostic accuracy.

**Results:** Twenty-seven studies were included in the review. Pooled sensitivity, specificity, AUC, and diagnostic odds ratio for the Mentzer index were 0.84, 0.88, 0.93, and 38, respectively. Fagan's plot showed increased post-test probability to 88% when positive, and reduced to 15% when negative. Meta-regression identified study design as a key factor influencing diagnostic accuracy.

**Conclusion:** The Mentzer index is an effective screening tool for beta-thalassemia, providing reliable differentiation from IDA. Its use in mass screening could help reduce the burden of thalassemia, particularly in resource-limited settings.

## INTRODUCTION

Microcytic and hypochromic anaemia is the indicator of both beta-thalassemia and iron deficiency anaemia (IDA). IDA is a very frequently occurring nutritional disorderaffecting approximately one billion people across the world, not specifically in the developing countries but also in the western world(1). Insufficient iron intake or menstrual loss in women of child bearing age or chronic blood loss in the gastrointestinal tract may result in IDA in case of elderly subjects(2,3), on the other hand IDA is one of the important factor causingimpairment of growth and intellectual development in infants and young children(4). Thalassemia is the most common genetic disorder worldwide, with 1.7% of the world's population carrying thalassaemic genes. Traditionally it has high prevalence in Mediterranean area(8%), Middle East countries (upto 10%), Southeast Asia (9%), Southern China(2.18%), and Indian subcontinent(3-15%).Nowadays thalassemia has spread over nearly the entire globe due to population migration, as a result non- endemic countries like Northern America, Northern Europe are also facing thalassemia related problem. According to an epidemiological survey,it has been reported that on an

average 15,000 subjects with transfusion dependent condition in Europe (2).Based on a report by WHO, about 12% of children born with transfusion-dependent β-thalassaemia are transfused, and less than 40% of those transfused obtain adequate iron-chelation therapy. About 100,000 patients are currently living with regular transfusions, and at least 3000 die annually in their early age (5).

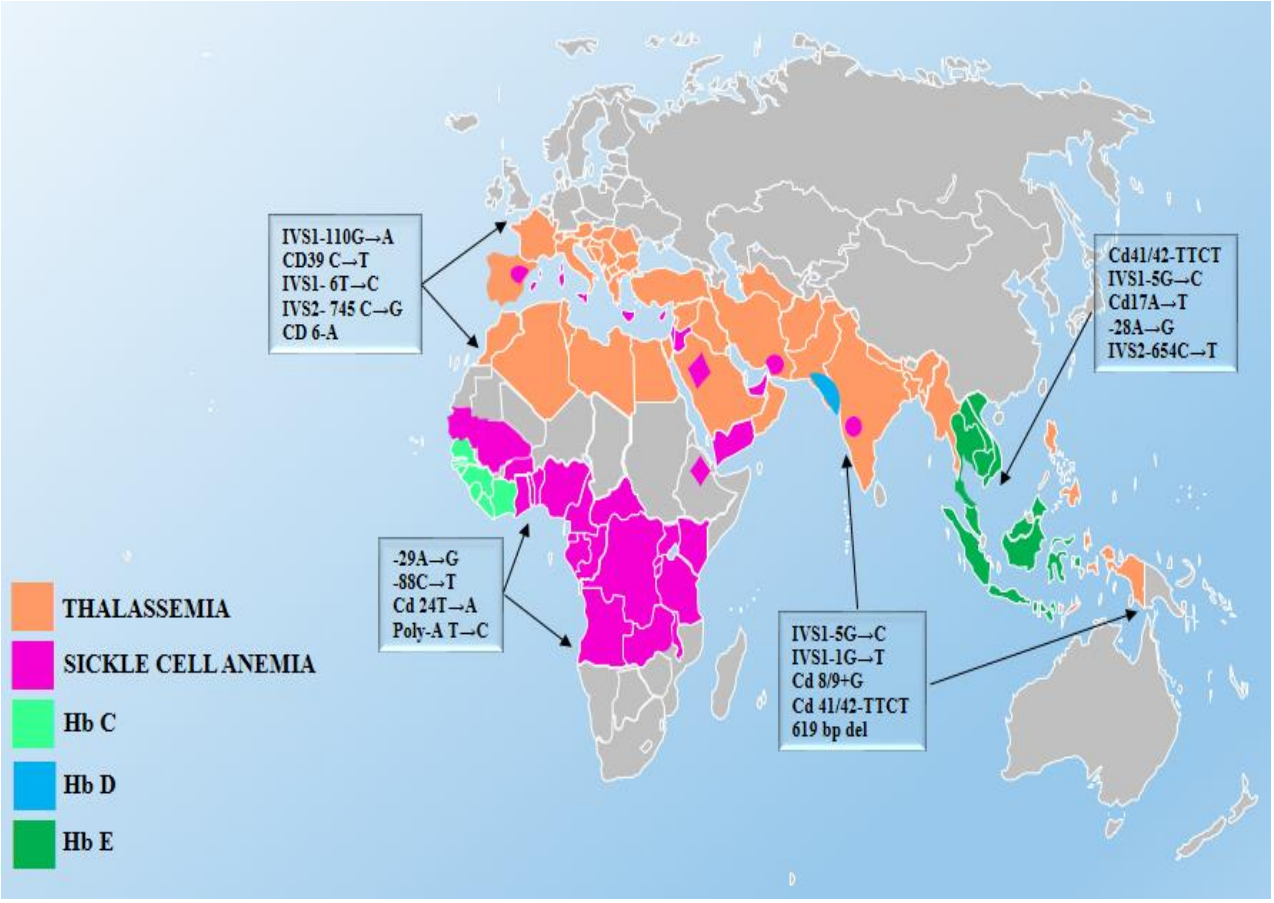


**Figure 1Global distribution of thalassemia(27)**

The inherited disorders of blood include haemoglobinopathies are the commonest hereditary disorders in India and poses a major public health problem (6). It has been estimated that in India with approximately 27 million births/year and a prevalence of pathological haemoglobinopathies 1.2/1000 live births would suggest about 32,400 babies born with a serious haemoglobin disorder (7).About 10 % of the world's thalassaemic population is born in India every year (8). In a more recent study addressing the burden of genetic disorder it has been reported that thalassemia (8.8%) is one of the severe haematological problem in India estimating beta- thalassemia has a frequency at birth of 1:2700, which means that about 9,000 cases of thalassemia major are born every year (9). InIndia,more than 3000 ethnic groups reside, but β-thalassemia trait is more common among certain Indian ethnic communities, such as Sindhis, Kutchis, Punjabis, Bengalis, Gujaratis, Assamese (10), the incidence varying from 1-17% (7,8) and the average frequency is 3.3%(11), this means on an average 1 in every 25 Indians is a carrier of β-thalassaemia.

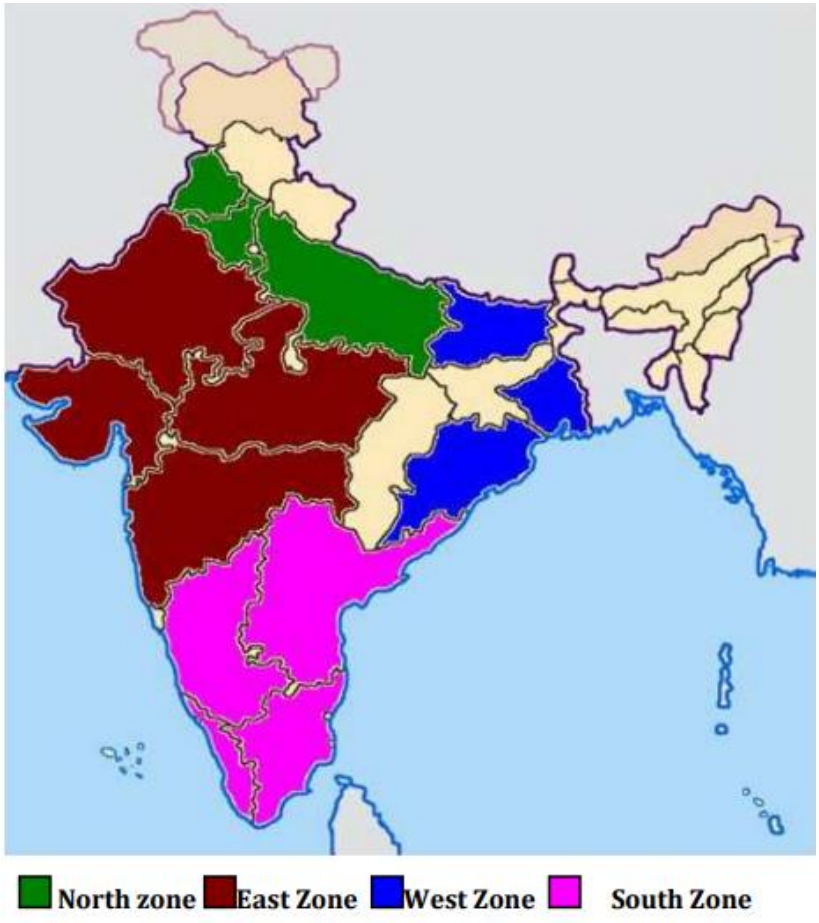


**Figure 2 Distribution of thalassemia in India (7)**

Thalassemia is an inherited autosomal recessive blood disorder in which the body makes an abnormal form of haemoglobin which is characterized by the absence or reduced synthesis of one or more of the globin chains (12). It is due to some genetic mutation or deletion of certain key gene fragments. Thalassemia is mainly of two types - alpha thalassemia and beta thalassemia. In beta-thalassemia, the beta globin genes are affected and in alpha-thalassemia,at least one of the alpha globin genes has mutation or shows abnormality(13). β- thalassemia is a heterogeneous group of genetic disorder in which, no β globin ($\beta^o$ thalassemia) chains are synthesized while in others ($\beta^+$ thalassemia) they are produced at a reduced rate (14). Moreover, it has been found that β- thalassemia mutations are relatively ethnicity specific i.e. each ethnic group has its own set of mutants (7). The severity of the disease depends on the presence of mutation on one or both alleles which in turn determine the clinical picture as follows-

- β- thalassemia major is caused by $\beta^o/\beta^o$ genotype, which is characterized by lack ofβ chain synthesis thus no HbA can assembled, HbA2 2-5%, HbF 95-98%
- β- thalassemia intermedia is caused by $\beta^+/\beta^o$ genotype or $\beta^+/\beta^+$ genotype in which some HbA is produced.
- β- thalassemia minor is caused by $\beta/\beta^o$ or $\beta/\beta^+$ genotype in which β chain production is not highly effected so that carriers may be clinically asymptomatic. (12,7)

They are the most common single gene disorders which inherited from parents and results in life threatening hereditary haemolytic anaemia. If one parent is the carrier ofβ- thalassemia trait and other

parent has normal haemoglobin level then with each pregnancy there is 50% chance of having a child with β- thalassemia trait (Fig.3) where as if both the parents are the carrier of β- thalassemia trait then with each pregnancy there is 25% chance of having a child with β- thalassemia trait(Fig.4). (8)
Primarily, treatment for thalassemia is lifelong blood transfusion at regular interval of 15-30 days along with iron chelation therapy and monitoring of blood parameters. Bone marrow transplant is only curative treatment which is largely unaffordable and available in very few centers. The estimated economic cost in India for each patient per year is around Rs. 90,000- 100, 000 which is unaffordable for most of the patients, as a result optimal treatment is available for only 5-10% patients. Most of the patients of thalassemia have a significant morbidity and mortality rate, in addition with high economic burden, produces huge emotional and psychological trauma on patients including their family members (6,8)

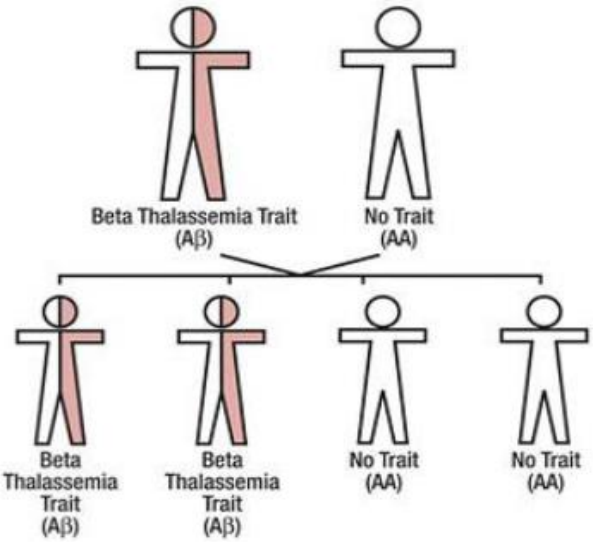


**Figure 3 Possible outcomes between beta thalassemia trait and no trait**

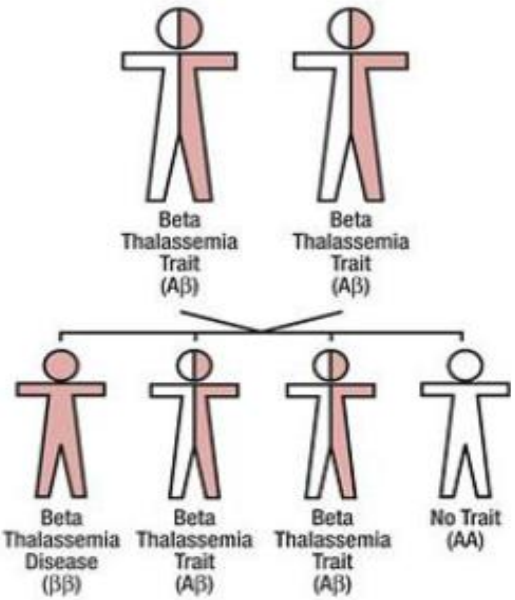


**Figure 4Possible outcomes between two beta thalassemia traits**

Consanguineous marriages increase the homozygosity in the community, hence the most effective approach to reduce the burden in the society is by implementing carrier screening and detection programme of pre-marital youth, antenatal diagnosis of women with first trimester, individuals belonging from high risk communities and selective termination of pregnancy of the affected foetus (15).Hba2 concentration of lysed RBCs using Hb electrophoresis and CE-HPLC (Cation Exchange High Performance Liquid Chromatography) are considered as "gold standard" method for the diagnosis of thalassemia (16). The next step for correct diagnosis include serum ferritin(SF), serum iron(SI),total iron binding capacity(TIBC), transferrin saturation(17,4). However, these are very expensive and time-consuming for public health economy in countries with high prevalence of microcytosis and hypochromic (18,19). Therefore, there is necessity of simple and basic blood test in public health laboratories to study the hematologic parameters for addressing haematological disorders. Microcytic and hypochromic anemia is the indicator of both IDA and β- thalassemia. Additionally, if a patient has both forms of anaemia, the investigations are likely to suggest iron deficiency (20), thereby missing out on diagnosis of βthalassemia.Thus, to controlthalassemia it is important to distinguish microcytosis of heterozygous β- thalassemia from non-microcytosis thalassemia(21).

In mass screening programme where resources are constraints the differentiation could be achieved mathematically through RBC parameters between IDA and β- thalassemia. Since the early 1970s, several indices and formulae of CBC parameters have been developed to discriminate IDA and β-thalassemia (22,23). The purpose of using these discriminating indices is to reduce the unnecessary cost of diagnosis(24). These indices include Mentzer index, England & Fraser index, Green & King index, Shine & Lal index, Srivastava index, Red cell distribution width index, Ricerca index, Bessman index, Ehsani index (1,25). It is widely accepted that none of these indices are 100 % specific neither 100 % sensitive even after using combination of indices. Moreover, these indices do not show consistent performance in all the studies; reasons behind these discrepancies are still not clear (1).

Considering the above situation, Mentzer Index which is the MCV/RBC count ratiocan be easily obtained from any laboratory settings. Therefore, it can be considered as a cost effective, simple, quick screening criterion to discriminate IDA & βthalassemia. If the quotient of the mean corpuscular volume (MCV, in fL) is divided by the red blood cell count (RBC, in Millions per microliter) and the value is < 13, thalassemia is said to be more likely and if the value is > 13, then iron-deficiency anaemia is said to be more likely. According to Afraz et.al, Mentzer Index has 91 % positive predictive value in discriminating these two kinds of anemia (26). Another study carried out in Turkey reported that Mentzer Index has good sensitivity,specificity and was found to be a very convenient and inexpensive screening procedure (23).

Costly treatment, repeated blood transfusion with iron chelation therapy, and high economic burden on family resources, suggests that prevention is better than cure. In a country like India, where thalassemia is endemic and resources are constrained,Mentzer index could be prove asan effective screening procedure not only in hospital setting but also in rural health care settings. Beside this there are different opinions regarding the diagnostic accuracy of Mentzer index. Several studies have conducted in other parts of the globe but they have done on different age group, varying samplesizes, different health care settingsand some on specific ethnic groups which indicate to undertook a systematic review to assess the diagnostic accuracy of Mentzer Index as a convenient and effective screening index for β-thalassemia.

## AIM OF THE REVIEW

To determine the diagnostic accuracy of the Mentzer index for differentiating β-thalassemia trait from iron deficiency anaemia in microcytic, hypochromic anaemic patientas a simple, quick and cost-effective screening procedure comparing with standard procedures.

## METHODOLOGY

We conducted a detailed and systematic online searchusing the key words "Beta thalassemia, Diagnostic accuracy, Mentzer index, MCV/RBC count ratio" with their corresponding MESH terms in combination with "OR" and "AND" wherever applicable. Using those identified MESH terms (Appendix-1)a comprehensive search strategy was built up to find out all the relevant articles. We did not apply any other search filters while searching for articles. Apart from these, we looked in the references of the searched articles to identify the relevant articles for this review. The search period was lied between month of February-March 2017.

**Inclusion criteria:**

- Studies where Mentzer index wasused as a discrimination index
- Studies focused on differentiationbetween beta thalassemia and IDA
- Studies conducted on any population irrespective of age group

**Exclusion criteria:**

- Studies were omitted from the review if Mentzer index was not mentioned as discrimination indices
- In-vitro and studies performed on animals

**Study design:**

All the study designs i.e. Cross-sectional, Cohort, Case-control were considered for this review purpose.

**Participants:**

Irrespective of age all the subjects with microcytic and hypochromic RBC were considered as participants.

**Index test:**

Mentzer index or MCV/RBC count ratio was considered as index test to differentiate beta-thalassemia from IDA, more precisely to reduce the uncertainty. We did not consider any comparator test

**Target condition:**

Patients with abnormal haemoglobin parameters were susceptible for the target condition. But there was clinical uncertainty about their status, either they were the carriers of the beta-thalassemia or they were anaemic because of iron deficiency.

**Reference standard:**

CE-HPLC and Hb electrophoresis was referred as the gold standard method to compare with the index test to assess its validity. It was the best available method for identifying the target condition.

**Search methods for identification of studies:**

Search engines like Cochrane library (Appendix-2) and MEDLINE (www.pubmed.com) (Appendix-3) were used to obtain a large number of studies to address the study objective. Search strategy was neither restricted to any specific study design nor the population, it was predominantly focused on index test and target condition. The search was limited to, articles written in English.No time frame restrictions were applied for the inclusion of the studies, all the studies published till March 2017 were considered for this review purpose.

## DATA COLLECTION AND ANALYSIS

Literature search, data collection and management, statistical analysis were conducted by the review authors.

**Selection of studies:**

Review authors discussed between themselves to resolve the discrepancies regarding the study eligibility criteria.

**Data extraction and management:**

All the relevant data were extracted from the included studies using a data extraction form (Appendix-4). In addition to accuracy data we also collected following information for each study:

- Name of the authors & journal, date of publication, study design, name of the country
- Characteristics of study subjects
- Accuracy data of other reported discrimination indices

We assembled all the extracted data in an Excel spreadsheet where each row represented a single study and each column a variable of interest.

**Assessment of methodological quality:**

Quality of the studies were assessed according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) (28). If there was more than one "no" or "unclear" response to the signalling question for a specific domain we marked risk of bias as "high" or "unclear" respectively.

**Statistical analysis and data synthesis:**

We performed statistical analysis according to the guidelines of Cochrane for Diagnostic Test Accuracy(29). Statistical software STATA and Review Manager 5.1 were used to carried out all the statistical performance.

Forest plots were used to display the sensitivity and specificity along with their 95 % CI for all the included studies. A summary receiver operating characteristics (SROC) plot was also used to show the individual studies in a ROC place where a single study plotted as a single sensitivity specificity point. Fagan's nomogram (based on Bayes' theorem) was used to calculate the pre-test probability (usually the incidence of the target condition) and post -test probability (patient's probability of having the disease). It is a graphical tool where a straight line is drawn from the left axis (pre-test probability) through the likelihood ratio of the test (middle test) intersects the right axis (post -test probability).We also performed meta regression including various covariates i.e. study design, geographical region, sample size, type of countries to assess the effect of these factors on the diagnostic accuracy of Mentzer index.

**Investigation of heterogeneity:**

We used bivariate box plot to assess the heterogeneity.

## RESULTS

**Results of the search:**

The literature search yielded a total of 493 potentially relevant articles based on keywords used. Out of which, we excluded39 articles as they were published in different languages other than English. We also excluded 9 articles as they were carried out on animals.By reading title and abstracts we omitted 405 articles andfinally 40 papers were retrieved for full review.We identified two citations as potentially meeting the inclusion criteria but could not assess them by the time.At last, 27articles were included in the review according study inclusion criteria (Fig.5). But due to insufficient information 5 studies were excluded from the meta-analysis.

In total, 27articles comprised of 16,548 results of patients among which 3194 were diagnosed with the target condition. Studies were excluded from the review if only Mentzer index was not mentioned as discrimination indices in. Most of the studies were published after 2005.73.91% (17/23) studies enrolled Asian population and they were mainly cross-sectional study designs (13/23,56.52%).

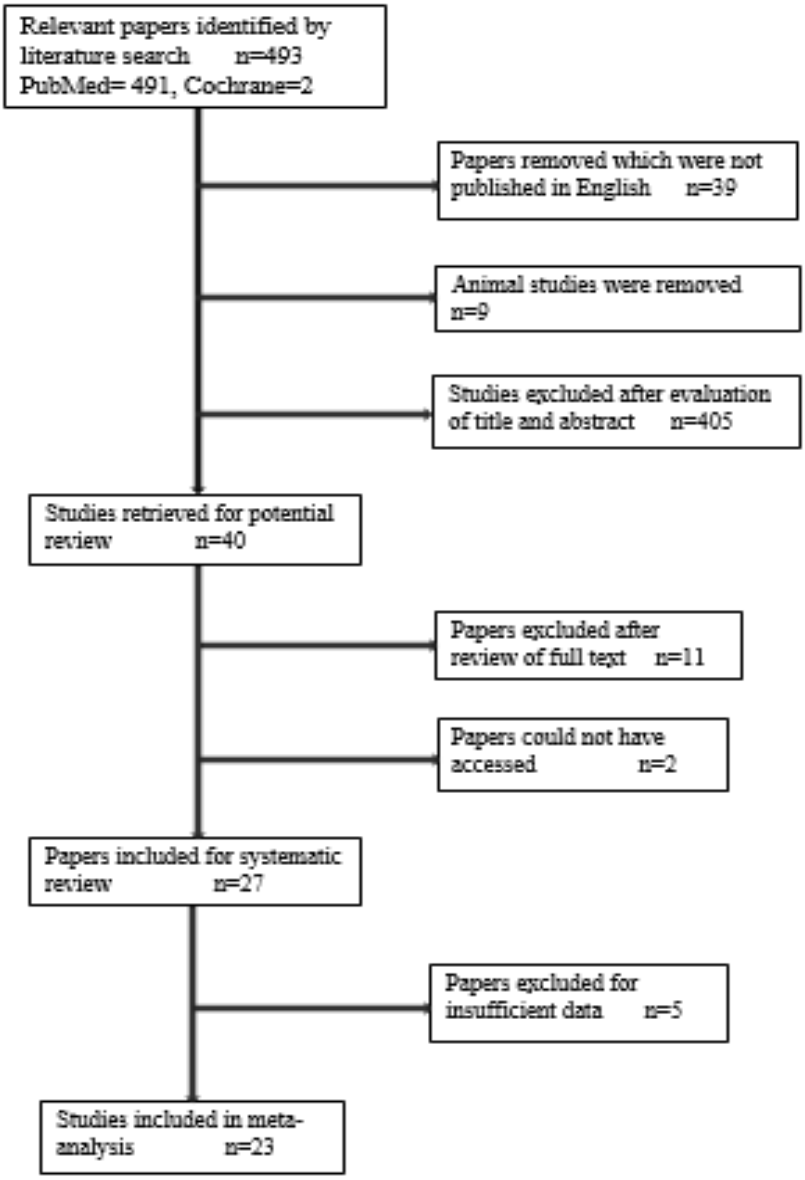


**Figure 5Study flow chart detailing identification of studies for inclusion in the review ** During analysis one study is used as two as it possesses two different groups**

**Methodological quality of included studies:**

The overall quality of the included studies was good according to the QUADAS-2(Appendix - 5) graph(Fig.6) and summary result (Fig.7). However, two studies presented high risk in patient selection (Ullah Z, Vehapoglu A),one study (AlFadhi SM) presented high risk in flow and timing as well as in index test (Miri-Moghaddam E), due to unclear reporting. In most of the studies, patient sampling and the interval between reference standard and index test was unreported.

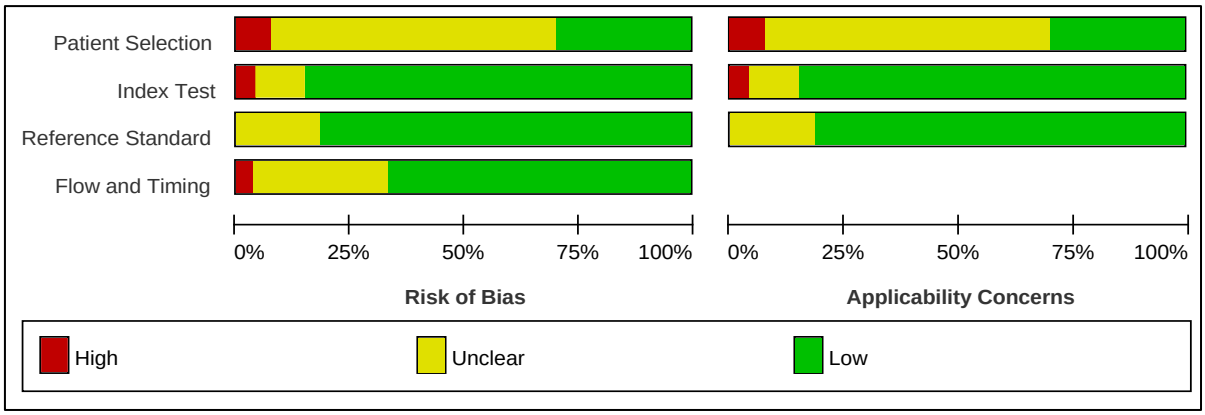


**Figure 6Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies**

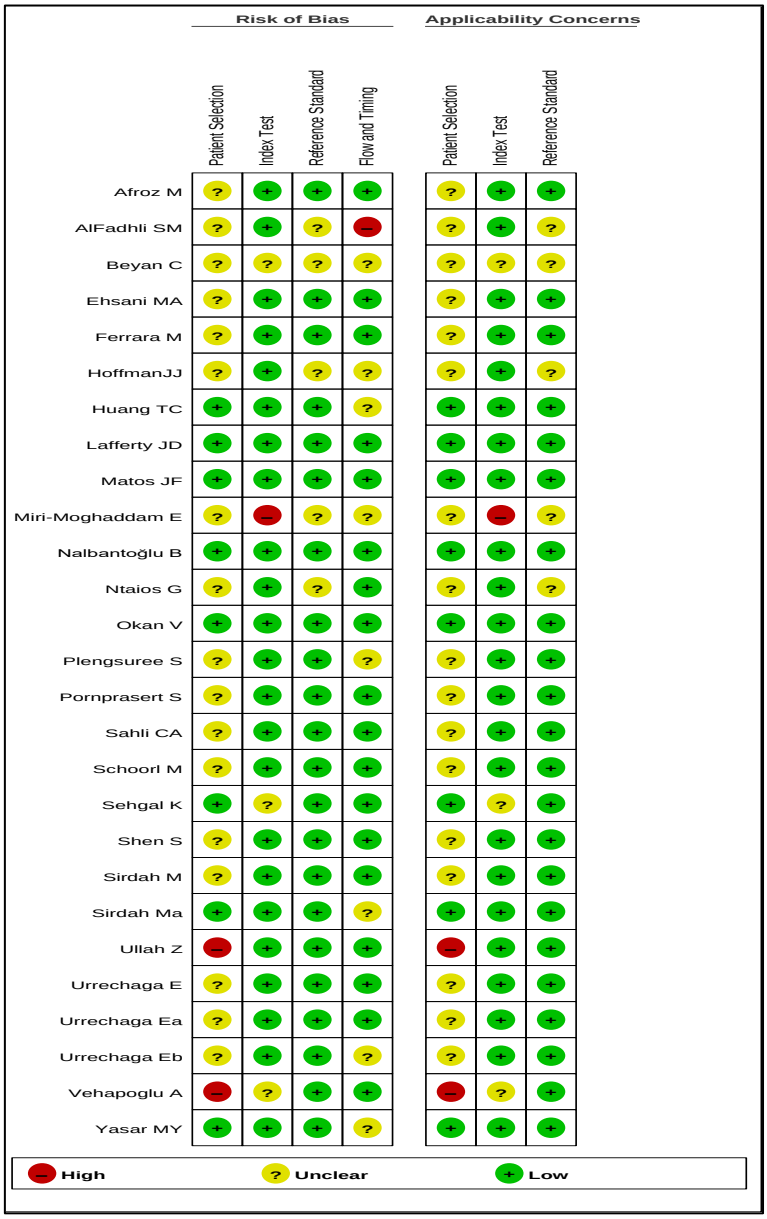


**Figure 7Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included stud**

**Findings:**

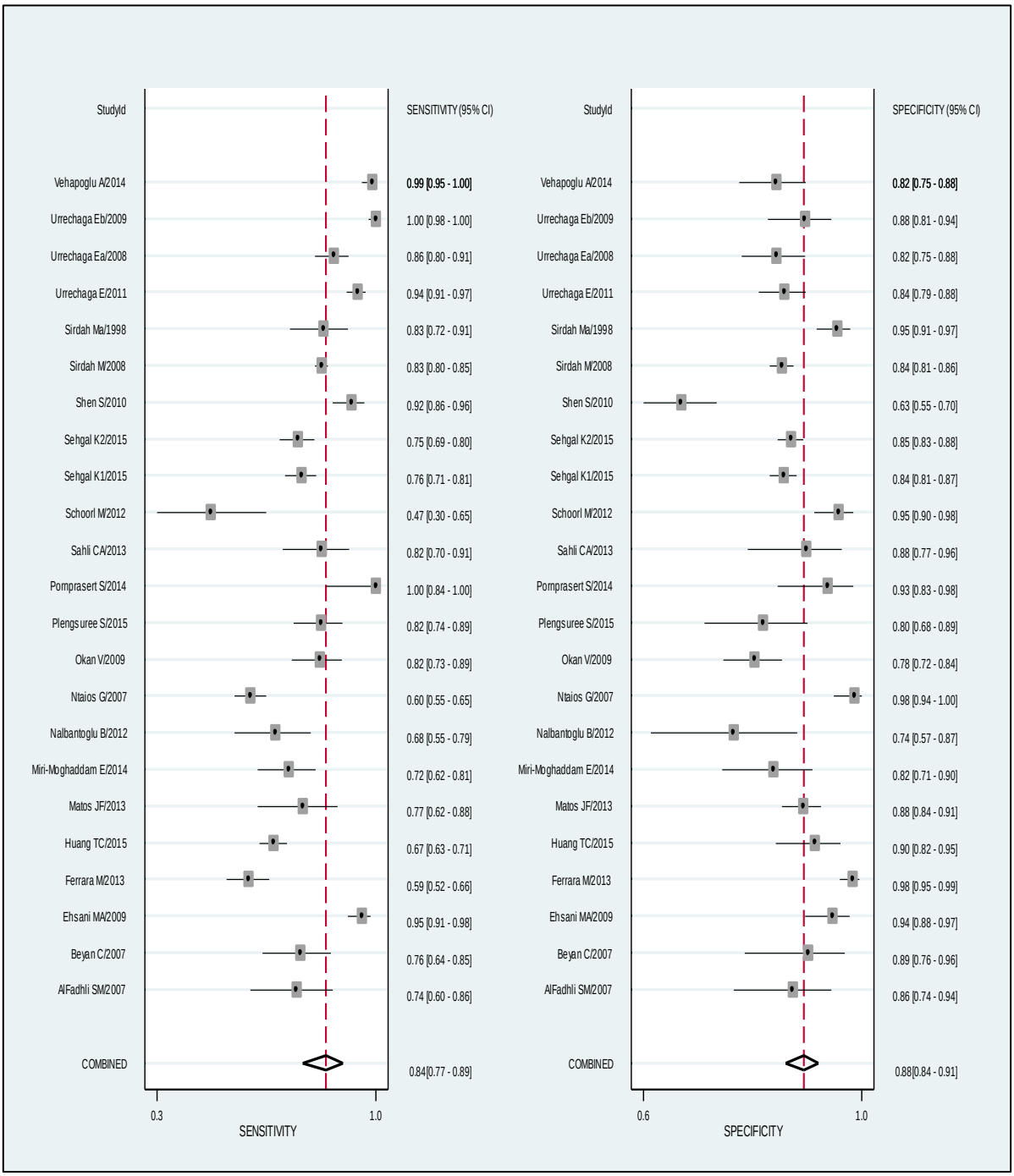


**Figure 8 Forrest plot**

For each study sensitivity and specificity with their 95 % CI was represented.The diamondshowed the summary point with 95 % CI for the sensitivity and specificity 0.84(0.77-0.89), 0.88(0.84-0.91) respectively.

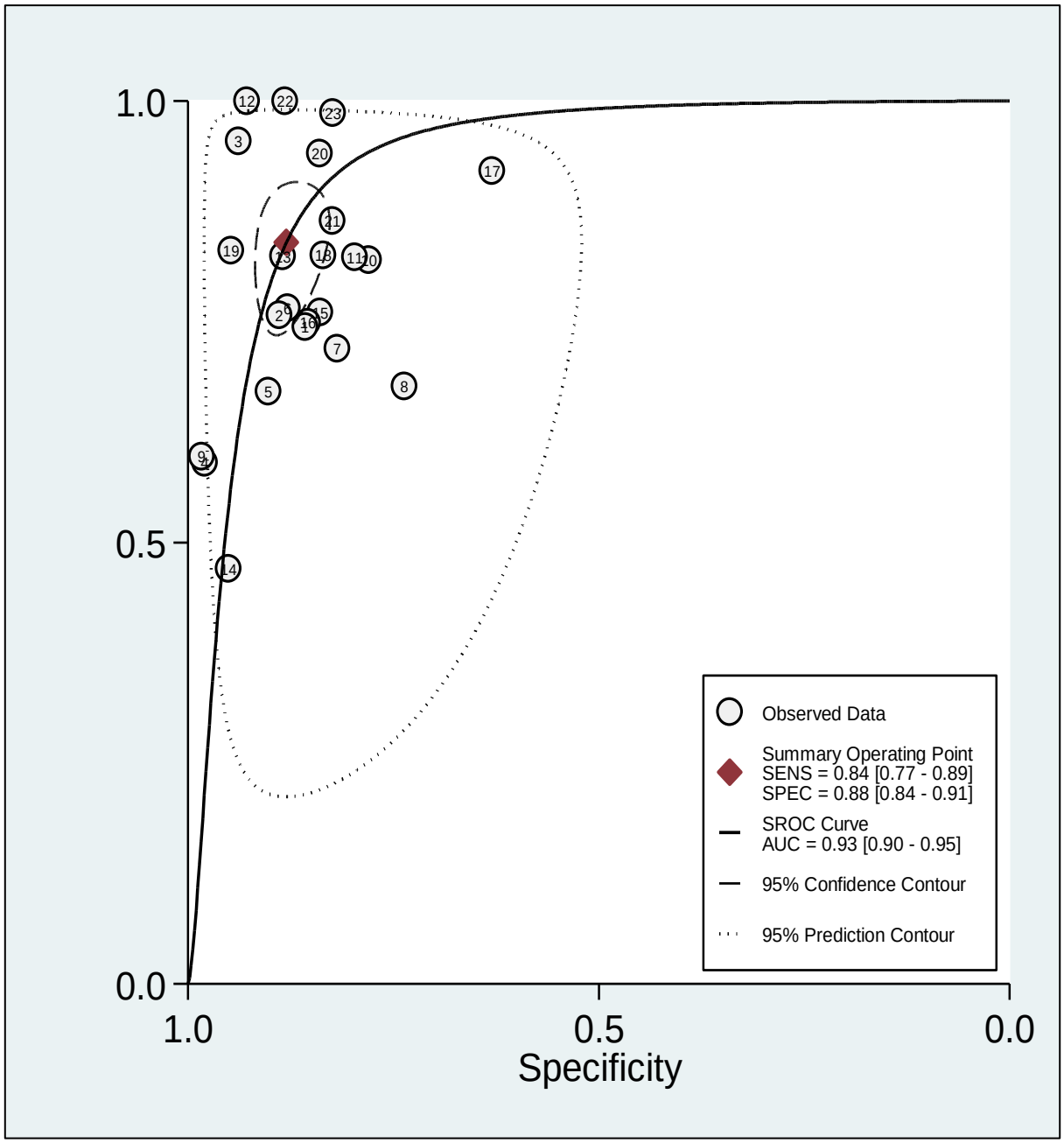


**Figure 9Summary Receiver Operating Characteristics Plot**

Summary ROC plot was used to assess the diagnostic accuracy of Mentzer index to discriminate beta-thalassemia from IDA. Sensitivity and specificity of each study was represented by the number. The summary point for the sensitivity and specificity was represented as the diamond. The nearby dashed line represented the 95 % confidence region where as the dotted line as the prediction region.The summary sensitivity, specificity and AUC were 0.84(0,77-0.89), 0.88 (0.84-0.91), 0.93(0.90-0.95) respectively.

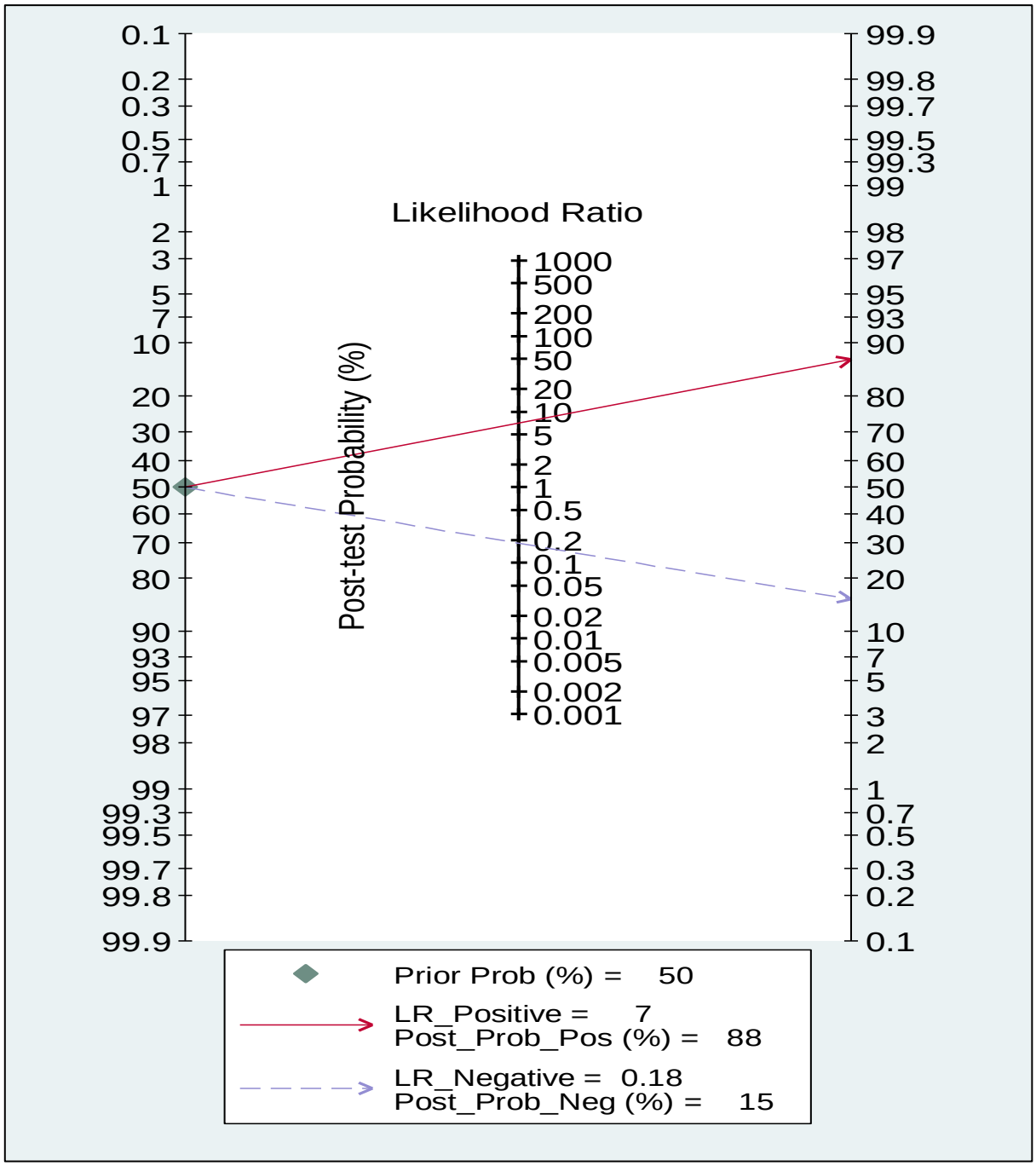


**Figure 10 Fagan's Nomogram (As an example at 50 %)**

Fagan's plot was used to evaluate the clinical usefulness of the index test. It illustrates the changes of probability considering a given pre-test probability. It can be clinically useful because it increases the previous probability from 50 % to 88 % when positive and lowers the same probability to 15% when negative.

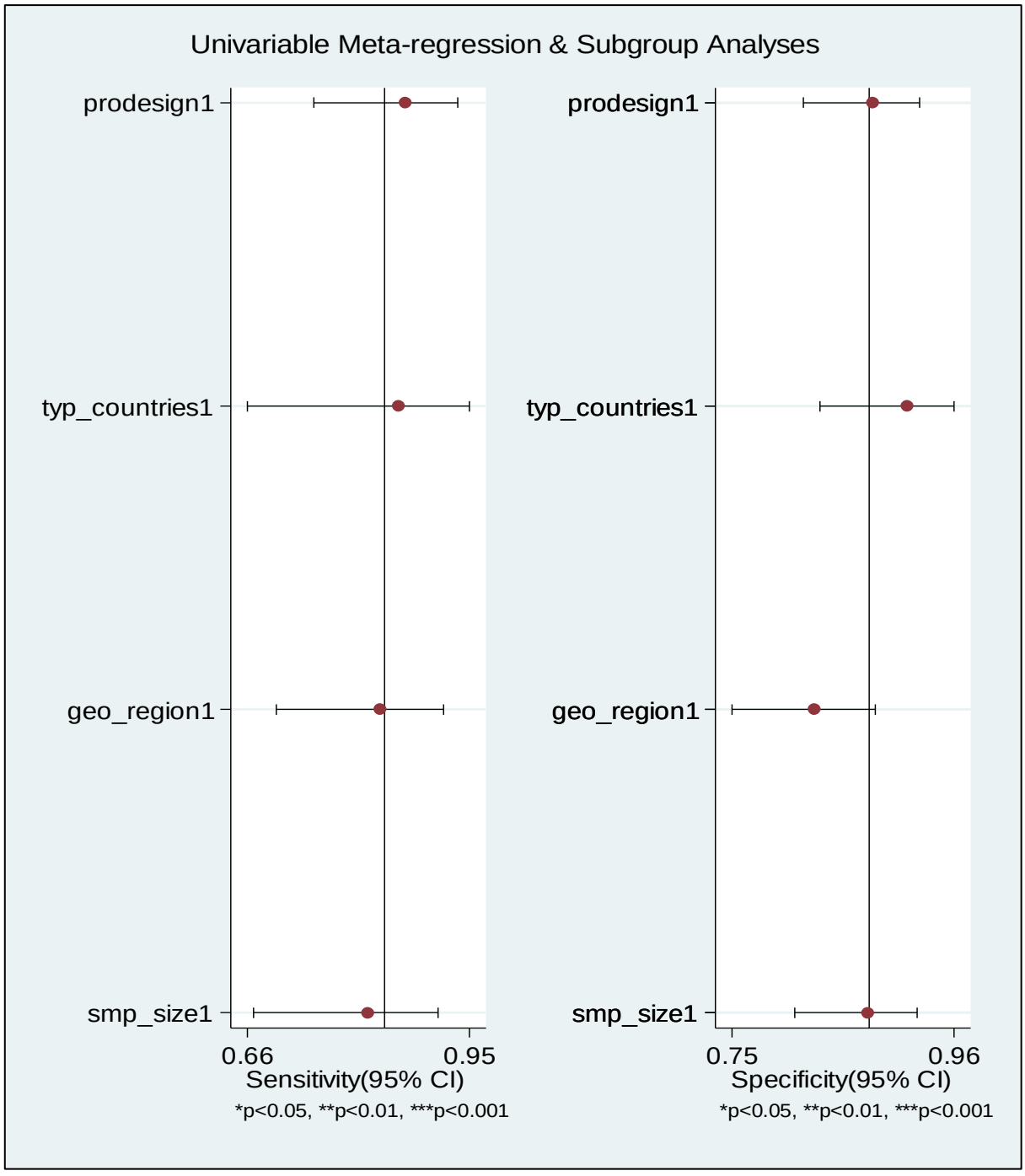


**Figure 11 Meta regression and sub group analysis**

Meta regression was performed including various covariates i.e. study design, geographical region, sample size, type of countries to assess the effect of this factors on the diagnostic accuracy of Mentzer index.From the above figure, it can be said that none of the factors show significant results as p value is > 0.05 in all cases.

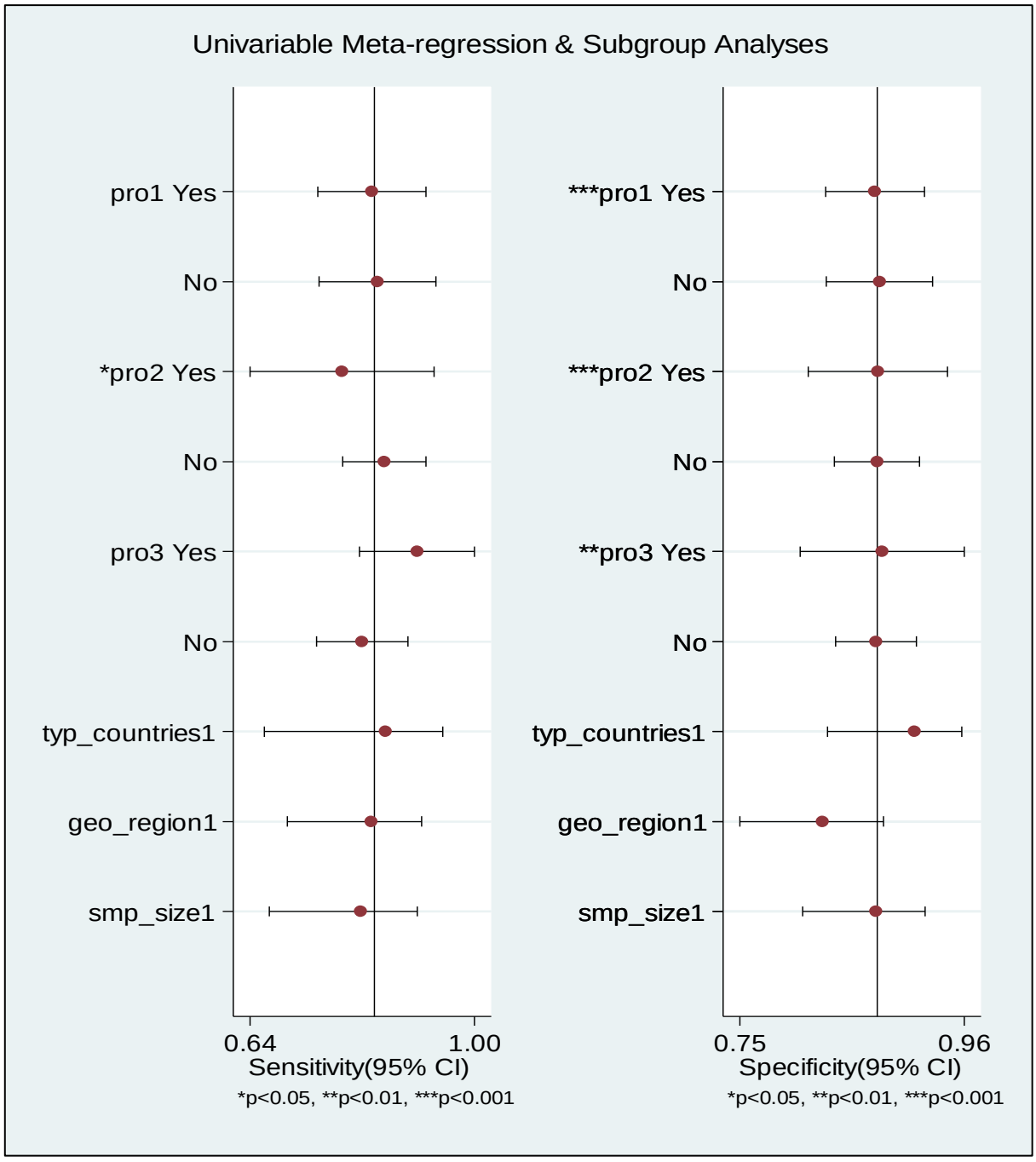


**Figure 12Meta regression and sub group analysis stratified for study design**

pro1= Cross sectional, pro2= Cohort, pro3= Case-control

After adjusting for other covariatesstudy design have significant impact on diagnostic accuracy of the Mentzer index. All the study designs i.e. Cross sectional, Cohort, Case-control are significantly associated with specificity as the p values are <0.001, <0.001, <0.01 respectively but only one study design with sensitivity p value is <0.05.

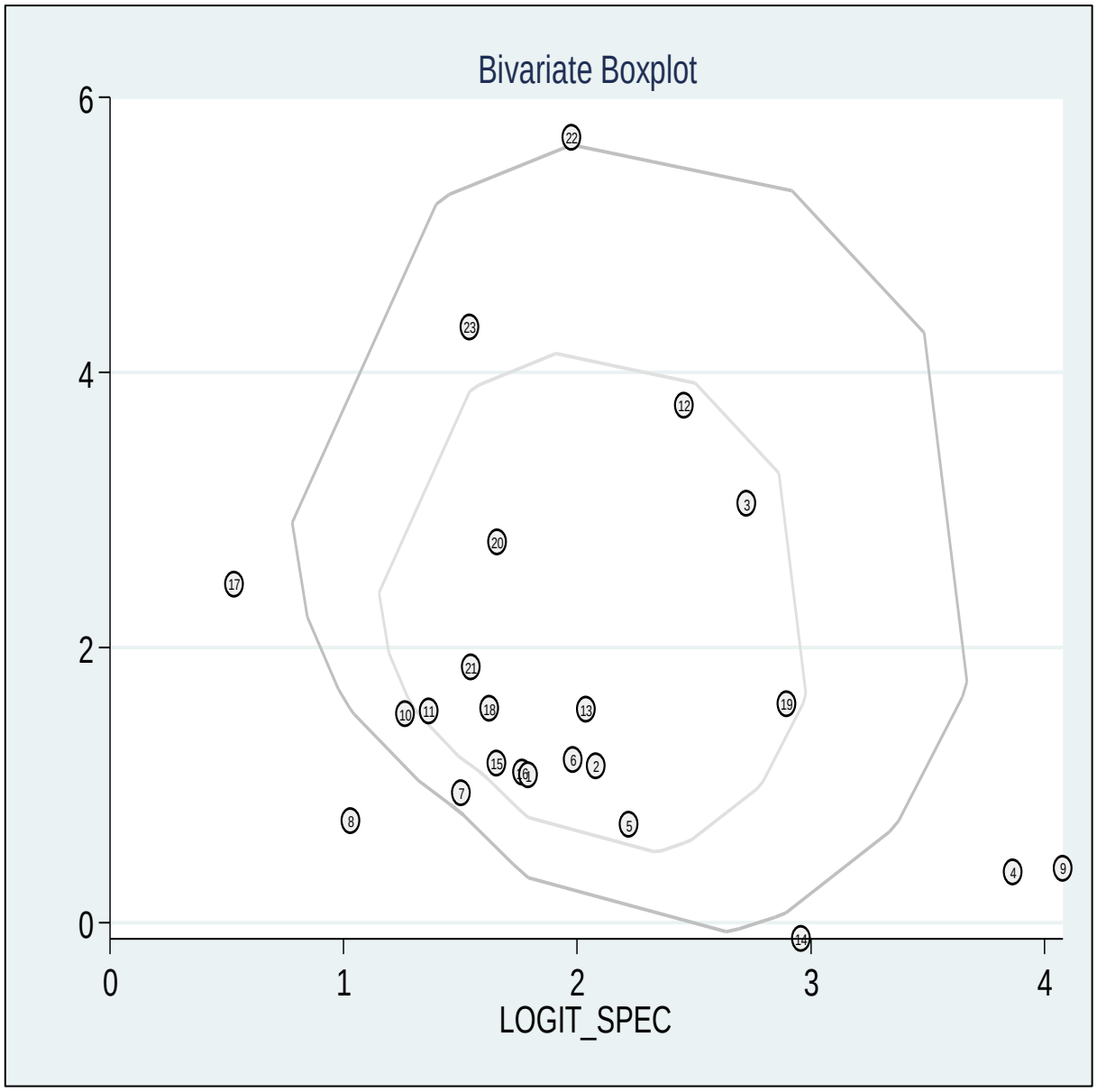


**Figure 13Bivariate Boxplot**

The bivariate box plot describes the degree of interdependence including the central location and identification of any outliers. The inner oval represents the median distribution of the data points. The outer oval represents the 95% confidence bound.Most studies clustering within the median distribution with six outliers suggesting a lower degree of heterogeneity.

**Summary of main results:**

- We collected data from 27 articles published till March 2017 comprised of 3194 patients with beta-thalassemia which represents the largest number reported on this topic.
- The quantitative analysis of the 23 studies showed the summary estimate of sensitivity, specificity, DOR and AUC of Mentzer index to discriminate beta-thalassemia were 0.84 ,0.88, 38 and 0.93 respectively.

- Heterogeneity between studies was negligible. Clustering of most studies within the median distribution with six outliers in bivariate box plot suggesting a lower degree of heterogeneity. Moreover, ICC value for sensitivity and specificity were 0.27 and 0.13 respectively.
- We also performed meta regression by including covariates (type of countries, geographical region, study sample size, study design) to assess the effect of the various factors on the diagnostic efficacy of Mentzer index but none of them shown significant result.
- After adjusting for other covariates study design have significant impact on the diagnostic accuracy of the Mentzer index
- Overall, it can be said that Mentzer index can be used as discrimination indices to differentiate beta-thalassemia from IDA

**DISCUSSION**

Thalassemia is one of the major chronic disease induced by the deficiency of one or more globin chain synthesis in the haemoglobin molecule (30).Beta thalassemia is the most frequent type of thalassemia which presents in three forms: thalassemia major, thalassemia intermedia and thalassemia minor.

- Thalassemia major: It is the most severe form and usually need medical attention within the first 2 years and require regular blood cell transfusions to survive. Affected individuals progressively become pale, diarrhoea, irritability, recurrent bouts of fever, splenomegaly may occur. Peripheral blood smear shows microcytosis, hypochromia, anisocytosis, poikilocytosis and nucleated red blood cells.
- Thalassemia intermedia: Patients having moderate anaemia and show markedly heterogeneous clinical picture. Principle symptoms are pallor, jaundice, cholelithiasis, enlargement of liver and spleen, leg ulcers, moderate to severe skeletal changes etc. Transfusions are occasionally required.
- Thalassemia minor: It leads to mild asymptomatic haemolytic anaemia (7,33,42)

Approximately 1.5 % of the global population are carriers of the beta-thalassemia and represent a substantial public health burden, since affected population usually have life-threatening anaemia (31). A series of workshops held under WHO reported that thalassemiapredominates in South -East Asia region and sub Saharan Africa has the second highest burden. Therapy for thalassemia primarily include blood transfusion, iron chelation and regular monitoring of blood parameters such as Hb, MCV, MCH, MCHC, TIBC (43) With adequate transfusion and chelating agent therapy, a child with thalassemia might grow well and can live a longer life. But unfortunately, chelators like desferrioxamine are expensive which results in inadequate receiving of the dosage (32). Worldwide, transfusion is available for a small fraction of those who need it, and most transfused patients will die from heart attack due to iron overload or early death in childhood in case of no transfusion (33).Bone marrow transplantation is a curative treatment but limited to those patients who have compatible sibling donors (32). Management of thalassaemic patients constitutes a heavy burden on affected families as well as for health care system. It accounts for significant mortality, morbidity and related health care expenses in addition to having significant emotional and psychological impact on the families of the patient (34). Prolonged medication in this patient group also affect different aspects of their life i.e. general health, psychological health, quality of life in those patients (31). In India, being a carrier of thalassemia leads to social isolation, marital tensions and stigmatization (34). Moreover, cost of the treatment is beyond affordability of most patients and available in few centres.Recent surveys suggest that annually there may be about 270 million carriers of inherited disorders of haemoglobin between 300,000 and 400,000

babies are born with a serious haemoglobin disorder each year (23,000 with β-thalassemia major) and that up to 90% of these births occur in low- or middle-income countries (31). It has been estimated that, globally, 9 million carriers become pregnant annually and 1.33 million pregnancies are at risk of thalassemia major (41). Prevention of the birth of children with thalassemia is therefore, could be a vital parameter to reduce the burden of the disorder. Hence, early (pre-marital/ pre-conception) screening and detection can play an important role to control thalassemia.Organizations like Thalassemia International Federation (registered under Cyprus Company Law Cap.113) were established to monitor the development and establishment of National Programs for the prevention and management of hemoglobinopathies in the affected countries (35).

Although treatment regimen is available for a small proportion of patient group, availability of potentially inexpensive oral iron chelator is licensed more widely. The patients' dilemmaemphasizes the need for combined treatment and prevention programmes. Wherever combined programmes exist survival is gradually improving, affected birth rates are falling, and numbers of patients are stabilizing. The policy is spreading because of its demonstrable cost-effectiveness, and thus thalassaemia is steadilycontrolled (5).

Accurate and early detection of beta-thalassemia trait can prevent occurrence of new cases among new-borns (36).Initial investigation includes a complete blood cell count (CBC) for anaemia, microcytosis and hypochromia. No separation technique can be solely relied upon for diagnosing beta-thalassemia, DNA analysis is the ultimate for the characterization of mutations. However, quantification of raised HbA2 is the hallmark diagnosis for routine identification of beta-thalassemia carrier (37).There are many chromatographic and electrophoretic approaches for estimation of HbA2 i.e. Haemoglobin electrophoresis, Cation exchange High Performance Liquid Chromatography (CE-HPLC).

- Haemoglobin electrophoresis:It was first discovered in 1949 by Pauling et al. for identification of HbS using moving boundary electrophoresis (15). The basic principle based on the migration of electrically charged molecules under an applied electric field. Electrophoresis at alkaline pH 8.5 allows for the separation of the major Hb molecules and a number of less common Hb variants. Under this situation all the haemoglobin molecules have negative charged and moved towards the cathode. HbS which have additional positive charge migrates slowly than HbA. The Hb bands can be visualized by automated staining of gel with amido back. The clear background of the gel enables measuring the concentration of the individual fraction by densitometric or spectrophotometry scanning. Accuracy largely depends on the experience and the training of the laboratory technicians and the levels of HbA2. Because of its simplicity and cost effective for lower to medium volume laboratories it is widely used for Hb screening (38,39).
- Cation exchange High Performance Liquid Chromatography: Today CE-HPLC has become the method of choice for quantification of HbA2. Basically, it follows the principle of HPLC and the column comprises of small 3.0×0.46 cm cation exchange cartridge. Collected samples should be stored at 2-8°C and be used within one weekof collection. Only 5µl of EDTA anticoagulant blood sample in 1 ml haemolysis solution is required for the analysis. Haemoglobin fractions are separated based on their ionic interaction with the cartridge material. The separated haemoglobin fractions pass through a flow cell where absorbance is detected at 415 nm then at 690 nm. The haemoglobin retention time (from insertion of the sample until the maximum point of each peak) is calculated and chromatogram is plotted. Each analytical cycle from sampling to printing of results takes about 6.5

minutes. It has been reported that in Iran after applying this technique there was 70 % reduction in infants born with thalassemia (38,39,40,15).

Although these techniques are very efficient for screening of beta-thalassemia these are very expensive and time-consuming, need expensive equipment and laboratory expertise (18). In this situation Mentzer index can be considered as cost effective, quick screening criterion for detection of thalassemia. Mentzer index or MCV/RBC count ratio is a simple mathematical formula which is not only easy to calculate also can be obtained through simple blood tests. Thereby, it could be an effective screening procedure not only in hospital settings but also in rural health care settings.It can be considered as efficacious, quick and cost-effective laboratory screening procedure in highly prevalent area and in a poor resource setting.

From the literature search, we obtained 27relevant articles among which one study was from India (3.84%), three from Pakistan (11.54%), nine from Middle East countries (34.62%), two from North & South America(7.69%), six from European countries (23.08%), two from East Asia (7.69%), two from South-East Asia (7.69%), one from North Africa (3.84%). Sixteen studies (61.53%) investigated adults, five studies (19.23%) included only children, four studies (15.38%)included mixed populations of adult and children, one study(3.84%) did not provide the patients age.

In this systematic review of the diagnostic accuracy of Mentzer index for differentiating beta-thalassemia we analysed data from the largest series of studies ever considered in this topic (n=23). Using the specific statistical methods dedicated to diagnostic meta-analysis we quantitatively summarized all the available evidence and found that overall Mentzer index is clinically useful for differentiating thalassemia trait from IDA.

After analysing the data, we identified the summary sensitivity and specificity of Mentzer index ranged from 0.77 to 0.89 and 0.84 to 0.91 respectively, which is significantly higher than 0.50 null value. Bayesian analysis (Fagan's nomogram) strengthen the study finding. If someone knows the prevalence of the disease, using the likelihood ratio value it would assign a predictive value to the patient. For an example if the proportion of beta-thalassemia is 50% (pre-test probability) it gives patient's probability of having the disease (post -test probability) which is to 88 % when positive and lowers the same probability to 15% when negative. This could be a very helpful approach for clinicians during the decision-making process.

To the best of our knowledge no systematic review had been reported till now on this topic.

The main strength of this review is the number of patients which is the highest number ever reported on this topic. To the best of our knowledge this is the first review paper on the diagnostic accuracy of Mentzer index. Along with summary estimate of diagnostic measures we also provide Bayesian analysis in the means of Fagan's plot which adds information on the clinical usefulness of the Mentzer index. The main limitation of the study is that, we could not include those studies which are published in languages other than English. Our search was limited to MEDLINE database.

**Applicability of findings to the review question:**

The number of studies identified 27 and the number of patients enrolled (12,628) were sufficient to address the review question i.e. diagnostic accuracy of Mentzer index to differentiate beta-thalassemia. Study setting, participants, index test, reference standard were homogeneous across all the studies.

**Authors' Conclusion**

Implications for practice:

Findings of the review support the use of Mentzer index to screen the people with beta-thalassemia. It

can be considered as clinically useful to guide physicians for mass screening of the population with hypochromic microcytic RBC. Early detection of the diseasereduces the cost of the treatment as well as the side effect of the iron-chelation therapy.

Implications of research:

Further investigation will be required to compare with the other diagnostic tools in order to investigate the diagnostic potential for early screening of beta-thalassemia. Globally,burden of thalassaemic major patients cannot be underestimated. Prevention of birth of children with beta-thalassemia major would thus spare a lot of distress, effort and expenses for the families involved and for the society.

**REFERENCES**

1. 1.Hoffmann JJ, Urrechaga E, Aguirre U. Discriminant indices for distinguishing thalassemia and iron deficiency in patients with microcytic anemia: a meta-analysis. Clin Chem Lab Med. 2015 Nov;53(12):1883-94
2. 2.Urrechaga E. Discriminant value of % microcytic/% hypochromic ratio in the differential diagnosis of microcytic anemia. Clin Chem Lab Med. 2008;46(12):1752-8.
3. 3.Urrechaga E. Red blood cell microcytosis and hypochromia in the differential diagnosis of iron deficiency and beta-thalassaemia trait. Int J Lab Hematol. 2009 Oct;31(5):528-34.
4. Shen C, Jiang YM, Shi H, Liu JH, Zhou WJ, Dai QK, Yang H. Evaluation of indices in differentiation between iron deficiency anemia and beta-thalassemia trait for Chinese children. J Pediatr Hematol Oncol. 2010 Aug;32(6): e218-22
5. 5.Global epidemiology of haemoglobin disorders and derived service indicators: Bernadette Modell a, Matthew Darlison. Available from: http://www.who.int/bulletin/volumes/86/6/06036673/en/
6. 6.Balgir, R.S. The burden of haemoglobinopathies in India and the challenges ahead. Current Science. 2000; 79(11):1536-47
7. Grow K, Vashist M, Abrol P, Sharma S, Yadav R Beta Thalassemia in India and Challenges Ahead - International Journal of Pharmacy and Pharmaceutical Sciences. Available from: http://www.ijppsjournal.com/Vol6Issue4/9093.pdf
8. 8.Help prevent thalassemia – Jago re. Dr Sharmila Ghosh, Available from: https://www.hindujahospital.com/communityportal/.../articles-download
9. Verma, I.C. Burden of genetic disorders in India. Indian Journal of Pediatrics. 2000; 67(12):89398.
10. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, et al. Prevalence of beta-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. Journal of Community Genetics. 2013; 4(1):33-42.
11. Madan N, Sharma S, Sood SK, Colah R, Bhatia LH. Frequency of beta-thalassemia trait and other hemoglobinopathies in northern and western India. Indian Journal of Human Genetics. 2010; 16(1):16-25
12. 12.Wikipedia. Thalassemia [29 May 2017] Available from: https://en.wikipedia.org/wiki/Thalassemia
13. Holm G. Thalassemia Healthline2012 [29 May 2017]. Available from: http://www.healthline.com/health/thalassemia#Overview1
14. Higgs DR, Thein SL, Woods WG, ed: The molecular pathology of the thalassaemias. In The Thalassaemia Syndromes. Oxford, England: Blackwell Science; 2001:133-191

15. 15.Wajcman H, Moradkhani K. Abnormal haemoglobins: detection & characterization. The Indian Journal of Medical Research. 2011; 134:538-46
16. 16.Urrechaga E, Borque L, Escanero JF. The role of automated measurement of RBC subpopulations in differential diagnosis of microcytic anemia and β-thalassemia screening. Am J Clin Pathol. 2011 Mar;135(3):374-9
17. 17.Pornprasert S, Panya A, Punyamung M, Yanola J, Kongpan C. Red cell indices and formulas used in differentiation of β-thalassemia trait from iron deficiency in Thai school children. Hemoglobin. 2014;38(4):258-61
18. 18.Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, Kontoninas Z, Nikolaidou A, Savopoulos C, Pidonia I, Alexiou-Daniel S. Discrimination indices as screening tests for beta-thalassemic trait. Ann Hematol. 2007 Jul;86(7):487-91.
19. 19.Ferrara M, Capozzi L, Russo R, Bertocco F, Ferrara D. Reliability of red blood cell indices and formulas to discriminate between beta thalassemia trait and iron deficiency in children. Hematology. 2010 Apr;15(2):112-5
20. 20.Wikipedia.Mentzer Index [30 May 2015]. Available from: https://en.wikipedia.org/wiki/Mentzer_index.
21. Kotwal J, Saxena R, Choudhry VP, Dwivedi SN, Bhargava M. Erythrocyte indices for discriminating thalassaemic and non-thalassaemic microcytosis in Indians. Natl Med J India. 1999 Nov-Dec;12(6):266-7
22. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008 Aug;30(4):324-330
23. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, Kacan A. Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiency anemia. Anemia. 2014; 2014:576738
24. 24.Ullah Z, Khattak AA, Ali SA, Hussain J, Noor B, Bano R, Jan Mahsud MA. Evaluation of five discriminating indexes to distinguish Beta-Thalassemia Trait from Iron Deficiency Anaemia. J Pak Med Assoc. 2016 Dec;66(12):1627-1631
25. Huang TC, Wu YY, Chen YG, Lai SW, Wu SC, Ye RH, Lu CS, Chen JH. Discrimination Index of Microcytic Anemia in Young Soldiers: A Single Institutional Analysis. PLOS ONE. 2015 Feb; 10(2): e0114061
26. 26.Afroz M, Shamsi TS, Syed S. Predictive value of MCV/RBC count ratio to discriminate between iron deficiency anaemia and beta thalassaemia trait. The Journal of the Pakistan Medical Association. 1998; 48(1):18-9.
27. Global distribution of thalassemia [2nd June 2017] Available from: https://www.seenso.com
28. QUADAS-2 [19 th June 2017] Available from: http://www.bristol.ac.uk
29. Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy [19 th June 2017] Available from: http://methods.cochrane.org/sites/methods.cochrane.org
30. 30.Kaheni S, Yaghobian M, Sharefzadah GH, Vahidi A, Ghorbani H, Abderahemi A. Quality of Life in Children with B-Thalassemia Major at Center for Special Diseases . Iranian Journal of Pediatric Hematology and Oncology. 2013;3(3):108-113.
31. De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, Daar S, Wali Y, Yassin M, Soliman N, Sobti P, Al Jaouni S, El Kholy M, Fiscina B,Angastiniotis M. β-Thalassemia

Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint. Mediterr J Hematol Infect Dis. 2017 Feb20;9(1)
32. Weatherall D.J, Clegg J.B. Inherited haemoglobin disorders: an increasing global health problem. Bulletin of the World Health Organization, 2001, 79: 704–712 [29 th June 2017] Available from: http://www.who.int/bulletin/archives79%288%29704.pdf
33. Safizadeh H, Farahmandinia Z, nejad SS, Pourdamghan N, Araste M. Quality of Life in Patients with Thalassemia Major and Intermedia in Kerman-Iran (I.R.). Mediterranean Journal of Hematologyand Infectious Diseases. 2012;4(1).
34. Wong LP, George E, Tan JAMA. Public perceptions and attitudes toward thalassaemia: Influencing factors in a multi-racial population BMC Public Health.2011,11:193
35. Soteriades ES, Weatherall D. The thalassemia International Federation: A Global Public Health Paradigm [ 1st July, 2017] Available from: http://www.pagepressjournals.org/index.php/thal/article/view/thal.2014.1840/5708
36. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: Report of 2600 cases. Indian J Pathol Microbiol 2010;53:57-62
37. Brants A. Detection of hemoglobinopathies and thalassemias using automated separation systems [1st July, 2017] Available from:https://www.mlo-online.com/detection-of-hemoglobinopathies-and-thalassemias-using-automated-separation-systems.php
38. Brants A. Hemoglobinopathy and thalassemia detection Traditional methods and a novel method – capillary electrophoresis technology [1st July, 2017] Available from:http://iacld.com/DL/elm/hemoglobinopathyandthalassemiadetection.pdf
39. Lt Col Gupta PK, Col Kumar H, Lt Col Kumar S, Brig Jaiprakash M. Cation Exchange High Performance Liquid Chromatography for Diagnosis of Haemoglobinopathies MJAFI 2009; 65: 33-37
40. Colah RB, Surve R, Sawant P, D'Souza E, Italia K, Phanasgaonkar S, Nadkarni AH, Gorakshakar AC. HPLC Studies in Hemoglobinopathies Indian J Pediatr 2007; 74 (7): 657-662
41. Cao A, Kan YW. The Prevention of Thalassemia. Cold Spring Harbor Perspectives in Medicine. 2013;3(2).
42. Cao A, Galanello R. Beta Thalassemia Gene Test Review. Genetics in Medicine (2010) 12, 61–76
43. Karim MF, Ismail M, Hasan AM, Shekhar HU. Hematological and biochemical status of Beta-thalassemia major patients in Bangladesh: A comparative analysis. International Journal of Hematology-Oncology and Stem Cell Research. 2016;10(1):7-12
44. AlFadhli SM, Al-Awadhi AM,AlKhaldi D.Validity Assessment of Nine Discriminant Functions Used for the Differentiation between Iron Deficiency Anemia and Thalassemia Minor. Journal of Tropical Pediatrics 2007 May 53(2):93-7
45. Beyan C, Kaptan K, Ifran A. Predictive value of discrimination indices in differential diagnosis of iron deficiency anemia and beta-thalassemia trait. Eur J Haematol. 2007 June78(6):524-6
46. Ehsani MA, Shahgholi E, Rahiminejad MS, Seighali F, Rashidi A. A New Index for Discrimination between Iron Deficiency Anemia and Beta-Thalassemia Minor: Results in 284 Patients. Pak. J. Biol. Sci. 2009 ;12(5): 473- 475
47. Matos JF, Dusse LMSA, RVB, Ferreira MR, Coura-Vital W, Fernandes APSM, Faria JRD, Borges KBG, Carvalho MDG. Comparison of discriminative indices for iron deficiency anemia and β thalassemia trait in a Brazilian population. Hematology. 2013 Jul;18 (3): 169-174

48. 48.Miri-Moghaddam E, Sargolzaie N. Cut off Determination of Discrimination Indices in Differential Diagnosis between Iron Deficiency Anemia and β- Thalassemia Minor. IJHOSCR. 2014 Apr 1;8(2):27-32
49. Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the beta-thalassaemia trait from those with iron deficiency anaemia.Journal of International medical research.2009 Feb; 37(1):25-30.
50. Schoorl M, Schoorl M, Linssen J,Villanueva MM, NoGuera JAV, Martinez PH, Bartels PCM. Efficacy of Advanced Discriminating Algorithms for Screening on Iron-Deficiency Anemia and β-Thalassemia Trait. Am J Clin Pathol.2012; 138:300-304
51. Nalbantoğlu B, Güzel S, Büyükyalçın V, Donma MM, Güzel EC, Nalbantoğlu A, Karasu E , Özdilek B. Indices Used in Differentiation of Thalassemia Trait from Iron Deficiency Anemia in Pediatric Population: AreThey Reliable? Pediatric Hematology and Oncology. 2012 Aug;29 (5): 472-478
52. Lafferty JD, Crowther MA, Ali MA, Levine M. The Evaluation of Various Mathematical RBC Indices and Their Efficacy in Discriminating Between Thalassemic and Non Thalassemic Microcytosis. AJCP.1996 Aug; 106 (2):201-205
53. 53. Sahli CA, Bibi A, Ouali F, Fredj SH , Dakhlaoui B, Othmani R, Laouini N, Jouini L, Ouenniche F, Siala H, Touhami I, Becher M, Fattoum S, Toumi NEH, Messaoud T Red cell indices: differentiation between β -thalassemia trait and iron deficiency anemia and application to sickle-cell disease and sickle-cell thalassemia. Clin Chem Lab Med. 2013 Jun;51 (11): 1-10
54. Sehgal K, Mansukhani P, Dadu T, Irani M, Khodaiji S. Sehgal index: A new index and its comparison with other complete blood count-based indices for screening ofbeta thalassemia trait in a tertiary care hospital. Indian J Pathol Microbiol.2015 Jul-Sep;58(3):310-5
55. 55.Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int Journal Lab Hematol, 2008 Aug; 30(4): 324-330
56. Sirdah M, Bilto YY, ElJabour S, Najjar KH. Screening secondary school students in the Gaza strip for β-thalassemia trait. Clin. Lab. Haem 1998; 20: 279-283
57. Plengsuree S, Punyamung M, Yanola J, Nanta S, Jaiping K. Red Cell Indices and Formulas Used in Differentiation of b-Thalassemia Trait from Iron Deficiency in Thai Adults. Hemoglobin,2015 Jun; Early Online: 1–5
58. Yasar MY, Khan S, Raziq F. β.-Thalassemia Trait: Haematological Parameters. J Ayub Med Coll Abbottabad;2010 22(4): 84- 86