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Abstract Title:- Genetic diagnosis of haemophilia A families leading to a time-saving two tier strategy along with insights on de novo cases: decadal data

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Aims:-Haemophilia A (HA) is one of the two most common inherited bleeding disorders; clinical manifestations include haemarthrosis, easy bruising or muscle and soft tissue bleeding culminating in significant morbidity and mortality. Predominantly, these patients fall below the poverty line, and the cost of treatment is high, with government-funded healthcare options being comparatively insufficient. Hence, carrier and antenatal testing hold paramount significance & ICMR-NIIH has been the primary center offering this service for over a decade, albeit it is both laborious and expensive. With our experience and data, this study has following aims:

1) To deduce a simple time saving strategy for HA genetic diagnosis.

2) Mutation analysis in possible sporadic cases of severe HA.

Methods:- HA families referred at our center for carrier and antenatal diagnosis were included.

Phenotypic assays & subsequent genetic analysis were done.

F8 gene mutation analysis: Intron 1 & 22 inversions were detected using multiplex and inverse PCR respectively followed by direct sequencing of gDNA using FVIII-specific oligonucleotide primers. Gross deletions were confirmed by MLPA technique.

Results:- 657/664 HA families were analyzed in the current series. 303(46.11%) families were found to be inversion positive i.e., Intron 22(n=287) & Intron 1(n=16); 30(4.5%) showed deletions of different exons. Mutations were not detected in 7 cases. A heterogeneous pattern of variations throughout F8 gene was seen in 324 (49.31%) patients. Based on the frequency of mutations in different exons we divided them into 2 sets of exons; first set: exons 2,4,7,8,9,11,13,14a,14e,14g,14j,14k,18,19,23& 24; mutations detected in 236 (72.84%) cases and second set: 1,3,5,6,10,12,14b,14c,14d,14f,14h,15,16,17,20,21,22,25&26 where only 88 (27.16%) could be detected. Thus, an effective strategy has been developed:

- Screening for F8 gene Intron 22 & 1 inversions
- Screening for gross deletions followed by sequencing of probable exons

In this series(n=657) 197 families had no prior family history of hemophilia or bleeding. 48.22% of these cases had inversions 1&22, 21.32% showed gross deletions and frame shift variants, 30.46% showed point mutations including nonsense, missense & splice-site variants compared to 45.22%, 16.3 % and 38.48 % respectively in those with a prior family history. 13 mothers of the propositi were found to be non-carriers, i.e., true de-novo cases.

Conclusions:- A time saving two-tier strategy has been developed and adopted in our laboratory for routine genetic diagnosis of HA families. Interestingly, we have found 29.9% of the cases to have no family history with 13 cases being truly de-novo and inversion 22 being more prevalent in this group.

Keywords:- haemophilia A, genetic diagnosis, two-tier strategy, de-novo, decadal insights