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Intron 1 Inversion among Hemophilia a Patients in Basrah at the South of Iraq

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Abstract

Background/Aim: Hemophilia A is an inherited bleeding disorder that results from a mutation in FVIII gene, intron 1 inversion is one of the mutations of FVIII gene. We aimed to detect the prevalence of Intron 1 inversion among hemophilia A patients in Basrha/ south of Iraq

Subjects and Methods: A screen for the presence of intron 1 inversion at the F8 gene in 95 hemophilia A patient from Basrah at the south of Iraq. All patients have A total genmic DNAwas extracted from EDTA fresh whole blood samples drawn from all participants,then detection of intron 1 inversion by conventional PCR and use of touchdown technique.

Results: Not there any one of Hemophilia A patients has Intron one Inversion

Conclusions: Absent of intron 1 inversion compare to other studies may be referred to geographic distribution of patients in spite of low prevalence percentage of intron 1 inversion, also more number of patients increase the accuracy of the research.

Keywords: intron 1 inversion, Basrah, Hemophilia, FVIII mutation, touchdown PCR.

Introduction

The most common congenital bleeding disorder is hemophilia A, which is caused by a lack of the clotting protein Factor VIII (Callaghan, Sidonio and Pipe, 2018).

Factor VIII (FVIII) deficiency is an X-linked recessive condition that affects one out of every 5000 male births, regardless of ethnicity.(Witmer and Young, 2013).

F8 genotype mutations included large deletions (single or multiple exons), intron 1 and 22 inversions, nonsense mutations (light chain or non-light chain), small deletions/insertions/combined deletions and insertions (in poly-A runs or outside poly-A runs), splice site (conserved or non-conserved nucleotide positions), and missense mutations.(Gouw *et al.*, 2012).

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Intron1 Inversion of factor VIII has been documented as one of the causes of severe hemophilia, and it appears to be responsible for roughly 2-5 percent of severe HA cases(Hart and Giangrande, no date; Hassan, 2017) and up to 7 % of HP in Asia (CHEN *et al.*, 2010).Intron 1 inversion mutations are caused by homologous recombination between a 1041-bp region (Int1h-1) and a 140-kb telomeric region (Int1h-2) that splices the FVIII gene promoter from exon 1.(Goodeve and Peake, 2003; Faridi, Kumar and Husain, 2012). This mutation occurs during male meiosis and results in the production of short mRNA, which results in the absence of FVIII.(Oldenburg, Pezeshkpoor and Pavlova, 2014).

Materia and method

Patients:

The study included 95 male patients with Hemophilia A, ranging in age from 2 months to 63 years. All of the patients were diagnosed and registered at the Basra Center for Hereditary Blood Diseases, where they were assessed throughout their follow-up visits.

Patients with hemophilia A were split into three categories based on the severity of the disease: mild 37 (39%), moderate 33 (34.7 %), and severe 25 percent (26.3 %)

Regarding clinical presentation of hemophilia A patients in relation to the severity of the condition,96% of the sever cases presented with heamarthrosis, and 88% of them had Mucous membrane bleeding.while(69.7%) of moderate Hemophilia A patient presented with Muscle bleeding and (32.4%) of mild cases had bleeding after circumcision

Sample collection and there processing:

Two milliliters of venous blood collected in an EDTA tube for Total genomicDNA extracted by wizard kit (Promega, USA). The extracted DNA was frozen in 30- C° until molecular analysis.

DNA loading and electrophoresis: 7 microliters of DNA were combined with 3 microliters of loading dye before being carefully placed in each gel well. The present power supply was switched on after that (70 volts for 30 minutes). From the cathode(-) to the anode(+) pole, the DNA band migrated. The Ethidium Bromide-stained bands inthe gel were visualized under UV transilluminator and photographed by adigital camera and the concentration of DNA was detected by Nanodrop. A Nano-dropSpectrophotometerwasusedtomeasuretheextractedDNAat260nm,whichwas thewavelengthofmaximum absorption forDNA.

PCR Amplification

To detect intron 1 inversion, a single PCR test run was employed to amplify a specific target using standard PCR and touchdown PCR techniques.The primers used in this study were

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described in**Table 1**according to study of Wed elbaharwith some modifications (Centre and Uk, 2016).

The PCR reaction tube contain 12.5µ of master mix, 4µ of primers mixture, 3µ of DNA and 5.5µ RNase-Free Water and use the condition of PCR reaction in the **Table 2.**In the reaction used two PCR tubes that just different in Primers mixture to detect of inversion in intron 1, that mean for each sample used 2 tubes in the reaction,first tube contain primers mix were (9F, 9cR, h-2F) produce region of 1908 bp for case without int1 inversion or 1776 bp for case with int1 inversion, while the second tube contain (h-2F, h-2R, 9F) primers mix that produce region of 1191 bp for case without int1 inversion or 1323 bp for case with int1 inversion according to **Figure 1.**The product of PCR diagnosed on agarose gel 2% after 1 hour at 70 Watt.

Table 1: primers used to detect of FVIII gene Intron 1 inversion

Fragment	Primer name	Primer sequence
Int1h-1	9F	5'-GTTGTTGGGAATGGTTACGG-3'
	9cR	5'-CTAGCTTGAGCTCCCTGTGG-3'
	Int1h-2F	5'-GGCAGGGATCTTGTTGGTAAA-3'
Int1h-2	Int1h-2F	5'-GGCAGGGATCTTGTTGGTAAA-3'
	Int1h-2R	5'-TGGGTGATATAAGCTGCTGAGCTA-3'
	9F	5'-GTTGTTGGGAATGGTTACGG-3'

Table 2 Procedure of conventional PCR reaction to detect of intron 1 inversion

Step	Temp (°C)	Time	Cycle
Initial Denaturation	94	5 minutes	1
Denature	94	30 second	10
Anneal	55-65	30 second	
Extension	72	2 minutes	
Denature	94	30 second	25
Anneal	55	30 second	
Extension	72	2 minutes	
Final Extension	72	5 minutes	1

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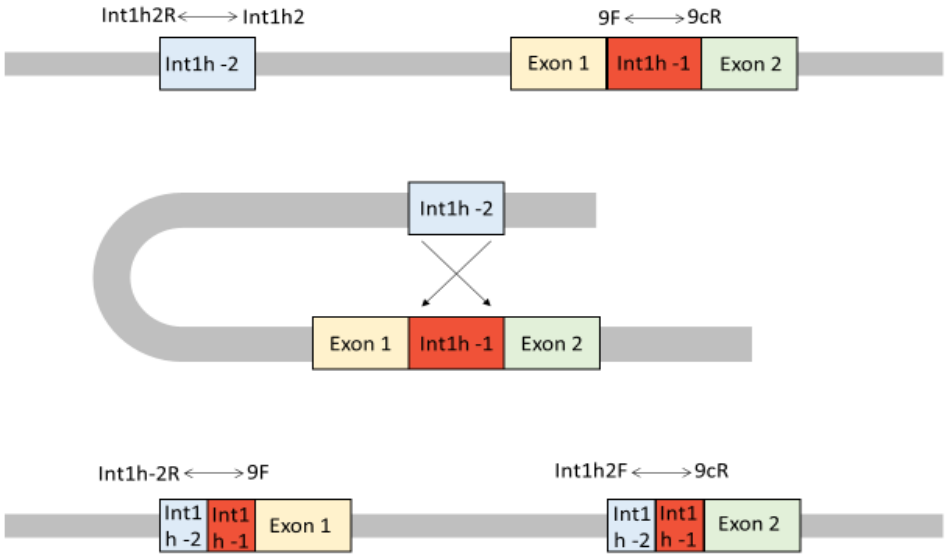

Figure 1: inversion of intron 1 and primers position

Result

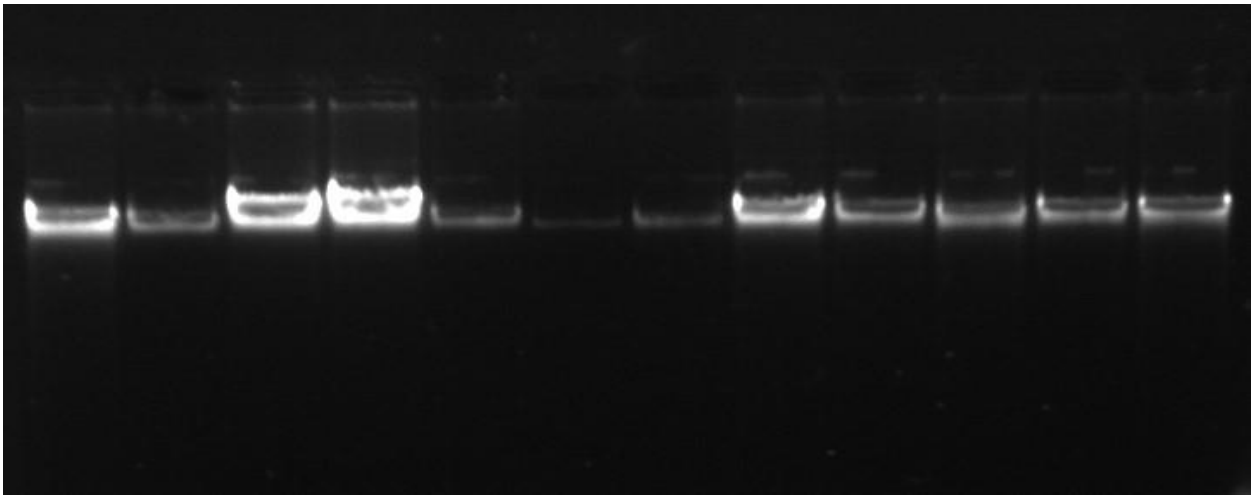

Figure 2: (0.8%)ofagarosewasusedfordemonstration of genomicDNA

There is no inversion in Intron I in any of the participants with hemophilia A (severe, moderate, or mild)as appear in picture 1 (9F,9cR and Int1h-2F)produce 1908 bp band while the second band appear 1191 bp with primersmixture (Int1h-2F, Int1h-2R and 9F). For that we not found direct relation between intron.

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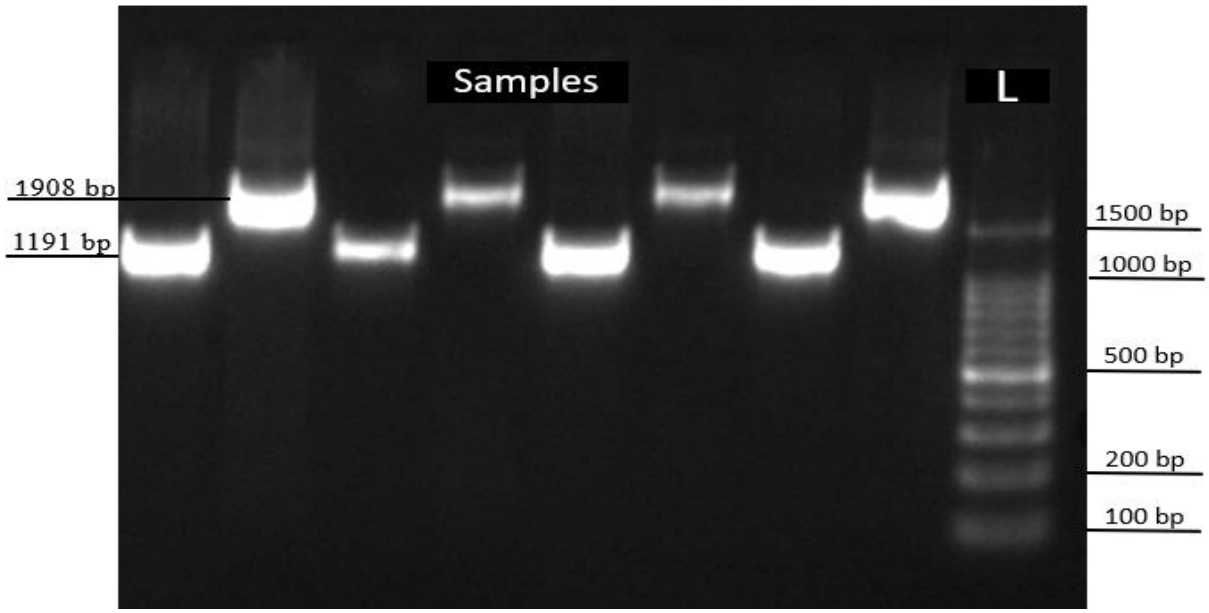

Figure 3: PCR product on agarose gel for 75 minutes at 70 volts, all hemophilia patients without inversion, L: ladder 1500bp.

Discussion

Intron 1 inversion (Inv1) is a large molecular defect in FVIII gene resulting in severe HA. The pathogenic mechanism associated with this inversion involves homologous recombination between a 1041 bp region of intron 1 (int1h-1) of the FVIII gene and an extragenic copy (int1h-2) of region 5 approximately 140 kb telomeric to the FVIII gene.

In this study all the 95 with hemophilia Apatients were analyzed for Intron 1 inversion and none of the severe casesnor the mild and moderate has this type of inversion. This result differs that found by Abdulqader*et al*., who reported 3.3% (2/60) had Inv1 among Iraqi kurdish patients with Hemophilia A (Abdulqader *et al.*, 2020), and Qadir *et al*., in Kurdistan, Iraq also, found intron 1 inversion in only one out of 28 (3.5%) patients with hemophilia A (Qadir *et al.*, 2020).

The reported frequency of Inv 1 among patients with hemophilia A varies in different countries. In Pakistan, Inv. 1 was reported in 0.77% of severe HA patients (Sattar *et al.*, 2017). In India, Inv. 1 was found in 3.6% of PWH (Faridi, Kumar and Husain, 2012).

The results of the current study are similar to Castaman*et al*., study in Albania (Castaman *et al.*, 2007) and Mantilla-Capacho*et al*. study in Mexico (Faridi, Kumar and Husain, 2012) who did not report Inv. 1 in their patients with hemophilia A. These variations in the results may be related to the sample selection, beside samples size and the geographical area of patients, also due to the immigration from the middle and north provinces to Basrah city in the last years

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