Title: Inversion 22 in Hemophilia A in the North Indian Population: a New cDNA Based Protocol.

Praveen Kumar*, Nuzhat Husain* Nuzhat Jahan Faridi*, Priyanka Soni*, Sudhir Kumar Goel#.

Institutions: **Department of Pathology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India. # Petroleum Toxicology Division, Indian Institute of Toxicology Research, Lucknow, India.*

OBJECTIVES

➢ To assess the frequency of Intron 22 Inversion Mutation (Inv 22) in North Indian Population and to develop and evaluate a new protocol for Inv22 mutation detection.

MATERIALS AND METHOD

➢Clinical assessment, family history was obtained in all cases.

➢Genetic studies included assessment of frequency of the Inv22 in a group (n=181) of 102 severe cases and 79 moderate cases with hemophilia A from North Indian Population by using Inverse PCR assay (*Rosette et al*).

➢A new cDNA based method was designed and evaluated to assess Inv 22.

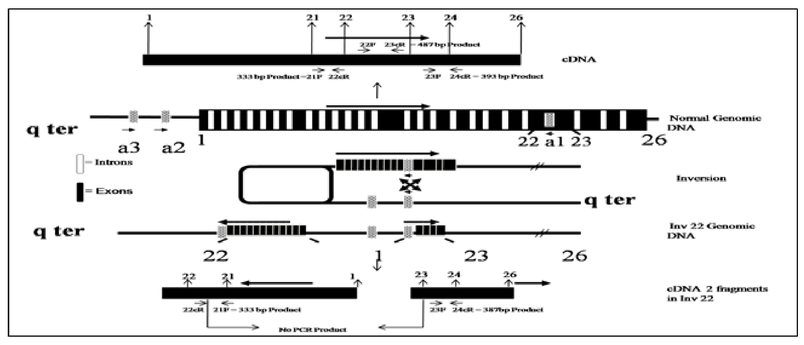

Fig 1: New Protocol : *cDNA prepared from F VIII gene in cases and controls was amplified across 21-22, 22-23, and 23-24.*

RESULTS

Clinical and Phenotypic profiles in Inversion 22 + vs Inversion 22 – cases with Hemophilia A :

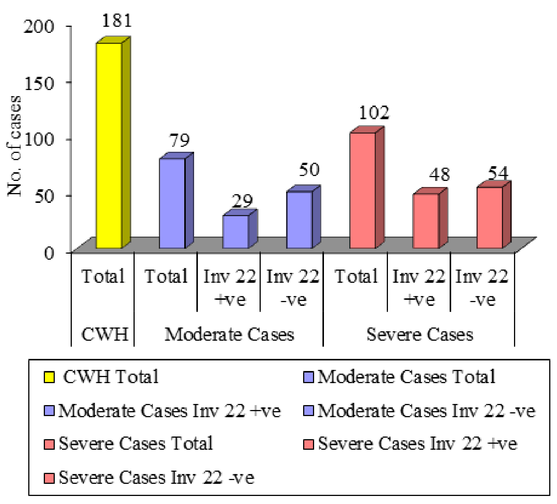

Fig 2: Phenotypic Division of Cases on the basis of Severity

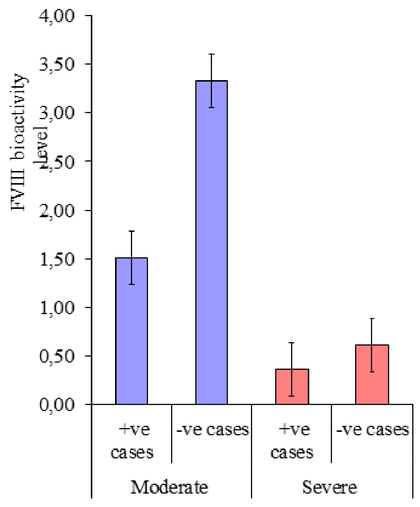

Fig 3: FVIII bioactivity in Severe and Moderate Phenotypes

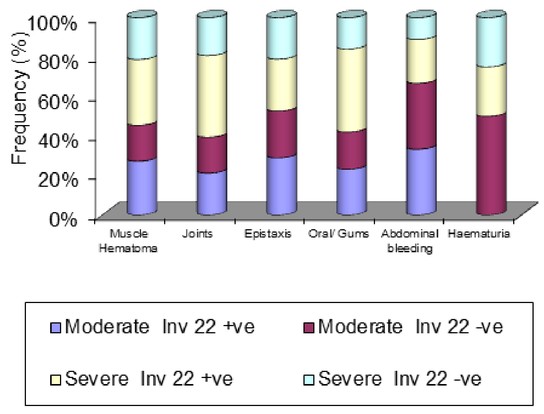

Fig 4: Sites of bleeding in CWH

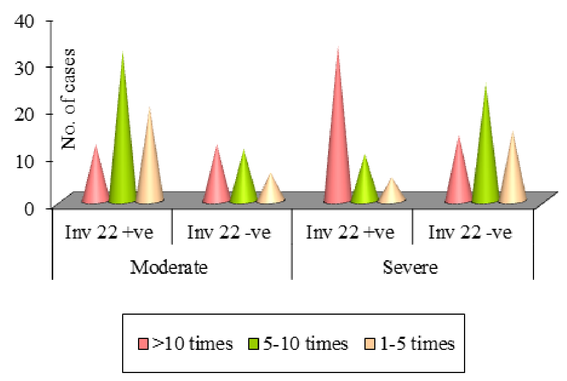

Fig 5: Annual requirement of F VIII infusion

➢Observed frequency of Inv22 in North Indian Population was 42.5 % by standard Inverse PCR.

➢In the new method of cDNA, Real Time PCR amplification was done in 20 CWH and 10 controls have been taken for the study in which 8 cases were Inv 22 Positive showing no amplification of Exon 22-23 and produced no cT values and 12 cases were Inv 22 Negative showed positive amplification with a cT value ranges from 21.6-24.3.

➢The newly designed cDNA protocol matched completely with the results of Inverse PCR. No abnormal results were obtained in controls as well as in cases. The new method appears to be valid for detection of Inv 22 Mutation in CWH.

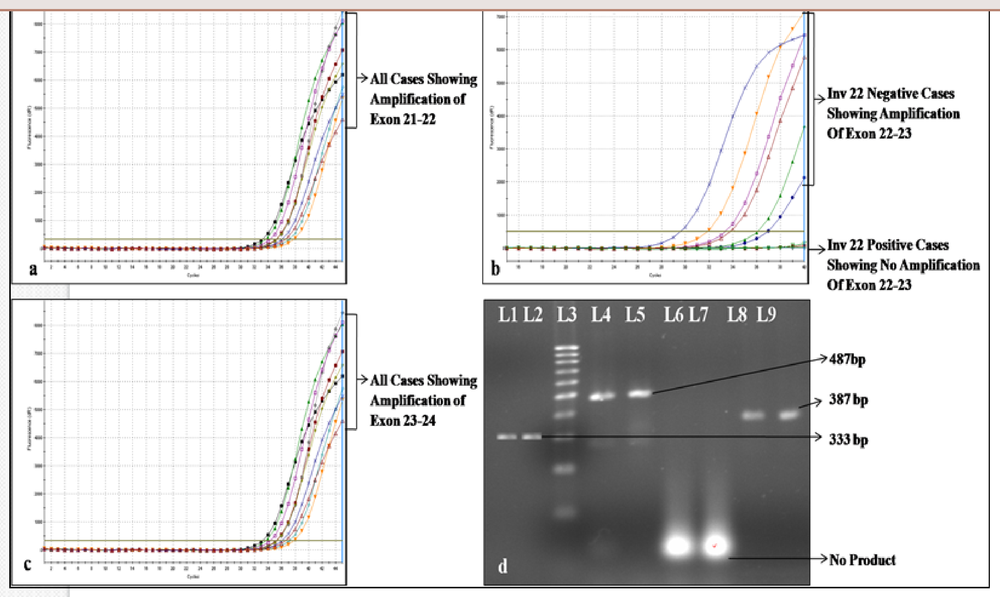

Fig 6: New cDNA protocol Results

a). Showing the RT PCR amplification of Exon 21-22.
b). Showing the RT PCR amplification in the cases negative for Inv22 and there is no amplification in the positive cases of Exon 22-23.
c). Showing the RT PCR amplification of Exon 23-24
d). Gel Electrophoresis of cDNA protocol: Lane 1&2 shows amplification of Exon 21-22 with a product of 333 bp, Lane 3 is a 100 bp ladder (Fermentas, Germany), Lane 4,5 shows the amplification of Exon 22-23 with a product size of 487 bp in Inv22 negative cases, Lane 6,7 Shows primer dimer formation of Exon 22-23 in Inv22 positive cases, Lane 8,9 shows amplification of Exon 23-24.

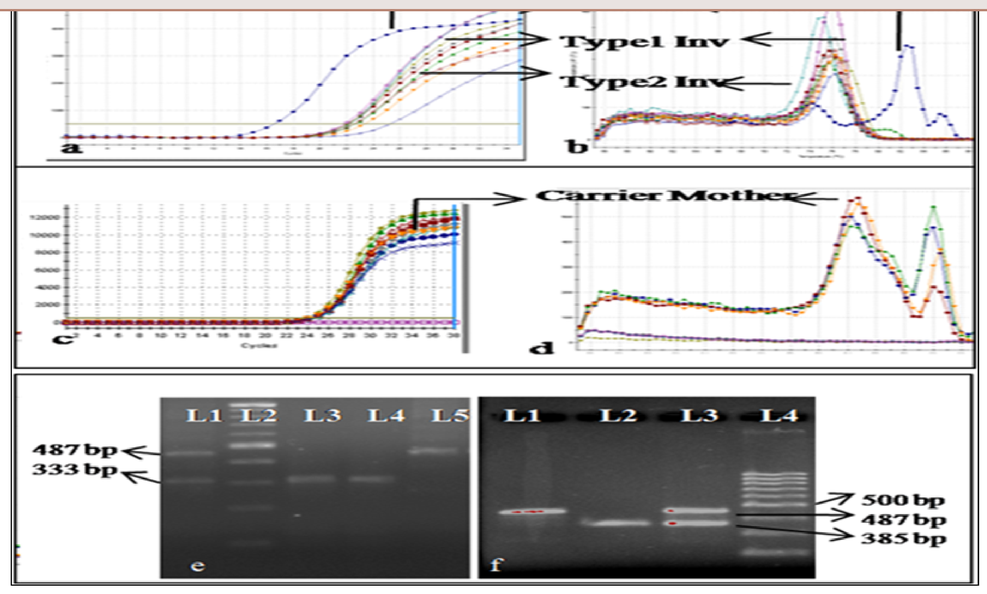

Fig 7: I-PCR Results

(a) Real Time PCR Amplification plot of control, Inv22 Type 1 and Type 2
(b) Dissociation plot of the figure 1(a) (c) Amplification plot of carrier mother
(d) Dissociation plot of figure 1(c) carrier mother with two peaks showing hetrozygosity
(e) Gel electrophoresis of Type 1 inv22: Lane 1 shows Type 1 carrier mother, Lane2: 100 bp Ladder (New England Biolabs, USA), Lane 3&4: Inv 22 Type 1 positive case, Lane 5 inv 22 negative case
(f) Gel electrophoresis of Type 2 inv22: Lane 1 shows Inv22 negative case, Lane2: Inv 22 Type 2 positive case, Lane 3: Type 2 carrier mother, Lane 4: 100 bp Ladder (Fermentas, Germany).

CONCLUSION

➢Inv 22 mutation lead to a more severe form of Hemophilia A in our population.

➢Inv 22 can be conveniently detected by using Inverse PCR method which is easy to standardize and lowest in cost.

➢The new cDNA based method is short, involves three short segment amplification and is easy to reproduce.

➢Further validation of the test and its use in carriers needs to be evaluated.

THANKYOU

DOI: 10.3252/pso.eu.WFH2012.2012