Study on the Clinico-Haematological Profile of Patients of Non-Transfusion Dependent Thalassemia (NTDT) Attending Thalassemia Clinic at a Tertiary Care Hospital

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BACKGROUND

Non-Transfusion Dependent Thalassemia (NTDT) is a term used to label patients who do not require lifelong transfusions for survival. The common conditions traditionally described as NTDT are beta thalassemia intermedia, haemoglobin H disease and haemoglobin E beta thalassemia. The major problem with NTDT patients remains that, as they do not require regular transfusions, so they often do not seek medical attention until they develop serious complications such as anaemia due to ineffective erythropoiesis, iron overload, hypercoagulability and hormonal imbalances like hypogonadism, hypoparathyroidism, renal dysfunction etc.

METHODS

Our study was conducted in the Thalassemia Out Patient Department, Institute of Haematology & Transfusion Medicine at Medical College, Kolkata, over a span of 1 year 6 months. It was a cross sectional observational study of 30 patients of Non-Transfusion Dependant Thalassemia (NTDT) selected randomly as per the inclusion criteria. Thorough history taking and clinical examination were performed. Blood samples were tested for haemoglobin levels, RBC indices, fasting glucose, serum calcium, serum phosphate, SGPT, serum creatinine, TSH, FT4, FSH and LH (3 pooled samples at 30-minute-intervals), serum testosterone (in males) and serum ferritin. Ultrasonography and echocardiography were done. The data was analysed by standard statistical methods, using MedCalc (version 3.0) software. The correlation of different complications of NTDT with serum ferritin levels was done using Mann-Whitney U test. An alpha level of 5% has been taken i.e. any p value < 0.05 has been taken as significant.

RESULTS

Serum ferritin levels were found to be quite high in the NTDT patients, with a range of 335.1 (min.) - 1300 (max.) ng/mL with a mean serum ferritin level of 568.78 ng/mL and SD of 224.9, despite the fact that nearly 87% (26 out of 30) of the patients had received less than 10 transfusions in their lifetime. Our study showed that, delayed puberty, renal dysfunction, liver dysfunction and pulmonary hypertension were found to be significantly related to the serum ferritin levels (serving as a marker of liver iron concentration).

CONCLUSIONS

Thus, we conclude that despite requiring much fewer transfusions than transfusion dependent thalassemia patients, the NTDT patients do develop iron overload as well as different complications, some of which are significantly related to the liver iron overload. Knowledge of such complications could help to initiate chelation therapy at the appropriate time for NTDT patients, thereby reducing morbidity and improving their quality of life.

KEY WORDS

Non-Transfusion Dependent Thalassemia (NTDT), Ferritin

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BACKGROUND

Thalassemias are a group of inherited haemoglobin disorders characterized by the defective synthesis of the alpha- or beta-chains of adult haemoglobin A. Transfusion dependence, from the patient’s as well as the clinician’s point of view, is a very important issue. However, at the other end of the spectrum lie the non-transfusion dependent thalassemias (NTDT). NTDT is a term used to label patients who do not require lifelong transfusions for survival, although they may require occasional or even frequent transfusions in certain clinical settings and for certain defined periods of time.[1] Hence, we surmised that a study of the most common complications and their co relation to the body’s iron load as measured by serum ferritin, would help in future, to identify those at risk at an earlier stage. The common conditions traditionally described as NTDT are[2]

- Beta thalassemia intermedia.
- Haemoglobin E beta thalassemia.
- Haemoglobin H disease.

As per the 2011 Census, India has a prevalence of beta thalassemia carriers is around 1-17% and that of Hb E carriers around 2-50%. [3] Regardless of specific subtype, all NTDT patients are prone to develop certain morbidities, which can be attributed to chiefly the following pathophysiologic processes:[4]

Transfusion independence does not mean freedom from complications in NTDT patients. NTDT patients also suffer from anaemia, although usually less severe than transfusion dependant thalassemia patients. As a consequence of chronic anaemia and ineffective erythropoiesis, there occurs growth retardation, facial dysmorphism and extramedullary haematopoeisis. Secondly, ineffective erythropoiesis results in increased gastrointestinal iron absorption.[5] Such iron overload, which may be reflected in the serum ferritin levels, can in turn, lead to hepatic or cardiac fibrosis, with increased risk of hepatocellular carcinoma or cardiac dysfunction. Iron loads are best reflected by MRI based liver iron assessment.[6] Thirdly, factors such as abnormal circulating RBCs & platelets may lead to hypercoagulable states, resulting in thrombotic episodes, chronic leg ulcers and pulmonary hypertension with secondary right ventricular dysfunction.[7]

In addition, NTDT patients may also develop many other complications such as hormonal deficiencies eg. hypothyroidism, hypoparathyroidism, diabetes mellitus or hypogonadism (mainly due to ineffective erythropoiesis, medullary expansion & iron mediated target gland damage), osteoporosis and renal dysfunction. The major problem with NTDT patients remains that, as they do not require regular transfusions, they often do not seek medical attention until they develop serious complications. Hence, we surmised that a study of the most common complications and their co relation to the body’s iron load would help in future to identify those at risk at an earlier stage, thereby improving the quality of life of NTDT patients with appropriate preventive or remedial measures. A recent longitudinal follow-up over a 10-year period confirmed these findings, and a serum ferritin level of ≥800 ng/mL was the threshold after which all patients became at risk of developing morbidity.[8]

Objectives
1. To assess the complications of NTDT patients.
2. To correlate the complications with their genotypes.
3. To correlate the iron load (serum ferritin level) with the complications present. which would enable us to predict or recognize such problems early.

METHODS

This cross-sectional observational study was done in Thalassemia OPD, Institute of Haematology and Transfusion Medicine (HTM), Medical College Hospital from Jan 2014 to July 2015 among thirty non-transfusion dependant thalassemia (NTDT) patients of both genders attending Thalassemia OPD through purposive sampling.

Total Study Population
Out of the total 42 patients assessed, 12 were excluded based on presence of different exclusion criteria. Rest 30 patients of NTDT, satisfying the inclusion criteria were analysed.

Parameters to be Studied
- History taking.
- Clinical examination.
- Laboratory investigations.
- Radiological investigations.
- Study of medical records.

Study Tools
A. History taking & Examination of Medical Records
- Number of transfusions in past.
- Presence of symptomatic anaemia (easy fatigability, generalized weakness, palpitations).
- Past history of non-healing leg ulcers.
- Past history of gallstones.
- Age of onset of puberty.

B. Clinical examination:
- Facial dysmorphism.
- Presence of hepatosplenomegaly.
- Presence of leg ulcers.
- Measurement of height & weight.
- Sexual Maturity Rating.

C. Blood Examination:
- Hb levels, RBC indices.
- Blood for fasting glucose, serum calcium, serum phosphate, SGPT, serum creatinine.
- Blood levels of TSH, FT4, FSH, LH, serum testosterone (in males).
- Serum ferritin levels.

D. Radiological Evaluation:
- USG abdomen for endometrial thickness (in females) and presence of gall stones & hepatosplenomegaly.
• Echocardiography for presence of pulmonary hypertension by TRV measurement.

Inclusion Criteria
• Age >2 yrs. at diagnosis.
• Hb values between 7 gm/dL and 9 gm/dL without the need for regular transfusion regimen (at diagnosis) in patients with or without splenomegaly.
• No transfusion within previous 6 months prior to study entry.
• Serum ferritin > 300 ng/mL.

Exclusion Criteria
1. HbS variants of thalassemia syndrome.
2. Anticipated regular transfusion during study.
3. Age <2 yrs. at diagnosis.
4. Lab values: Cr Cl <\= 40 mL/sqm BSA/min, serum creatinine levels > upper limit of normal, SGPT > 5 times upper limit of normal.

Study Technique
Meeting the inclusion and exclusion criteria, 30 patients of Non-transfusion dependant thalassemia were selected. Thorough history taking and clinical examination were performed, and previous medical records examined.

Blood samples were drawn and tested for various parameters, viz. haemoglobin levels, RBC indices, fasting glucose, serum calcium, serum phosphate, SGPT, serum creatinine, TSH, FT4, FSH and LH (3 pooled samples at 30 minutes interval), serum testosterone (in males) and serum ferritin. All the investigations were done in the hospital itself.

Patients also had an ultrasonography of abdomen done to determine liver and spleen size, presence of gallstones and endometrial thickness (in females). Echocardiography was done to determine presence of pulmonary hypertension by estimation of TRV. All radiological investigations were done by the same operator for all patients in order to minimize variability in interpretation.

The results were interpreted according to thresholds well accepted for clinical use and predefined on the basis of published literature.

Statistical Methods
The data was analysed by standard statistical methods, using MedCalc (version 3.0) software. The categorical variables were expressed as number of patients and percentage of patients and compared across the groups using Pearson’s Chi Square test for Independence of Attributes. Continuous variables were expressed as Mean +/- Standard deviation. P-value was calculated using the Chi-square test for testing the significance of the tests. A p-value of <0.05 was considered “significant” and >0.05 as “not significant” The co relation of different complications of NTDT with serum ferritin levels was calculated using Mann Whitney U test. The statistical software MedCalc version 3.0 has been used for the analysis. An alpha level of 5% has been taken i.e. any p value < 0.05 has been taken as significant.

Ethical Considerations
An informed consent was obtained from each participant prior to their enrolment in the study. The study was approved by Ethics Committee.

Descriptive Statistics of Various Parameters Studied in NTDT Patients

| Gender | Total No. of Subjects | Mean Serum Ferritin | SD   | P Value | Significance |
|--------|-----------------------|---------------------|------|---------|-------------|
| Male   | 16                    | 507.5               | 272.72 | 0.9     | Not significant |
| Female | 14                    | 524.8               | 158.97 |         |             |
| Total  | 30                    |                     |       |         |             |

| Age     | Total No. of Subjects | Mean Serum Ferritin | SD   | P Value | Significance |
|---------|-----------------------|---------------------|------|---------|-------------|
| <18 yrs | 11                    | 393.0               | 83.67 |         |             |
| >18 yrs | 19                    | 559.3               | 241.18| 0.001   | Significant |
| Total   | 30                    |                     |       |         |             |

| No of transfusions | Total No. of Subjects | Mean Serum Ferritin | SD   | P Value | Significance |
|--------------------|-----------------------|---------------------|------|---------|-------------|
| <10                | 26                    | 507.05              | 141.08|         |             |
| >10                | 4                     | 946.0               | 273.36| 0.004   | Significant |
| Total              | 30                    |                     |       |         |             |

| Symptomatic anaemia | Total No. of Subjects | Mean Serum Ferritin | SD   | P Value | Significance |
|---------------------|-----------------------|---------------------|------|---------|-------------|
| Present             | 17                    | 546.1               | 263.19| 0.07    | Not Significant |
| Absent              | 13                    | 430.2               | 108.56|         |             |
| Total               | 30                    |                     |       |         |             |

| Facial dysmorphism  | Total No. of Subjects | Mean Serum Ferritin | SD   | P Value | Significance |
|---------------------|-----------------------|---------------------|------|---------|-------------|
| Present             | 23                    | 524.1               | 232.02| 0.339   | Not Significant |
| Absent              | 7                     | 427.0               | 203.8 |         |             |
| Total               | 30                    |                     |       |         |             |

Descriptive Statistics of Various Parameters Studied in NTDT Patients
Of the 30 patients studied, 16 (53%) were male and 14 (47%) female, with Male: Female ratio of 1.14:1. Of the 11 patients of age <18 yrs. studied, 5 (45%) had height above the 3rd centile, while 6 (55%) had height below 3rd centile. Of the 14 female patients studied, 10 (71%) had normal endometrial thickness for age while 4 (29%) had low endometrial thickness. All the 16 male patients (100%) were found to
have normal serum testosterone levels. Of the 14 female patients studied, 10 (71%) had normal endometrial thickness for age while 4 (29%) had low endometrial thickness. Several other parameters were studied in the 30 patients as shown in the multiple bar diagram below.

| Hepatomegaly | Total No. of Subjects | Mean Serum Ferritin | SD | P Value | Significance |
|--------------|-----------------------|---------------------|----|---------|--------------|
| Present      | 15                    | 546.1               | 263.27 | 0.187   | Not Significant |
| Absent       | 15                    | 430.2               | 160.31 |          |              |
| Total        | 30                    |                     |      |         |              |

| Splenomegaly | Total No. of Subjects | Mean Serum Ferritin | SD | P Value | Significance |
|--------------|-----------------------|---------------------|----|---------|--------------|
| Present      | 21                    | 536.5               | 189.13 | 0.129   | Not Significant |
| Absent       | 9                     | 424.1               | 305.2  |          |              |
| Total        | 30                    |                     |      |         |              |

| BMI | Total No. of Subjects | Mean Serum Ferritin | SD | P Value | Significance |
|-----|-----------------------|---------------------|----|---------|--------------|
| >18.5 | 20                   | 503.3               | 195.18 |       |              |
| <18.5 | 10                   | 510.6               | 286.94 | 0.941   | Not Significant |
| Total  | 30                   |                     |      |         |              |

| SMR | Total No. of Subjects | Mean Serum Ferritin | SD | P Value | Significance |
|-----|-----------------------|---------------------|----|---------|--------------|
| Apt for age | 8                | 386.6               | 123.08 | 0.002   | Significant |
| Not apt for age | 22               | 541.3               | 237.73 |          |              |
| Total  | 30                   |                     |      |         |              |

| Parameters with Serum Ferritin Levels | Total No. of Subjects | Mean Serum Ferritin | SD | P Value | Significance |
|--------------------------------------|-----------------------|---------------------|----|---------|--------------|
| Hb                                    | >7 gm/dL | 21                | 509.0              | 199.77 | 0.354        | Not Significant |
|                                      | <7 gm/dL | 9                 | 546.1              | 281.62 |              |          |
|                                      | Total    | 30                |                     |      |              |              |

### DISCUSSION

Our study was conducted in the Thalassemia Outpatient Department, Institute of Haematology & Transfusion Medicine at Medical College, Kolkata over a span of 1 year 6 months. It was a cross sectional observational study. Overall 30 patients of Non-Transfusion Dependant Thalassemia (NTDT) who attended the Thalassemia OPD during this period and fitted into our inclusion criteria, were studied. In our study, we tried to determine the prevalence of different complications in NTDT patients and to co relate these complications with the serum ferritin level, which is a cheap and convenient way to estimate iron load in resource poor settings.

In our study, age of patients ranged from 6 yrs to 50 yrs., of whom 16 were male and 14 were female. The mean serum ferritin level in males was 59.46 ng/mL, with a SD of 272.7; while the mean serum ferritin level in females was 53.92 ng/mL, with a SD of 158.9. There was no significant difference in serum ferritin levels between the sexes. (p value = 0.9). Mean serum ferritin levels were found to increase significantly with age. [mean SF in those <= 20 yrs of age 181.74 ng/mL, SD 159.58; mean SF in those >20 yrs of age 368.25 ng/mL, SD 261.96; p value = 0.007]. Of the total 30 patients studied, 28 were E beta thalassemia and 2 were beta intermedia thalassemia. The E beta thalassemia population had a mean SF of 577.73 ng/mL, SD 229.71. The beta intermedia thalassemia group had a mean SF of 443.4 ng/mL, SD 92.77. Our study showed that, among the various complications studied, the following parameters were found to be significantly related to the serum ferritin levels (serving as a marker of LIC) delayed puberty as determined on the basis of history, physical examination and ng/mL, SD 123.3; mean SF level of those with delayed puberty 624.26 ng/mL, SD 237.73; p value = 0.011) liver dysfunction (as determined by serum medical records) [mean SF level of those with timely onset of puberty was 439.3. SGPT levels >35 IU/mL [mean SF of those with normal liver function 479.68 ng/mL, SD 124.63; SF of those with SGPT >35 IU/mL 646.73 ng/mL, SD 263.76; p value = 0.04]. Renal dysfunction: Mean SF of those with creatinine clearance greater than 90 ml/sqm/min was 465.65 ng/mL, SD 24.11; mean SF of those with creatinine clearance 60-89 ml/sqm/min was 569.92 ng/mL, SD 186.29; p value = 0.043

However, the difference in serum ferritin levels between those with creatinine clearance greater than 90 ml/sqm/min and those with values less than 60 ml/sqm/min was found to be not significant (p value=0.485); possibly because the number of subjects in the latter group was too small. Pulmonary hypertension (as determined by echocardiography with TRV >2.5 m/sec). [Mean SF of those with TRV <2.5 m/sec 458.89 ng/mL, SD 76.71; mean SF of those with TRV <2.5 m/sec 758.58 ng/mL, SD 271.18; p value =0.001]

Serum ferritin levels were found to be quite high in the NTDT patients, with a range of 335.1 (min) -1300 (max) ng/mL, with a mean serum ferritin level of 566.78 ng/mL and SD of 224.9; despite the fact that nearly 87% (26 out of 30) of the patients had received less than 10 transfusions in their lifetime. Although NTDT patients accumulate iron at lower rates than those receiving regular transfusions, a significant number of them do develop a clinically significant liver iron concentration (LIC).

In contrast, beta thalassemia major patients in whom cardiac iron overload is the major factor guiding therapeutic decisions, liver iron overload is a far greater concern in NTDT patients. Current data indicate that LIC and serum ferritin levels reach pathogenic levels in NTDT patients by >10 years.
of age. Elevated liver iron load is associated with hepatic fibrosis and increased risk of hepatocellular carcinoma; as well as other non-malignant complications like thrombosis, pulmonary hypertension, cerebrovascular accidents, hypothyroidism, hypogonadism, osteoporosis and renal dysfunction.\(^{[3]}\) Ideally, LIC should be estimated by histopathology, but it is not feasible in majority of situations. In such cases, alternative methods like MRI and serial measurement of serum ferritin levels every 3 months may be used. Serum ferritin levels (SF) >800 ng/mL found to be associated with increased mortality.

Nowadays safe and efficacious iron chelators are easily available, and the institution of chelation therapy at the appropriate time can minimize morbidity, thus improving the quality of life of the NTDT patients.\(^{[10]}\)

The other parameters, i.e. presence of symptomatic anaemia, haemoglobin levels, facial dysmorphism, hepatomegaly, splenomegaly, BMI, height (in those <18 yrs. of age), serum glucose, calcium, phosphate, thyroid function, FSH, LH, testosterone (in males), endometrial thickness and presence of gallstones or leg ulcers were not found to be significantly related to serum ferritin levels.

The population of beta intermedia patients was too small to find out the differences in the rates of prevalence of complications in between the two groups.

**CONCLUSIONS**

Among the various parameters studied, 4 were found to be significantly related to the serum ferritin levels- delayed puberty, liver dysfunction, renal dysfunction and pulmonary hypertension. Thus, we conclude, that despite requiring much fewer transfusions than transfusion dependent thalassemia patients, the NTDT patients do develop iron overload as well as different complications, some of which are significantly related to the liver iron overload. Knowledge of such complications could help us initiate chelation therapy at the appropriate time for NTDT patients, thereby reducing morbidity and improving their quality of life.

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