ROLE OF XMNIᴦᴳ POLYMORPHISM FOR HYDROXYUREA RESPONSE IN CLINICALLY AND GENETICALLY VARIED BETA-THALASSEMIA PATIENTS IN PUNJAB

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INTRODUCTION

- Beta thalassemia is one of the major health problems in Pakistan and also a cause of significant healthcare burden.

- The β-thalassemia syndromes are autosomal recessive hereditary disorders

TYPES WITH RESPECT TO CHAIN DEFECT

- Alpha thalassemia
- Beta thalassemia
- phenotypes are variable, ranging from severe, transfusion-dependent thalassemia major to mild, asymptomatic thalassemia trait.
- This interpatient clinical variability triggers researcher toward identifying genetic modifiers for these disorders.

To improve the quality of life in thalassemic patients, multiple pharmacological agents are in use.

- HYDROXYUREA (ANTINEOPLASTIC DRUG)
- Suppose to increase HbF level / altimately compensate the defective chain

  α/β chain disequilibrium.

NEED OF THE STUDY

- Effect of hydroxy urea on HbF level among beta thalassemia major patients with different XmnI genotypes.
- Association between the XmnI genotype and hydroxiurea treatment
- Evaluate the effect on transfusion frequency

MATERIALS AND METHODS

80 Patients were included in study with (08-29 year old)

DEVIDED INTO THREE GROUPS

Responders (Transfusion one time in 55-65 days)

Mild responders (Transfusion frequency became decrease 05-10 days)

Non responders (No effect on transfusion frequency)

After approval from Institutional Ethical Review Committee and written informed consent dully signed patients/parents.

5cc EDTA blood sample was collected.

MATERIAL AND METHODS

DNA extraction & quantification

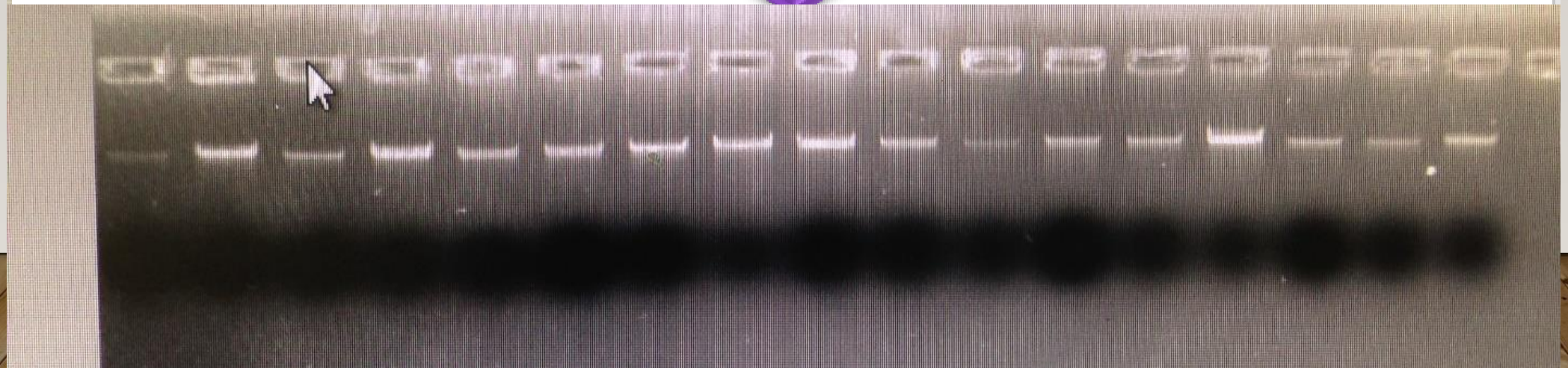
Blood collected from beta thalassemia patients getting hydroxy urea treatment (n=46).

DNA isolation from blood samples performed by favourgen Mini kit. Genomic DNA run on 2% agarose and done nonodrop to check purity and yield

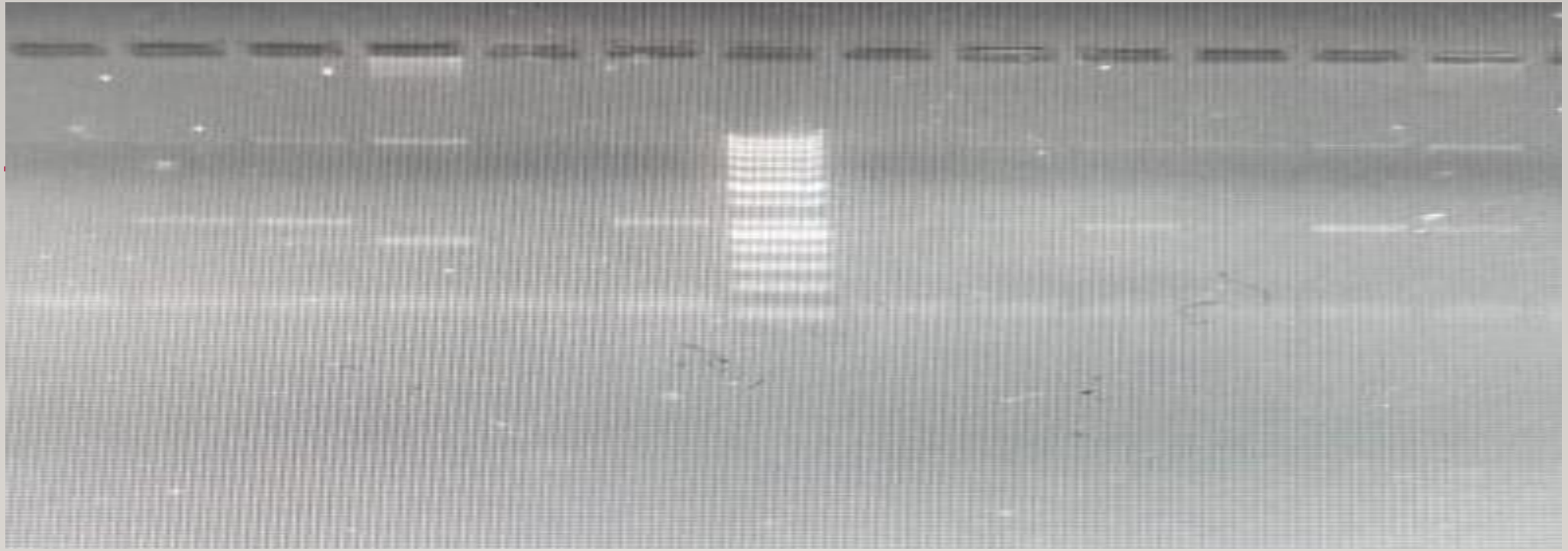
Multiplex ARMS PCR

ARMS Multiplex PCR is performed by extracted material for common beta thalassemia. Particular sample also checked for XmnI polymorphism.

Amplified 641 bp product by XmnI primer then applied pdmI XmnI (RFLP)to restrict amplified product into 418 bp and 223 bp if heterozygous or homozygous for XmnI.



METHDOLOGY ADOPTED



50 bp ladder run with 5 sample. lane I there is no sample. Lane II and III sample with IVSI-5 mutation 280bp. IVSI 5 (forward primer) while common A work as reverse primer. Lane IV contain mutant primer Fr 8-9. (forward primer) while common A work as reverse primer. Lane V contain also IVSI-5. While on right side of the ladder IVSI-I and CD 30 GC (forward primer) while common A work as reverse primer. Lane III, V and VI respectively. All lanes having 1000bp control band by (control F and Control R primer).

RESULTS AND DISCUSSION

- 80 subjects were included
- 60 getting hydroxy urea treatment
- 20 patients without hydroxyurea treatment

Respopnse Among trated patients

- out of 60 treated patients15 found to be good responder for hydroxyurea
  7 heterozygous(-/+)
- 1 was found to be homozygous (+/+) for XMNL polymorphism.

Mild responder group

- 25 patients on hydroxy urea showed mild effect with decrease transfusion frequency for approx.10 days
- out of 25 mildly responded patients 7 were heterozygous (-/+)and 2 found to be homozygous(+/+).

Results and Discussion

Non-responder group

- 20 patients on hydroxy urea showed no response for drug and transfusion frequency persisted the same duration as before.(25-30days)
- out of 20 non-responder patients 9 were heterozygous (-/+)and 1 found to be homozygous(+/+).

Untreated group

- 20 patients with out hydroxy urea (included on the basis of transfusion frequency and HbF level) no significant difference was observed regarding their hetrozygousty and homogzygousty of xmnl genotyping.

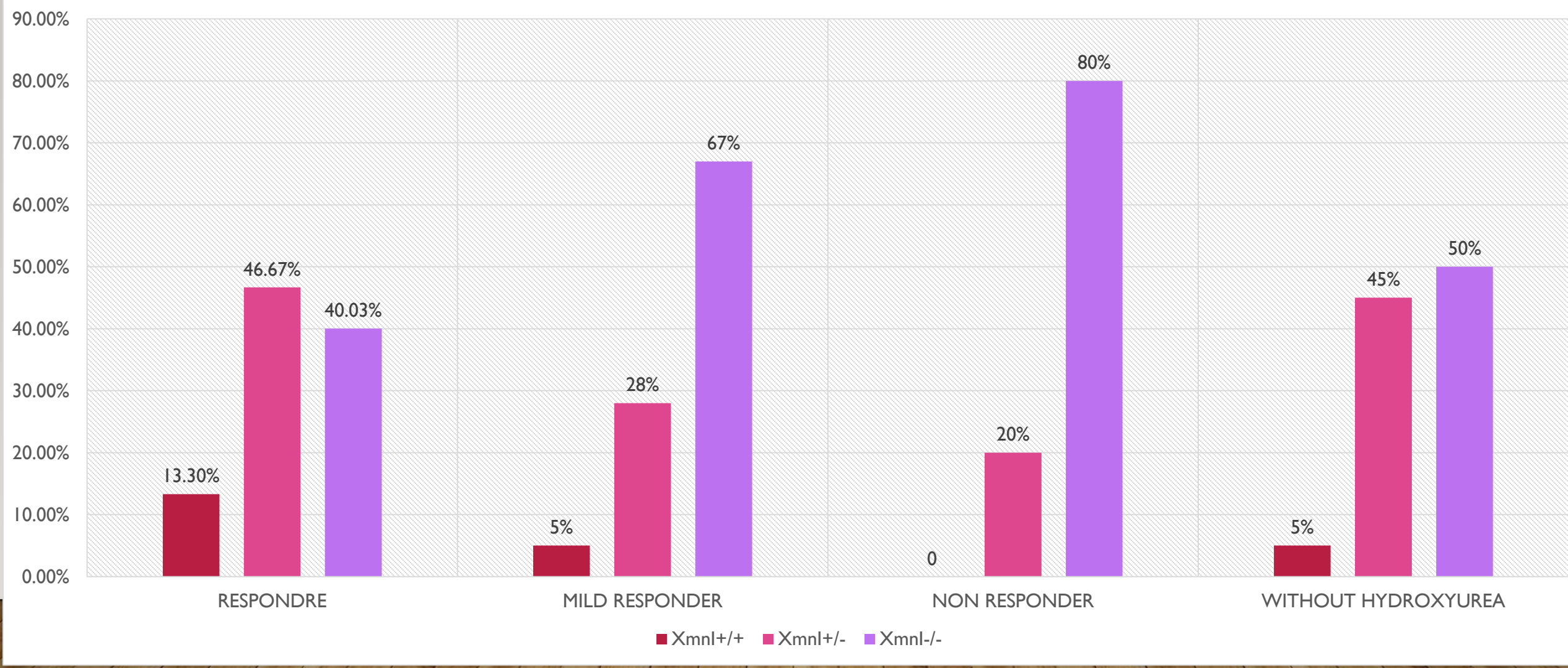
Beta thalassemia mutational status

IVSI-5 found to be commonest mutation in our selected patients. How over no significance correlation foumd between HBB gene common mutation and XmnI Polymorphism with respect to the response to hydroxy urea

Association with drug

with *p=0.127, showed no association between hydroxy urea responders and nonresponders and untreated patients with respect to the XmnI genotype.* respect to their XMNI genotyping

RESULTS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28%
67%
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20%
80%
5%
45%
50%
RESPONDRE
MILD RESPONDER
NON RESPONDER
WITHOUT HYDROXYUREA
Xmnl+/+
Xmnl+/-
Xmnl-/-

LIMITATION OF THE STUDY

SAMPLE SIZE

COFOUNDING FACTORS (INFECTED WITH HCV, HBV)

IRON OVERLOAD AND CHELATION THERAPY

HBS1-MYB INTRAGENIC REGION AND BCL11A GENE SHOULD STUDY PARTICULARLY

Future prospectives

- Study will Help us to understand There is considerable clinical variability between patients inheriting identical beta globin mutations

- Support the idea of exploring the role of XmnI in modulation HbF and hydroxy urea treatment. and exploit it and other modifiers as potential therapeutic target.


ThANk yoU