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Journal of Bioscience and Applied Research
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Assessing Gene Expression of factor XIII-A in Iraqi patients with FXIII deficiency

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DOI: 10.21608/jbaar.2024.268467.1038

Abstract

Coagulation factor XIII (FXIII) circulates in plasma as a pro-transglutaminase heterotetrameric complex (FXIIIA2B2), which upon activation by thrombin and calcium covalently crosslinks preformed fibrin polymers. The heterotetrameric complex is composed of a catalytic FXIIIA2 subunit and a protective/regulatory FXIII-B2 subunit coded by F13A1 and F13B genes, respectively. The study aimed to assess the level of expression of the FXIII-A gene in seven Iraqi families affected with FXIII deficiency. Samples of whole blood were collected from seven Iraqi families that were characterized with F13 deficiency register in the Hemophilia Ward, Children Welfare Teaching Hospital, Medical City, Baghdad. These samples were divided into three groups according to the genotype, which included affected homozygous recessive and heterozygous with control healthy group. RNA was isolated from whole blood samples and gene expression levels of the F13 A1 gene was determined using quantitative Real-Time polymerase chain reaction (RT-qPCR), the result revealed that the expression levels of the F13A gene in heterozygous parents were significantly increased compared with the homozygous affected and control group. The (ROC) curve was analyzed in this study to determine the optimal cut-off value for F13 expression and showed a very good predictor for FXIII deficiency diagnosis with "Area under the ROC Curve." AUC equals 0.844 and $p< 0.001$.

Keywords: Rare Bleeding Disorder, Coagulation factor, Factor XIII deficiency, Gene expression.

Introduction

Coagulation factor XIII (FXIII) is a protein from the group of transglutaminases (TGase) that has two catalytic A subunits (FXIII-A) and two transporter noncatalytic B subunits (FXIII-B) in form (A2B2) (1). It is a proenzyme that is activated by thrombin that is generated in the final stage of the blood coagulation cascade (2). The A subunit has the main function, and it includes the catalytic core domain, activation peptide, calcium ion binding site, and other structure domains. The B subunit mainly acts as a carrier protein to stabilize the A subunit, connects the A subunit to fibrinogen, and down-regulates the activity of FXIII (3). FXIII-A is synthesized primarily in cells of bone marrow origin, while FXIII-B is synthesized in the liver. The formation of tetrameric complex probably occurs in plasma (4). Coagulation Factor XIII (FXIII) plays an important role in wound healing by stabilizing fibrin clots and cross-linking extracellular matrix proteins (5). Mature FXIII-A is a 731-amino acid protein without a hydrophobic leader sequence (6). FXIII deficiency is one of the blood bleeding disorders that results from the deficiency or functional abnormality

Received: February 1, 2024. Accepted: March 27, 2024. Published: March 31, 2024

of one of the plasma proteins involved in providing normal coagulation that poses a high risk for severe hemorrhage (7). Bleeding usually includes umbilical bleeding, prolonged bleeding after an injury or surgical procedure, subcutaneous bleeding, gum bleeding, intracranial bleeding, joint bleeding, and muscle bleeding (8). The deficiency of FXIII, inherited or acquired, is known to cause bleeding predispositions, where congenital FXIII deficiency is due to defects either of the F13A1 (at genetic locus 6p24-25) or F13B genes (at genetic locus 1q32-32.1). The global prevalence of inherited FXIII deficiency is low (1–4 cases per million), which brings it under the category of rare bleeding disorders (9). Most defects associated with severe bleeding symptoms in inherited FXIII deficiency are largely due to defects in the F13A1 gene, whereas gene defects in the F13B gene cause mild to moderate symptoms (10). The gene coding for the A subunit has been localized to chromosome 6p24-25. Its 15 exons - and 14 introns span over 160 kb (11). Hereditary deficiency of coagulation factor XIII is inherited as an autosomal recessive trait (1). This study Assesses the mRNA level of coagulation factor XIII-A in whole blood using a one-step real-time-quantitative polymerase chain reaction (q-Rt PCR) and compares the assessing Gene Expression among three groups that were identified.

Material and methods

Sample Collection

Whole blood was collected from seven Iraqi families, whose members that diagnosed and registered with congenital FXIII deficiency in the Hemophilia Ward, Children Welfare Teaching Hospital, Medical City, Baghdad. The demographic and clinical features and treatment have been collected for included patients, and they were diagnosed by having bleeding tendency and normal standard coagulation tests (normal platelet count, normal prothrombin time, no normal partial thromboplastin time, and normal bleeding time), and the diagnosis was confirmed by clot solubility test in 5 m urea (qualitative test for FXIII deficiency).

All the laboratory tests were performed at the Hemophilia Ward, Children Welfare Teaching Hospital, Medical City, Baghdad laboratory. Five ml of blood was collected from seven families via venipuncture and transferred to a tube containing triazole in volume (1:2), the general characterization features of the seven families are shown in Table 1.

Real-Time Polymerase Reaction (RT-qPCR)

Total RNA was extracted from the blood of available families' individuals and healthy controls using the protocol of TRIzol™ Reagent, Quantus Fluorometer was used to detect the concentration of extracted RNA to detect the quality of samples for downstream applications. Analysis and Calculation of gene expression levels of one or more genes depend on RNA concentration after conversion to cDNA. By using GoTaq® 1-Step RT-qPCR System, MgCL2, Nuclease Free Water; Promega, USA. primers for a target gene and housekeeping gene were supplied by Macrogen Company in a lyophilized form. and the desired sequence was blasted in the relevant database (http://www.ncbi.nlm.nih.gov/ pubmed), the sequence and details of primers are shown in Table -2.

The results of RT-qPCR were analyzed by the relative quantification of gene expression level (fold change) according to Livak (12). Based on comparing the distinct cycle determined by threshold values Ct at a constant fluorescence level. The target gene was normalized to an endogenous control (HKG) and relative to the calibrator which is the target gene in the healthy control group. The fold-change was calculated for each sample using the following equations: ΔCt sample = Ct gene - Ct HKG, ΔΔCt sample= (ΔCt sample)- (average ΔCt control group), Fold-change sample= 2-ΔΔCt.

Table 1: The General Features of the Seven Iraqi Families with FXIII Deficiency.

	family symbol	paternal relationship	family member	F 13 def	sex/ familial arrangement	family history	symptoms date
family No 1	A	Consanguinity	A1	Affected	male/ son	Proband	from birth
			A2	Affected	male/son		through the first 6 month
			A3	Carrier	female / mother		non
family No2	B	Consanguinity	B1	Affected	male/son	Proband	from birth
			B2	Affected	female/ daughter		from birth
			B3	Carrier	female/ mother		non
family No3	C	Consanguinity	C1	Affected	male/ son	Proband	from birth
			C2	Affected	female/ daughter		from the first 6 month
			C3	Carrier	male/ father		non
			C4	Carrier	female/ mother		non
family No4	D	Consanguinity	D1	Affected	male/ son		from birth
			D2	Affected	male/son		from birth
			D3	Carrier	male/father		non
			D4	Carrier	female/ mother		non
family No5	E	Consanguinity	E1	Affected	male/ son	Proband	from birth
			E2	Affected	female/ daughter		from birth
			E3	Carrier	male/ father		non
			E4	Carrier	female/ mother		non
family No6	F	Consanguinity	F1	Affected	female/ daughter	Proband	from the first 6 month
			F3	Carrier	male/ father		non
			F4	Carrier	female/ mother		non
family No7	R	Consanguinity	R1	Affected	granddaughter		from birth
			R2	Carrier	Mother		non
			R3	Carrier	grandfather		non

Table 2: the primer sequence that was designed for the target gene and housekeeping gene.

Primer Name	Sequence 5`-3`	Annealing Temp. (ºC)
β-Globin-F β-Globin-R	ACACAACTGTGTTCACTAGC CAACTTCATCCACGTTCACC	65
F13A_exp-F F13A_exp-R	CAACAGCCACAACCGTTACACC CTTGGATCAGCACCGCCTCTTT	60

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 22 has been used in the current study for dealing with obtained data. Mean (median) and interquartile range have been used to calculate parametric results(quantitative) while non-parametric data(qualitative) was calculated by means and standard deviation. Additionally, the Pearson Chi-square test was used for comparisons, and the Spearman correlation was used to test the correlation between different study parameters. The P-value is significant < 0.05 at a confidence interval of 95%.

Result and Discussion

General characteristic of the study

The study included a total of seven families, containing parents and children, in addition to thirty healthy individuals. Table 3 shows all the details of the families.

The families under study were classified according to the clinical condition into affected individuals who were characterized by bleeding symptoms as a result of factor 13 deficiency, while the other category included healthy parents without symptoms with a bleeding disorder called heterozygosity because the disorder is a pattern of recessive inheritance.

Expression of F13A gene

The current study analyzed the mRNA expression levels of the FXIII A1 gene in whole blood samples of all families with FXIII deficiency patients and controls by Rt q PCR. Relative FXIII A1 expression levels of patients were obtained by using β-Globin as a reference for the gene for normalization and HCs as a calibrator sample. Cycle threshold Ct values were calculated by the 2-ΔΔCt method for evaluation of the expression levels.

The results indicated that the expression levels of the F13A gene found in patients were high expression. The median expression fold of the control group was 1.00, but it was observed that the median expression fold of affected and heterozygous individuals increased (1.3, 3.6) with a property value equal to 0.001. It is important to note that the median expression fold of heterozygote individuals was higher than that of those affected with FXIII deficiency compared to the control group, as shown in Table 4.

Table 3: General Characteristics of patients with FXIII deficiency and control

Characteristic		Patients		Controls	
		N	%	N	%
Group		24	100	30	100
Clinical state	**Affected**	12	50	0	0
	Heterozygote	12	50	0	0
Gender	**Male**	12	50	30	100
	Female	12	50	0	0

N= Frequency, %= Percentage

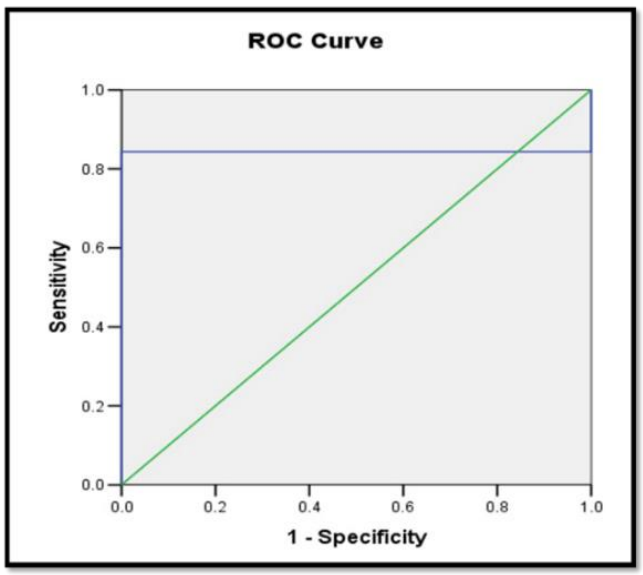
Table 4: Relative expression of F13A levels (fold) in blood samples of the studied groups.

Group	Affected (N=12 Median IQR(Min-max)	Heterozygote (N=12 Median IQR(Min-max)	Controls (N=10) Median IQR(Min-max)	p-value
B. globin	17.59 (13.0-23.6)	19.2 (12.1-21.7)	10.4 (9.4-12.9)	**0.003
F13A	31.8 (27.5+35.2)	32.5 (25.0-33.1)	26.2 (23.7-27.2)	**0.003
ΔCt	14.0(12.8-14.4)	12.5 (11.3-13.4)	14.0 (13.2-16.2)	*0.02
ΔΔCt	-.043(-1.6- 0.01)	-1.8 (-3.1- -0.9)	0.00 (0.00-0.00)	**0.001
Folding	1.3 (0.9-3.1)	3.6 (2.0-10.4)	1.00 (0.00-1.00)	**0.001

***Significant, **very significant**

Diagnostic value of the expression of FXIII gene

To understand whether the expression level of the FXIII gene in blood could differentiate patients from healthy controls, a Receiver Operating Characteristic (ROC) curve analysis was conducted. The result showed that the gene expression levels of F13A in patients had a very good ability to differentiate patients from healthy individuals. The AUC, sensitivity, and specificity were 0.844, 84.37%, and 100.00 %, respectively, at the cutoff value of >1 fold, which was the good value of FXIII deficiency correct prediction, as shown in Figure 1. Our results of ROC curve analysis showed that gene expression levels of F13A in patients could represent a very good predictor for FXIII deficiency diagnosis.



Test Result Variable(s): Folding to gene expression F13A gene	
Positive group (a)	32 (76.19%)
Negative group (b)	10 (23.81%)
Area under the ROC curve (AUC)	0.844
Standard Error	0.0652
95% Confidence interval	0.699 to 0.937
z statistic	5.271
Significance level (P-value) (Area=0.05)	<0.001
Accuracy (Youden index J)	0.8438
Associated criterion (cutoff)	>1
Sensitivity	84.37
Specificity	100.00

Figure 1: Receiver operator curve (ROC) analysis for the predictive value of relative expression of FXIII A1 levels (fold) in patients versus healthy controls. AUC= area under the curve, SE= standard error, CI= confidence interval, PPV = positive predictive value, NPV= negative predictive value.

Discussion

Coagulation factor XIII was expressed in a variety of tissues, mostly in monocytes. (21). FXIII deficiency is an autosomal recessive disease (13), so consanguineous marriage is a risk factor for this disorder (14). Due to mandilion migrations, single-gene recessive disorder has a clinical state phenotype when the genotype is homozygote and results from an affected parent with genotype heterozygote (15). The samples that were collected were divided into three groups due to genotype and clinical state: the affected group with recessive homozygote genotype and the carrier group with recessive heterozygous genotype in front of the healthy control group. In comparison to the median index of folding of gene expression among the three groups, there is evidence that gene expression of coagulation factor XIII (FXIII) can be overexpressed in patients with FXIII deficiency. This is thought to be a compensatory mechanism to try to increase the amount of FXIII protein that is produced. Patients with FXIII deficiency had considerably greater levels of FXIII gene expression in both their liver and peripheral blood cells compared to controls (16). Another study found that FXIII gene expression was increased in the synovial tissue of patients with rheumatoid arthritis (RA), a condition that is associated with an increased risk of FXIII deficiency (17). It is important to note that not all patients with FXIII deficiency have overexpressed FXIII gene expression (18). The AUC value (area under the curve) is a performance metric for classification problems. Gene expression levels of F13A in patients might serve as an ideal predictor for FXIII deficiency diagnosis, as the AUC value of the folding gene expression of the FXIII A1 gene is 0.844 (19). Many genetic disorders Use RT-qPCR as a detection tool, use RT-qPCR as a useful indicator for early detection of pediatric acute leukemia (20), In other research, the genetic foundation of acute myeloid leukemia is discovered by gene expression (22).

Conclusion

FXIII A1 deficiency is an autosomal recessive disorder therefore consanguineous marriage acts as a risk factor for this disorder. The results indicated over-expression of the FXIII A1 gene in the heterozygous group and homozygous patients than healthy control group. The AUC, sensitivity, and specificity indicate gene expression of the FXIII A1 gene has been a correct prediction with FXIII deficiency.

Acknowledgments

This work was supported in the Hemophilia Ward, Children Welfare Teaching Hospital, Medical City, Baghdad, Iraq, and we appreciate the support of the University of Baghdad, College of Sciences, and the Biotechnology Department.

Ethical Clearance

This research was ethically approved by the Research Ethical Committees of the Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research, Iraq.

Funding: No funding.

Conflict of interest:

The authors declare that they have no conflict of interest.

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