Thalassemia prevention In India – Novel approach by post martial counseling

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Hemoglobin disorders constitute the most common lethal inherited disorders worldwide. They are common in populations in not only India but also in Asia, tropical Africa, and the Mediterranean region.

It is estimated that In India every year more than 12,000 Thalassemia children are born. We know the mutations that cause Thalassemia, there is reliable technology for carrier detection and molecular technology for prenatal diagnosis but it is little used in India. Unlike many other genetic disorders, when carriers cannot be easily identified, the Thalassemia give us enormous opportunity to implement effective national screening programmes, to control affected births by community information, screening, counselling and prenatal diagnosis.

Our aim should be

1. No Thalassemia MAJOR Child should be born.
2. Safe, cost effective and reliable screening method in identifying the couple at risk of "producing the Thalassemia major child "(Both thal minor partners).
3. Coordination between government and NGOs to carry out the projects more effectively and efficiently.
4. Availability of accurate prenatal diagnostic services to all and at lower cost.

A FACT TO BE ACCEPTED - Social taboo for declaring their daughter / son as Thalassemia Minor.

Thalassemia is prevalent in the Mediterranean area, the Middle East and South East Asia, and the Pacific. The carrier rates range from 2-19% in the different populations. The birth prevalence of the haemoglobin disorders in countries affected by migration of populations varies according to the geographic location and the origin of the populations.

Treatment of Thalassemia involves lifelong treatment. Management includes regular blood transfusions, iron chelation treatment, management of complications including osteoporosis, cardiac dysfunction, endocrine problems, hepatitis B and C infection, HIV infection. Life expectancy for Thalassemia has improved significantly with modern medical treatment in developed countries but it has been estimated that only 5-10% thalassaemic children born in India receive optimal treatment. Without access to regular chelation treatment and medical care, the majority of children with Thalassemia major do not reach the age of 20.

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Screening for beta Thalassemia.

Madan and colleagues from the ICMR at the KEM Hospital in Mumbai and University College of Medical Sciences in Delhi, highlight a major public health burden in India. They analyze data of a two-centre study of 11,090 schoolchildren and determine the frequency of beta Thalassemia in India. **Their data shows an overall frequency of 4.05%, with the population at 1.2 billion and birth rate of 23/1000, and using the Hardy-Weinberg equation estimates the affected Thalassemia births to be 11,316 per year added to the existing affected patients.** This number is more than previously estimated.

They note that beta Thalassemia is present in the majority of castes, religious groups and population groups.

Looking at the birth rate of Thalassemia birth rate in India, suitable control measures are urgently to be taken in India. It is urgently required that India needs national prevention programmes for Thalassemia to be planned and carried out.

Although the joint collaborative project with British council division in Mumbai (1989-1994) provided some stimulus to the problem of Thalassemia in India and set up an prevention centre in Mumbai, a national prevention programme was not achieved.

Couples who are at risk for producing children with haemoglobin disorders have the option of avoiding the birth of an affected child with prenatal diagnosis. The births of affected children have been reduced in many Mediterranean countries by screening, counselling, and the offer of prenatal diagnosis and selective termination of affected fetuses.

However, worldwide only a very small proportion of affected births are prevented by prenatal diagnosis. We know that beta Thalassemia carriers are easily detected using automated cell counters and automated High Pressure Liquid Chromatography (HPLC) or similar methods.

An important constraint in effective large screening, especially in countries where the prevalence of Thalassemia is high are limited resources, India is one such country. Therefore, prevention of Thalassemia in the majority of these countries will depend on effective screening strategies and the use of strategies to optimize the cost-benefit ratio of mass screening.

Studies have shown that the naked eye single tube red cell osmotic fragility test (NESTROFT) can be a very useful screening tool for beta Thalassemia trait and is particularly attractive in a screening programme for India because of its low cost. Many research papers emerge from India; the beta Thalassemia mutations present at the regional level are known. So the platform has been more than ready for a long time to implement a prevention programme. The technology for prenatal diagnosis is available in many parts of India, but is carried out in an ad hoc manner, the majority of women already having an affected child. Below are the details of the possible mutations and common mutations in India.

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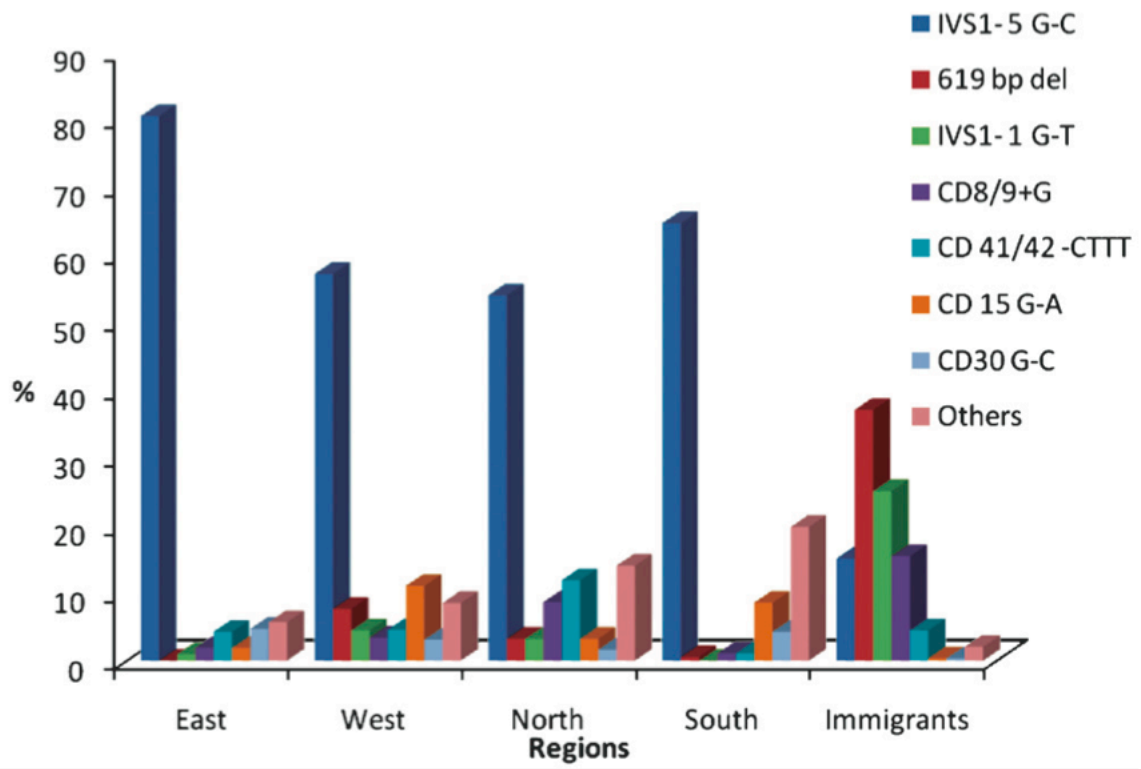

Fig. 1. Regional distribution of β-thalassaemia mutation in India.

Common mutations	States of India
IVSI-5(G→C)	Uttar Pradesh, Maharastra, Gujarat, West Bengal, Punjab
Codon 8/9(+G)	Uttar Pradesh, Punjab, Hariyana, Maharastra, West Bengal
IVS 1-1(G→T)	Maharastra, Gujarat, Uttar Pradesh, Punjab
Codon 5(-CT)	Gujarat, Uttar Pradesh, Maharastra, Andhra Pradesh, Kerala
Codon 41/42 (-TCTT)	Uttar Pradesh, West Bengal, Gujarat
Cap+1(A→C)	Punjab, Hariyana, Bihar, Rajasthan, Gujarat West Bengal, Uttar Pradesh
619 bp deletion	Gujarat, Uttar Pradesh
Codon 15(G→A)	Maharastra, Gujarat, West Bengal.Uttar Pradesh, Andhra Pradesh, Karnataka,Tamil Nadu
-88(C→T)	Punjab, Uttar Pradesh

Table I. Spectrum of β-thalassaemia mutations in India

S.No	Type of mutation	Phenotype
A. Transcriptional mutations		
1.	-90 (C>T)	β^+
2.	-88 (C>T)	β^+
3.	-87 (C>T)	β^+
4.	-80 (C>T)	β^+
5.	-29 (A>G)	β^+
6.	-28 (A>G)	β^+
7.	-25 (A>G)	β^+
B. Cap site		
1.	+1 (A>C)	β^+
C. Initiation codon		
1.	ATG > ACG	β^0
D. RNA processing mutations		
i) Splice junction site		
1.	Codon 30 (G>C)	β^0
2.	Codon 30 (G>A)	β^0
3.	IVS 1-1 (G>T)	β^0
4.	IVS 1-1 (G>A)	β^0
5.	IVS 1-129 (A>C)	β^0
6.	IVS 1-130 (G>C)	β^0
7.	IVS 1-130 (G>A)	β^0
8.	IVS II-1 (G>A)	β^0
(ii) Consensus site		
1.	IVS 1-5 (G>C)	β^+
2.	IVS 1-128 (TAG > GAG)	β^+
3.	IVS II-837 (T>G)	?
(iii) IVS changes		
1.	IVS I-110 (G>A)	β^+
2.	IVS II-591 (T>C)	β^+
3.	IVS II-613 (C>T)	β^+
4.	IVS II-654 (C>T)	β^+
5.	IVS II-745 (C>G)	β^+
iv) Coding region changes		
1.	Codon 26 (G>A) Hb E	β^+
E. RNA translational mutations		
i) Nonsense		
1.	Codons 4,5,6 (ACT CCT GAG> ACA TCT TAG)	β^0
2.	Codon 5 (-CT), Codon 13 (C>T), Codon 26 (G>C), Codons 27/28 (+C) in cis	?
3.	Codon 6 (GAG > TAG) and on the same chromosome Codon 4 (ACT> ACA), Codon 5 (CCT>TCT)	β^0
4.	Codon 8 (A>G)	?
5.	Codon 13 (C>T), Codon 26 (G>A), Codons 27/28 (-C) in cis	?
6.	Codon 15 (TGG > TAG)	β^0
7.	Codons 62-64 (7 bp del)	β^0
8.	Codons 81-87 (22 bp del)	β^0
9.	Codon 121 (G>T)	β^0

(ii) Frameshift

1.	Codon 5 (-CT)	β^0
2.	Codons 7/8 (+G)	β^0
3.	Codon 8 (-AA)	β^0
4.	Codons 8/9(+G)	β^0
5.	Codon 13 (C>T)	β^0
6.	Codon 15 (-T)	β^0
7.	Codon 16 (-C)	β^0
8.	Codon 16 (C>T)	β^0
9.	Codon 17 (A>T)	β^0
10.	Codons 22-24 (7 bp del)	β^0
11.	Codon 26 (G>T)	β^0
12.	Codon 35 (A>G)	β^0
13.	Codons 36/37 (-T)	β^0
14.	Codons 36-39 (8 bp del)	β^0
15.	Codon 39 (C>T)	β^0
16.	Codon 44 (-C)	β^0
17.	Codons 47/48 (+ATCT)	β^0
18.	Codon 55 (+A)	β^0
19.	Codon 55 (-A)	β^0
20.	Codons 57/58 (+A)	β^0
21.	Codon 88 (+T)	β^0
22.	Codons 106/107 (+G)	β^0
23.	Codon 110 (T>C)	β^0
24.	Codon 111 (-G)	β^-
25.	Codon 135 (C>T)	β^0

F. RNA cleavage and polyadenylation mutation

1.	AATAAA>AACAAA	β^-

G. Deletional mutations

1.	619 bp deletion; 3'end	β^0
2.	10.3 kb deletion	β^0
3.	Codons 126-131 (17 bp deletion)	β^0

In recent years both government agencies and nongovernment organizations (NGOs) in India have initiated programmes to deal with the problem. However, there is still no coordinated national Thalassemia control policy.

When is the best time to offer screening in India?

Ideally all affected births should be prevented if at-risk couples are identified, may be prior to marriage or just after the marriage.

Marriage can be a complex social phenomenon that involves many other family members besides the prospective couple, and marriage partners usually are selected either because of a strong personal preference, or for valid family or traditional reasons, or a mixture of all three. If a planned marriage is broken because both partners carry Thalassemia this can cause social embarrassment or stigma to the young couple and their families, and there is a risk that the problem will recur if the new partners found are also carriers for the same disorder.

For example, if population carrier frequency is 6 to 10 %, the chance that one or both new partners will be a carrier is 12 to 20 %. Therefore, the recurrence risk for the couples is 12 – 20 %.

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Being a carrier in India may render an individual unfit as a suitable marriage partner and testing after marriage or prenatal counseling would be more acceptable to the majority.

The stigma associated with being a carrier can only be reduced significantly through greater awareness and public education perhaps by involving community leaders and people who are involved with arranging marriages. Reduction of stigma will take years and years of public education.

India cannot afford to wait this long before a programme is established, there is the urgent need to start a prevention programme now, education will need to go hand in hand with a prevention programme.

Experience in other countries:

Recently, mandatory premarital screening for Thallasemia and sickle cell has been conducted in **Saudi Arabia** with the objective of decreasing at-risk marriages. However, following counseling almost 90% of couples married despite being aware of their risk.**In Iran** without prevention there would be approximately 1,200 affected children born annually, there are over 20,000 children attending treatment centers. Iran has taken on the vast task of providing national premarital screening and genetic counselling. By the end of 2001, over 2.7 million prospective couples had been screened and 10,298 at-risk couples identified and counseled. Fifty-three per cent of these couples proceeded with their marriage plans, 29% of at-risk couples separated, and the remainder were still struggling with their decision. Further recent data shows that the number of couples proceeding with marriage has increased even. Therefore the majority of couples find it unacceptable to select a partner on the basis of genetic screening information and there is a high demand for prenatal diagnosis. A similar approach was tried in **Cyprus** much earlier, when marriages between carriers were actively discouraged. This approach proved to be unacceptable to the population and was soon abandoned.

Once prenatal diagnosis became possible for Thalassemia, it was made available within the Cypriot health service. Soon after, confidential premarital screening was made mandatory among Greek Cypriots by the Greek Orthodox Church and among Turkish Cypriots by the civil authorities. It was then found that 98% of at-risk couples detected just prior to marriage proceed to marry. Nevertheless, the annual number of new births of children with Thalassemia major has decreased almost to zero in Cyprus, because couples use the information on genetic risk in a variety of ways to obtain a healthy family. Less than 5% of the decrease in Thalassemia major births is due to separation of engaged couples.

Another option is to test all school or college students. Several programmes are taking place in India today. We spend huge amount of money for this screening procedure today. At present, we mainly depend upon the premarital counseling.We expect that the school or

college girl / boy, who are thalassaemia minor, will ask their parents not to choose the partner who is also Thalassemia minor.In spite of putting up such a huge efforts and spending for the evaluation of thalassemia status of school and college students, still today in India more than 12000 Thallasemia children are born. This figures itself suggest that we need some change in the strategy.

Colah and colleagues attempted to assess the impact of screening and counselling high school children for beta Thalassemia on a programme that was undertaken between 1984-1988 on 5682 schoolchildren. One hundred and fifty-three individuals were found to be carriers and counselling was provided to the families of 71 children; after a gap of 20 years an attempt was made to follow them up. Forty-seven of the 71 families were contactable but none of the 41 individuals who were married had revealed carrier status or had their partners tested before marriage. Eleven had their spouses tested after marriage. One couple had a thalassaemic child.

From the recent survey by the Gujarat Red Cross, society had carried survey in six of the general hospitals in Ahmedabad.

They investigated almost 100,000 patients in last year and found out 12000 thal minor and 68 Thallasamia couple. Prenatal investigations for thal major was offered and eight were found to Thal major and legal termination were carried out.Let us make the economical evaluation for the period of 1 year. All calculations are made at the cost occurring to Lab itself, not to the patients…..

	hospital	No Of delivery	No of antenatal screening done	Thal minor found only patient	Thal minor couple	Screen negative	Prenatal testing Amount of money	
1	CHA	6700						
2	VS hospital	4900						
3	LG hospital	6200						
4	Shardaben Hospi	3400						
5	Ahmedabad	1,04,000	Rs 450 per test cost for the electrophorasis Makes the cost and screening cost very high				46,800,000 468 lakhs	5000 thal minors patients and about 250 thal couple…. We end up paying 1,87,000 for identifying one thal couple …

Therefore screening one time during antenatal period or school / college children with one-time counselling is insufficient to make an impact and seems to be very costly affair. As I believe that mass screening tool for any disease should be cheap, cost effective, accurate and reproducible.

A study by Colah and colleagues where antenatal screening was offered to women attending a hospital in Mumbai catering mainly to the lower social economic groups, found that only 15.4% of women booked in the first trimester of pregnancy. The women who were found to be carriers were counseled but only 29.5% attended for follow-up with their husbands. Two at-risk couples were identified and both opted for prenatal diagnosis. **Therefore antenatal screening is not the ideal solution (may be useful) in India firstly because of late booking and secondly because pregnancy is not the ideal time to inform women that they may be at risk of producing children with a serious genetic disease.**

Here we do NOT offer prenatal screening after 20 weeks of gestation as termination after 20 weeks is illegal in India!

Nevertheless, it is practiced in several countries like UK. The data show that a combination of screening approaches may be suitable for India.

Interestingly, prevention programmes are supported by the majority of Thalassemia major patients. It is possible that the continual birth of affected patients will ultimately have a detrimental effect on the treatment of the existing patients.

For instance, if there were no prevention program in Cyprus there would be approximately 50 affected births per year. By 2021, 70,000 units of blood will be required and 17.5% of the possible 400,000 possible donors (out of a population of 600,000) could need to donate blood at least once per year. [40] There is also the cost of iron chelation treatment including the cost of the remainder of the treatment, which will ultimately become prohibitive to the economy of Cyprus. There are also unspoken reasons why patients support prevention programme, like the financial burden, the difficulty in obtaining safe blood (infected with Hepatitis B, C, and HIV.) The pain and anxiety that families go through is insurmountable and the Quality of Life for most patients is dismal. Therefore, it is not surprising that prevention programmes are supported by the majority of Thalassemia major patients. For India, the introduction of a prevention programme will give more hope to the thousands of existing patients.

Resources do not allow effective treatment for Thalassemia, what hope is there for these patients if a prevention programme is not implemented in India. Unlike many other genetic disorders, when carriers cannot be easily identified, the Thalassemia give us enormous opportunity to implement effective national screening programmes, to control affected births by community information, screening, counselling and prenatal diagnosis.Unless we all concentrate, our efforts to implement a screening

programme in India, so that at-risk couples can be offered a choice of preventing the birth of affected children, the wealth of information we have is rendered useless. **The only solution for India is to implement a National Screening Programme immediately, but this can only be successful if there is political will.** There appear to be many NGO efforts to deal with the problem, but these efforts have to be brought together and a policy evolved and submitted to the government for a fundable comprehensive programme.

What is the possible better / best solution for the situation?

I was vice president of FOGSI in 2012– federation of obstetrics and gynecological societies of India having 27,000 members across India. My primary aim is" Thalassemia FREE India.

We want as a common goal to all of us

1.No Thalassemia MAJOR Child should be born.
2. Safe, cost effective and reliable screening method in identifying the couple at risk of "producing the Thalassemia major child "(Both thal minor partners).
3. Coordination between government and NGOs to carry out the projects more effectively and efficiently.
4. Availability of accurate prenatal diagnostic services to all and at lower cost.
5. A FACT TO BE ACCEPTED - Social taboo for declaring their daughter / son as Thalassemia minor.

Hospital	No of deliveries In 2010 year	Antenatal patients Evaluated in April to Jan 2010	Minor in April to Jan 2010	Normal in April to Jan 2010	Couple Screen positive in April to Jan 2010	Husband Screening Total/ positive	Opted for prenatal in April to Jan 2010		
CHA	6791	5055	273	4782	28	256/28	23		
VS	4954	3021	132	2889	8	129/8	5		
LG	6276	5011	252	4759	14	246/14	5		
Shardaben	3248	3346	139	3207	10	130/10	5		
Sola CH		1430	66	1364	4	64/4	2		
Bapunagar general		778	26	752	1	25/1	0		
Ahmedabad in total	1,04,791	18641	888	17753	65	850/65	40		
Corrected figure for the complete	**1,04,791**	**23302**	**1110**	**22191**	**81**	**1062/ 81**	**50**		

year									

Conclusion:

1. 4.76 % Thalassemia carrier rate
2. 62.5 % opted for the prenatal evaluation despite of at risk of producing Thalassemia major child.
3. 0.33 % - couple at risk could be identified where both were Thalassemia major.
4. If we continue the similar way, we will be able to screen only 21 % of population taking ANC in govt hospitals….
5. CONCEPT OF THE PREMARITAL EVALUATION NEEDS TO BE CHANGED TO POSTMARITAL EVALUATION…

Thalassemia major child may be born to one of the following group:

1. Thalassemia minor couple identified after couple evaluation.
2. Couple already having one Thalassemia major child

How to screen?

Step 1:confirmation of thalassemia minor status by doing Hb electrophoresis by HPCL.

Step 2 : Take 3 cc of blood in to EDTA tube and send it to the genetic lab for the identification of the genetic mutation listed above

Step 3: do prenatal test of the fetus either CVS / Amniocentesis and identify the mutations inherited from couple. If child do not have any of them – child is normal. If child has ONLY one of them – child is Thal minor and if BOTH the mutations are present in the child , it is thalassemia MAJOR.

GET MARRIED TO A PARTNER OF YOUR CHOICE BUT GET YOUR SELF TESTED FOR THALLASSEMIA JUST AFTER THE MARRIAGE.
IF BOTH OF ARE " THALASSEMIA MINOR " (YOU ARE THALLASSEMIA COUPLE!), THAN GET YOUR FETUS TESTED BETWEEN 12 TO 18 WEEKS FOR THE POSSIBLE THALLASSEMIA MAJOR FETUS.