Original Article

DOI: 10.7860/JCDR/2013/5286.3149

Genetics Section

The Prevalence of the Beta Thalassemia Trait among the Pregnant Women who attended the ANC Clinic in a PHC, by using the NESTROF Test in Bangalore, Karnataka

PRAVEEN KULKARNI, N R RAMESH MASTHI, SR NIVEDITHA, R SUVARNA

ABSTRACT

Contaxt: Every year in India 6000 to 8000 children are born with thalassaemia major. The birth of such a child produces considerable physical and economic strain on the affected child, its family and the community at large. Thus, the emphasis must shift from the treatment to the prevention of such births in the future.

Aims: To find out the prevalence of the Beta Thalassaemia trait among the pregnant women who attended the antenatal clinics in a Primary Health Centre, by using the NESTROF test; to describe the socio-demographic characteristics of the study subjects, to find out the pregnancies which were 'at risk' of delivering babies with Thalassaemia major and to find out the 'awareness' of the pregnant women regarding Thalassaemia.

Material and Methods: This exploratory study was conducted in a PHC which was attached to the Department of Community Medicine of a medical college which was situated in Bangalore, India, for a period of 3 months. All the pregnant women who attended the antenatal clinic and the husbands of the NESTROF positive women were included in the study. The details regarding the sociodemographic characteristics of the women were collected on a structured proforma and the NESTROF test was performed.

Results: Out of the 210 pregnant women who were tested, 18 (8.5%) were thalassaemia carriers. 12 (66.6%) of them were between 20 – 25 years of age. 5 (27.7%) were born out of 2nd degree consanguineous marriages. 7 (38.8%) had a history of abortions, among which 6 (33.3%) were in the 1st trimesters of their pregnancies. Out of the 18 positive women, 9 (50%) had turned up with their husbands. All of the husbands were negative for the Thalassaemia carrier status. Thus, there was no pregnancy which was at a risk of delivering babies with thalassaemia major. None (100%) of the pregnant women were aware of the disease, thalassaemia.

Conclusions: The prevalence of the Beta Thalassaemia trait among the pregnant women was 8.5%.

Key words: Beta Thalassaemia trait, Thalassaemia major, the NESTROF test, Prenatal diagnosis, Awareness

INTRODUCTION

The implementation of the National Rural Health Mission in the past few years, has resulted in decline of the Maternal Mortality Rate and the Infant Mortality Rate in the entire country, with most of the better performing states reaching the targets. This achievement will allow us to focus on the other important causes of the morbidity and the mortality which are seen in the children in these states. Beta-thalassaemia is the commonest type of haemoglobinopathy which is seen in India. Almost 30 million people in India are the carriers of Beta thalassaemia, with a mean prevalence of 3.3% [1]. 6000 to 8000 children are born every year with thalassaemia major, of which only 10-15% receive optimal treatment. The cost of treating one thalassaemic child amounts to Rs. 90,000 (1875$) to 1, 00,000 (2100$) annually at around 3 years of age, which increases further as the child grows [2]. The only cure which is available today is bone marrow transplantation, which is beyond the affordability of most of the patients. Thus, the birth of a thalassaemic child places considerable physical and economic strain, not only on the affected child and its family, but also on the community and the nation at large. Thus, the emphasis must shift from the treatment to the prevention of such births in the future [2,3].

The most effective and feasible approach for solving this problem is preventive genetics (screening). A prospective prevention, which includes population education, mass screening, genetic counselling and prenatal diagnosis, is the only effective way of coping successfully with such a disease. An antenatal diagnosis is considered to be very useful as a short term program, which can include the identification of the carriers and a prenatal diagnosis of the couples who are "at risk" of delivering babies with thalassaemia. The various screening methods for the antenatal mothers, that are available are viz., a peripheral blood smear examination, evaluation of the red cell indices, free red cell porphyrins, the osmotic fragility (quantitative), etc. All these tests, which include the HbA2 estimation (a confirmatory test for the beta thalassaemia trait) are expensive and time consuming and they require sophisticated equipments. They are thus not feasible at the field level [2,4,5].

The efforts need to be directed towards applying simple and inexpensive screening tests. A simple, cost effective, rapid and a reliable test which can be applied for a mass screening at the field level is the Naked Eye Single Tube Red cell Osmotic Fragility (NESTROF) Test [3,4]. This test is based on the fact that the

microcytic red cells of the subjects with the thalassaemia trait have a decreased osmotic fragility when they are compared to the normal red cells [6]. This test has a sensitivity, a specificity and positive and negative predictive values of 91%, 95%, 55% and 99% respectively among pregnant women [7,8].

Most of the studies which had been conducted previously with regards to this problem were hospital based and they were done among urban communities. Very few studies were conducted at the field level and no published study reports are available among the rural population. With this background, the present study was conducted in the rural field practice area of a medical college which is situated in Bangalore, India, with the objective of finding the prevalence of the Beta Thalassaemia trait among the pregnant women who attended the antenatal clinics in a Primary Health Centre, by using the NESTROF test, to describe the socio-demographic characteristics of the study subjects, to find out the pregnancies which were 'at risk' of delivering babies with Thalassaemia major and the extent of the 'awareness' about Thalassaemia among pregnant women.

SUBJECTS AND METHODS

This exploratory study was conducted in a Primary Health Centre which was attached to the Department of Community Medicine of a medical college which was situated in south Bangalore, India, for a period of 3 months (June – August, 2010). 210 pregnant women who attended the ANC clinic for the first time during the study period and the husbands of the NESTROFT positive women were the study subjects. All the recruited women were residents of the rural catchment area of the PHC. Ethical clearance was obtained from the Institutional Ethics Committee for conducting the study. Signed informed consents were obtained from the pregnant women who were willing to participate in the study and the details regarding the socio-demographic characteristics, the obstetric history, the past menstrual history and the family history were obtained on a structured proforma by using the interview method. With regards to the pregnant women who were positive for the NESTROF test; their respective husbands were also screened for the thalassaemia carrier status by using the same test. If both the husband and the wife were positive for the NESTROF test, then they were considered to be "at risk" of delivering a child with thalassaemia major. If the gestational age was less than 20 weeks, then the couple was counselled to undergo Hb electrophoresis to make a confirmatory diagnosis and subsequently, to make a prenatal diagnosis. If the gestational age was more than 20 weeks, the couple was counselled to undergo electrophoresis for the confirmation of the diagnosis and to make a prenatal diagnosis for the subsequent pregnancies.

The Procedure of the NESTROF Test: Two mililiters (2 ml) of 0.36% buffered saline was taken in one tube (10cm x 1cm diameter) and 2 ml distilled water was taken in another tube. 2 – 4 drops of anticoagulated blood (which was routinely collected for ANC) was added to each of the tubes. The tubes were left undisturbed for half an hour at room temperature. After half an hour, the contents of both the tubes were shaken and the tubes were held against a white paper on which a thin black line was drawn. The line would be clearly visible through the contents of the tube which contained distilled water due to complete lysis of the blood cells. If the line was visible through the contents of the tube which contained buffered saline, the test would be considered as negative, while the test would be considered as positive when the line was not visible [3,4,8].

The haemoglobin level was determined by Sahli's haemoglobinometer method. As per the WHO, the standard cut off for the Hb concentration of pregnant woman.

RESULTS

As can be observed from [Table/Fig-1], out of the 210 pregnant women who were enrolled in the study, a majority 100 (47.6%) were in the age group of 21-25 years, 190 (90.5%) were literate, 148 (70.5%) were Hindus by religion and 83 (39.5%) had an upper lower socioeconomic status as per the B G Prasad classification. Among the 210 pregnant women who were subjected to the NESTROF test, 18 were positive. Thus, the Prevalence of the Beta Thalassaemia trait among the pregnant women who attended the ANC clinic in the PHC by using the NESTROF Test was 8.5%.

Character	Frequency among pregnant women N= 210	Frequency among Beta Thalassemia traits N=18	z	p	95% CI	
					Upper bound	Lower bound
Age (years)						
< 20	72 (34.3)	6 (33.3)	0.0858	0.93	0.238	0.218
21-25	100 (47.6)	7 (38.9)	0.7098	0.4778	0.3272	0.153
26-30	29 (13.8)	3 (16.7)	0.36	0.71	0.1331	0.1931
>30	9 (4.3)	2 (11.1)	1.27	0.2	0.036	0.17
Education						
Illiterates	20 (9.5)	1 (5.5)	0.563	0.572	0.179	0.099
Literates	190 (90.5)	17 (94.5)	0.563	0.572	0.179	0.099
Occupation						
Labourers	31 (15)	4 (22.2)	0.786	0.431	0.104	0.244
Housewife	179 (85)	14 (77.8)	0.897	0.369	0.254	0.09
Religion						
Hindu	148 (70.5)	16 (88.9)	1.667	0.0954	0.032	0.4002
Muslim	58 (27.6)	2 (11.1)	1.52	0.12	0.37	0.04
Christian	4 (1.9)	0				
SESS						
Upper	10 (4.8)	2 (11.1)	1.12	0.25	0.045	0.169
Upper Middle	32 (15.2)	5 (27.8)	1.38	0.16	0.051	0.3
Lower Middle	72 (34.3)	5 (27.8)	0.6	0.54	0.29	0.15
Upper lower	83 (39.5)	6 (33.3)	0.542	0.587	0.29	0.16
Lower	13 (6.2)	0 (0)				

[Table/Fig-1]: Sociodemographic characteristics of pregnant women attending ANC clinic and Beta thalassemia traits
Note: Figures in parenthesis indicate percentage

Among the 18 women with the beta thalassaemia trait, a majority 7 (38.9%) were in the age group of 21-25 years, 17(94.5%) were literate, 16 (88.9%) were Hindus by religion and 6(33.3%) belonged to the upper lower socioeconomic status as per the B G Prasad classification. 5(27.8%) of the pregnant women with the Beta Thalassaemia trait belonged to the Vokkaliga community and 5(6.8%) of them were born out of 2nd degree consanguineous marriages (p=0.55) [Table/Fig-2]. 9(50%) of the pregnant women with the Beta Thalassaemia trait were primigravidae. 7(28%) had a history of one or more abortions in the past, out of which, 6(85.7%) had undergone first trimester abortions (p >0.05).

Among the pregnant women who attended the ANC clinic 118 (56.7%) had a Haemoglobin concentration of <11gm%, with a mean Hb% of 9.7+1.4gm% [Table/Fig-2]. A majority i.e 10 (55.5%) pregnant women with the Beta Thalassaemia trait had a Haemoglobin concentration of <10gm%, with a mean Hb% of 10.06+1.00gm% (p=0.88) and 8(44.4%) of the pregnant women with the Beta Thalassaemia trait belonged to the B Positive blood group.

Among the 18 pregnant women with the Beta Thalassaemia trait, only 9 (50%) had turned up with their husbands for the NESTROF test. None of the husbands of the NESTROF test positive pregnant women were found to be positive. Among the couples which were tested in the present study, no pregnancies were at a risk of delivering babies with Thalassaemia major. None (100%) of the pregnant women were aware of the disease, Thalassaemia.

Character	Low Hb (< 11gm%)	Normal Hb (>11gm%)	Total	p
Beta thalassemia trait				
Present	10 (55.5)	8 (44.5)	18	0.955
Absent	108 (56.2)	84 (43.8)	192	
Consanguinity				
Consanguineous	5 (6.8)	68 (93.2)	73	0.55*
Non consanguineous	13 (9.5)	124 (90.5)	137	
H/o abortion				
Abortion	7(28)	18 (72)	25	>0.005**
No abortion	11 (6)	174 (94)	185	

[Table/Fig-2]: Distribution of study subjects based on consanguinity, H/o abortion and Hemoglobin concentration

Note: Figures in parenthesis indicate percentages
* Chi–square test
** Fisher's exact probability test

DISCUSSION

In the present study, the prevalence of the beta thalassaemia trait among the pregnant women who attended the antenatal clinic at a primary health centre on using the NESTROF test was 8.5%, which is consistent with the findings of Bani Gajra et al., who had used the same test in a hospital based study which was done among pregnant women at Kolkata, India, where it was observed to be 7.1% [1]. In another study which was conducted by Deepa V et al., the overall prevalence of the beta thalassaemia trait among the Sindhi community was found to be 16.81% [3]. The results of the present exploratory study throw light on a higher existence of the carrier status, even in south Indians, which needs special attention, as much of the available published studies have been concentrated among the north Indian population.

The practice of consanguineous marriages, preferably with maternal kindreds, is an accepted socio-cultural phenomenon, irrespective of the religion, caste and the economic status, which also has an effect on the transmission of thalassaemia [9]. In the present study, it was observed that 6.8% of the carrier pregnant women were born out of 2nd degree consanguineous marriages. In contrast, the consanguinity was found to be higher in the studies of Shivashankara AR et al., at Dharwad, north Karnataka (20%) [9] and of Hafeez M et al., in Lahore, Pakistan (56.7%) [7].

In the present study, it was observed that 55.5% of the beta thalassaemia carrier women had Hb concentrations of less than 10gm%. Similar results were observed by Deepa V et al., in their epidemiological study which was done on the beta thalasssemia trait, where 22.7% of the carrier women were diagnosed to have anaemia. The chances of misdiagnoses of the carriers of beta thalassaemia are high, as Iron deficiency anaemia itself may hinder the detection of the thalassaemia carrier status, thus giving more false negative NESTROF results. This is considered as one of the limitations of the NESTROF test.

The thalassaemia carrier status may result in abnormal pregnancy outcomes like spontaneous abortions. In the present study, 39% of the carrier women had histories of one or more abortions, of which 85.7% were first trimester spontaneous abortions. A multicentric study which was conducted by ICMR, reported a 2.8% incidence of spontaneous abortions among women with thalassaemia traits [10].

Only 50% of the women with the beta thalassaemia trait had turned up with their husbands for the NESTROF test. A similar observation was made by Bani Gajra, where only 60.3% women had turned up with their husbands for the screening to make a prenatal diagnosis for [1]. Only an improvement in the awareness of the people regarding the causes and the consequences of thalassaemia and the modes of its prevention can bring about a desirable change in the attitude of the family members, especially among the the male partners, who can volunteer for these kind of tests and procedures.

In the present study, none of the pregnancies were at a risk of delivering a Thalassaemia major child. On the contrary, Bani Gajra reported 15.8% couples to be at a risk of delivering thalassaemic children [1]. This could be because the husbands of only 50% of the antenatal mothers had turned up for the NESTROF test. Similarly, regarding the awareness about thalassaemia, we observed that none of the subjects had any knowledge about thalassaemia. This indicated that there was need to improve the awareness of the people regarding thalassaemia.

In the present study, the cost which was incurred in conducting the NESTROF test was only Rs 1.5/- only per subject, which implied that the NESTROF test was a simple and a low cost screening tool which could be used for the identification of the carrier status of beta thalassaemia. It is useful for screening large populations, especially in the remote village areas and at the primary health care centres, where laboratory facilities are not available. However, there is a need for carrying out similar studies which cover larger sample sizes and wide geographic areas, for generalizing the results, as well as the cost effectiveness of the test.

REFERENCES

[1] Bani Gajra, Shila Chakraborti and Bani Sengupta. Prenatal Diagnosis of Thalassaemias. *Int J Hum Genet.* 2002;2(3):173-8.
[2] Mamta Manglani, MR Lokeshwar, Vani VG, Nishi Bhatia and Vijay Mhaskar. 'NESTROFT'-an effective screening test for beta thalassemia trait. *Indian Pediatrics.* 1997;34:702-7.
[3] Deepa V Mulchandani, MB Fulare, SP Zodpey, ND Vasudeo. Prevalence and some epidemiological factors of Beta Thalassemia trait in Sindhi community of Nagpur city, India. *Indian Journal of Public Health.* 2008;52(1):11-15.
[4] SK Bhobate, ST Gaikwad, T Bhaledrao. NESTROFT as a screening test for the detection of beta thalassemia trait. *Indian J. of Pathol. Microbiol.* 2002;45(3):267-68.
[5] Sunil Gomber, Sanjeev, Nishi Madan. Validity of Nestroft in Screening and Diagnosis of ß-Thalassemia Trait. *Journal of Tropical Pediatrics.* 1997;43(6):363-66.
[6] BC Mehta. NESTROFT: A screening test for beta thalassemia trait. *Indian Journal of Medical Sciences.* 2002; 56(11):537-44.

[7] Hafeez M, Aslam M, Ali A, Rashid Y, Jafri H. Regional and ethnic distribution of beta thalassemia mutations and effect of consanguinity in patients referred for prenatal diagnosis. *J Coll Physicians Surg Pak.* 2007;17(3):144-47.
[8] Manjula Maheshwari, Sadhna Arora, Madhulika Kabra and PSN Menon. Carrier Screening and Prenatal Diagnosis of b-Thalassemia. *Indian Pediatrics.* 1999;36:1119-25.
[9] Shivashankara AR, Jailkhani R, Kini A. Hemoglobinopathies in Dharwad, North Karnataka: A hospital-based study. *J Clin Diagn Res.* [serial online] 2008;2:593-9.
[10] Indian Council of Medical Research (ICMR) Collaborating Centers and Central Technical Coordinating Unit ICMR, New Delhi, India. Feasibility of introducing genetic services in the National Family Welfare Programme in India. *Indian J Med Res.* 2009; 130: 404-12.

Author(s):
1. Dr Praveen Kulkarni
2. Dr NR Ramesh Masthi
3. Dr SR Niveditha
4. Dr R Suvarna

Particulars of Contributors:
1. Assistant Professor, Department of Community Medicine, JSS Medical College, Mysore, India.
2. Associate Professor, Department of Community Medicine, Kempegowda Institute of Medical Sciences (KIMS), Bangalore, India.
3. Professor, Department of Pathology, Kempegowda Institute of Medical Sciences (KIMS), Bangalore, India.
4. Professor, Department of Obstetrics and Gynaecology, Kempegowda Institute of Medical Sciences (KIMS), Bangalore, India.

Name, Address, E-Mail Id of The Corresponding Author:
Dr Praveen Kulkarni,
Lecturer, Department of Community Medicine,
JSS Medical College, SS Nagar, Near Bannimantap,
Mysore-570015, Karnataka, India.
Phone: 91-9008926878
E-mail: prakulfi@gmail.com

Financial or Other Competing Interests: None

Date of Submission: **07 Nov, 2012**
Date of Peer Review: **23 Feb, 2013**
Date of Acceptance: **16 Apr, 2013**
Date of Publishing: **01 Jul, 2013**