

**AN APPROACH FOR THE PREVENTION OF  
THALASSAEMIA IN PAKISTAN**

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## ABSTRACT

The basic aim of this thesis was to identify a suitable approach for prevention of thalassaemia in Pakistan. The overall carrier rate for  $\beta$ -thalassaemia was 5.3%. The rate varied by ethnic group, i.e. Punjabi: 4.5%, Pathan: 5.2%, Sindhi: 1.3%, Baluchi: 9% and Mohajir: 5.2%. The annual birth rate of affected children was estimated at 1.35 per 1000. Screening for  $\alpha$ -thalassaemia showed 6% carrier rate for  $\alpha^{-3.7}\alpha/\alpha\alpha$  genotype. Approaches for identifying carriers and at risk couples were investigated in pregnant females, but proved to be technically difficult and cost ineffective. By contrast screening in ten index families with haemoglobin disorders identified 21-70% (mean 31%) carriers per family. No carrier was identified in five control families without a history of haemoglobin disorder. Follow-up for one year in the families screened showed a significant effect on the marriage choices.

Molecular basis of thalassaemia was investigated in 1240 mutant alleles in all ethnic groups. 19 different mutations were found including one novel allele. The five commonest mutations accounted for 81% of the alleles. The pattern of mutations was significantly different in the ethnic groups studied. 7.5% of patients on blood transfusions had thalassaemia intermedia, and its genetic basis included Xmn-1 +/+ genotype (36%) and mild mutations (31%). Coincidental  $\alpha$ -thalassaemia was found or suspected in 38% of cases of thalassaemia intermedia. Prenatal diagnosis of thalassaemia was introduced for the first time in Pakistan and in the two years of the study 158 couples used the test. All couples, except two, already had at least one affected child. They had better education and socio-economic status than the controls and 93% requested termination of pregnancy when the fetus was affected. Over 98% of the diagnoses were done by direct mutation analysis. A multiplex polymerase chain reaction for mutation analysis was developed and significantly reduced the total cost and time required for prenatal diagnosis.

Consanguineous marriage and recessive disorders were studied in the nine index and the five control families. In 319 couples studied 46% were consanguineous, 52% were Biradri/Tribe members, and only 6% were completely unrelated. Consanguineous marriage (2<sup>nd</sup> cousins or closer) had increased from 12% in the 1st generation to 45% in

the 2nd generation. Antecedent consanguinity had significant effect on the kinship coefficient of the close as well as distant relatives. There was only a marginal difference in coefficient of inbreeding, calculated in a random population sample by conventional methods (0.0257) and by allele frequencies at the D21S11 locus (0.0272). Morbidity and mortality from genetic causes was significantly higher in the children of consanguineous than the non-consanguineous couples living under similar socio-economic conditions.

This pilot study appears to have identified a suitable approach for prevention of thalassaemia in Pakistan.

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## ABBREVIATIONS USED

A	Adenine
AFIP	Armed Forces Institute of Pathology
ARMS	Amplification Refractory Mutation System
bp	base pairs
C	Cytosine
Cd	Codon
CI	Confidence Interval
CVS	Chorionic Villus Sampling
Del	Deletion
DGGE	Denaturing Gradient Gel Electrophoresis
dl	decilitre
DNA	Deoxy-ribo Nucleic Acid
dNTP	deoxynucleoside triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EMR	Eastern Mediterranean Region
fl	femtolitre
Fr	Frame shift
G	Guanine
g	gram
Hb	Haemoglobin
HIV	Human Immune Virus
ID	Identification
IVS	Intervening Sequence
LCR	Locus Control Region
MCH	Mean Cell Haemoglobin
MCV	Mean Cell Volume
mRNA	messenger Ribo Nucleic Acid
µl	microlitre
ng	nanogram
NWFP	North West Frontier Province
PCR	Polymerase Chain Reaction

pg	picogram
pM	picomole
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribo Nucleic Acid
Rs	Rupees
SD	Standard Deviation
SDS	Sodium Dodecyl Sulphate
SSCP	Single Strand Conformation Polymorphism
STR	Short Tandem Repeat
T	Thymidine
Taq	Thermus aquaticus
Thal	Thalassaemia
TI	Thalassaemia Intermedia
TM	Thalassaemia Major
TRBC	Total Red Blood Cell Count
UCH	University College Hospital
UV	Ultra Violet
WHO	World Health Organization
yrs	years