

Genealogical, Biochemical Analysis And Correlation Of A Negative Blood Group With Hemophilists

Naser Kamberi¹, Hyzer Rizani^{2*}, Fitlm Alidema³, Edona Tasholli⁴, Zahide Arifi⁵

Abstract

This paper includes 10,000 individuals from the population of the Municipality of Presev, which is located on the E-75 corridor in southeastern Serbia bordering northern Macedonia. During the physical interviews in the family, we came across several genetic diseases where we singled out hemophilia type A. During the direct observation, we found that only males were infected with this disease while females were not affected with this type of hemophilia. Also based on biochemical, molecular analyzes we noticed that all cases with hemophilia were with blood group A and with rhesus factor negative. Also based on the statements of the scientist Bar that one X chromosome in the early embryonic stages becomes inactive, then women must also be affected by this disease. These two cases of negative R-factor in all hemophiliacs led us to another study about the type of inheritance and their possible correlation. Analyzing the genetic results we did not have cases of females being hemophilic and the other phenomenon was why these hemophiliacs have group A negative. This gives us an idea that antigen A is not related to blood coagulation factor (enzyme). This leads us to think that the possibility of men having hemophilia may be related to a lack of antigen A. The alleles for blood groups are known to be on chromosome 9 while the alleles for the rhesus factor are on chromosome 1. Negative blood group A and clotting factor VIII are Missense mutations (Ala723Thr and Lys439Ser) and protein shortening changes (Trp1854 * and Arg2326 *) have been observed in 2 each of these cases. Of the 3 hemophiliac cases having incompatible antigen-activity ratios, most of those mutations produced FVIII with low specific activity for FVIII and for antigen A with rhesus negative blood factor. Regarding the body of Bari from many genealogical,

¹Direction Dentistry-Prishtina, University of Business and Technology (UBT), Calabria, 10000 Prishtina, Kosovo. E-Mail: naser.kamberi@ubt-uni.net-

² *University of Business and Technology (UBT) - Food Sciences and Biotechnology- Calabria, 10000 Prishtina. R. of Kosovo- E-Mail: hyzer.rizani@ubt-uni.net

³ Direction Dentistry-Prishtina, University of Business and Technology (UBT), Calabria, 10000 Prishtina, Kosovo.. E-Mail: fitim.alidema@ubt-uni.net-

⁴ Direction Dentistry-Prishtina, University of Business and Technology (UBT), Calabria, 10000 Prishtina, Kosovo.

⁵ Social Center in Gjilan-Kosovo.
Email. Zaid erexhepi06@gmail.com.

biochemical analyzes we came to the conclusion that this body of Bari is genetically active, but morphologically changes its original shape. From biochemical analysis and knowing that the egg cell is approximately 10000 times larger than the spermatozoid made us analyze the biochemical structure of the egg cell. In the egg cell it was observed that inside the cytoplasm is rich in numerous enzymes which at the time of fertilization will promote the division of the zygote into two new cells. In this case we found out that the cause of condensation of X chromosome DNA in sperm is the factor of the egg cell environment.

This means that enzymes, mitochondrial DNA have many redox enzymes that can affect the condensation of the X chromosome coming from the father. Assuming that the sex X chromosome is inactive we did not have the opportunity for women to be hemophilic as well. By doing the genealogical analysis of these families we will present the thoughts about the role of Bar's body when it comes to hemophilia type A. For these cases of laboratory hemophilia, biochemical and genetic analyzes were made to observe the level of penetration and expressivity of factor VIII. We will present through the genetic tree the origin or genealogy of the gene for this disease. Based on biochemical and genetic analyzes using PCR, we will present the level of factor I, IX, XI, aTTP, vWF- Von Willebrandov factor, locus Xq28, ccddee genotype, fibrinogen, blood group, rhesus factor in hemophiliacs included in this study [1]..

Keywords: PCR, Bar Body, Blood Group A, ccddee, hemophilia VIII, Xq28

Introduction

Factor VIII (FVIII) is an essential blood clotting protein, also known as anti-hemophilic factor (AHF). In humans, factor VIII is encoded by the F8 gene. Defects in this gene result in hemophilia A, a recessive X-linked coagulation disorder. Barry and Bertram observed in 1949 that the X chromosome became inactive in the embryonic stage at the time of implantation, at the end of the first week of pregnancy. This X chromosome from the father is condensed in the form of a spot (black dot) and for which the genes in it are thought to be inactive. Hemophilia A is inherited through the sex X chromosome. Women have a 50% chance of transmitting the factor VIII mutation in any pregnancy. Boys who inherit factor VIII from their mother will be affected by hemophilia. If the sex X chromosome is inactive in females then the dose of the chromosome is equal to the male sex. For this reason the likelihood of getting hemophilia would be equal, does not in reality this stand. So with this paper we will give our thoughts about the body of Bar or gender heterochromatin regarding the dilemma of this case (Fig.1). A deletion that occurs in the RHD gene during the evolution of hominids, so many modern people completely lack the RHD gene. This haplotype (vocabulary) is the leading cause of negative D phenotype worldwide and is marked as nonsense mutation.

Therefore, we can say that the correlation is supported by the absence of coagulation factor or the F8 gene - as a mutation and in the absence of a mutation. RHD-CE-D hybrid alleles are often formed by gene conversion.

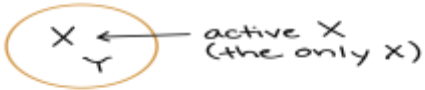
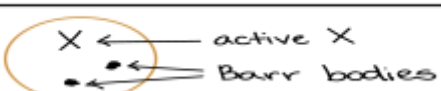
XX female	
XY male	
XXY male (Klinefelter)	
XXX female (triple X)	

Fig.1. The Barr body

Material and methods

The material was collected in the form of a questionnaire directly from families both in Preševo and in the villages. During this questionnaire, we encountered cases of hemophilia A, where out of 10,000 inhabitants, we identified 4 cases of male hemophilia A, but one of them died. From detailed analysis we learned that these are the three first cousins from the mother's line who are genetically close in the 4th degree of consanguinity. For this study, we did the genealogical research of the family that was affected by factor VIII of hemophilia. In order to know the level of action of hemophilic factors and to know their genotype for this disease, laboratory, biochemical and genetic analyzes were carried out at the Institute of Nish. For the molecular analysis, we have the results from the PCR with which the genotype for hemophilia ccddee was determined, the location of the location of the hemophilia gene on the sex chromosome X in the Xq28 region. Analysis of aTTP coagulation factors, factor VIII level, vWF-Von Willebrandov factor and other analyzes shown in Table 1 were performed [5].

Results and discussion

a) Genealogical analysis of hemophilia A

Through the genealogical analysis with hemophilia A in three families it is seen that the source of this disease is from female generation III-8. We ascertain this from the analysis of previous generations where we have no cases with this disease. Doing the analysis it is clear that

we have a death from hemophilia which is in generation IV- and serial number 10 (IV-10). Of the three cases with hemophilia we also have the son with hemophilia disease in the 5th generation and with serial number 9 (V-9) and two brothers with hemophilia in the 5th generation with serial number 11 (V-11) who is dead and the brother of the same generation V with serial number 12 (V-12) alive but hemophilic. From the genealogical analysis (Fig.2) it is clear that the carrier of factor VIII for hemophilia type A is the individual in generation III with serial number 8 (III-8) which is inherited from her mother stock II with serial number 3 (II-3). The generation IV-10 individual presented as the proposition with the arrows is a dead male. According to genealogical cases, this dead brother IV-10, has two sisters who are carriers of type A hemophilia who are underlined with arrows in generation IV with serial numbers 14 and 15. Both married sisters (14 and 15) in different families have three hemophilic sons one dead in generation V with serial number 11 (V-11). While the other two hemophiliacs are alive of the V-9 and V-12 generation. These are in the 4th degree of kinship 1/16. If they marry in this family with fourth degree relatives, every 16th born will be hemophiliac [6].

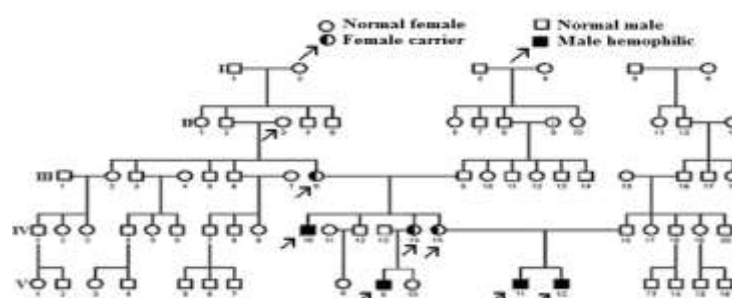


Figure 2. Genetic tree of hemophilia in cousins- type A-VIII

b) Biochemical and genetic analysis of hemophiliacs with factor VIII-A.

From the analyzes made by PCR it is concluded that it is about factor VIII of hemophilia A. From the results obtained in the case of hemophilia A-VIII it results that it is about the average type of hemophilia with these values 1.5%, 1.7% and 2%. Level of hemoglobin in type A-VIII of hemophilia is 10.4, 10.6, and 10.7. Thromboplastin activation time (aPTT) is clearly seen to be much later 71.7s, 68s and 75s. The prothrombin test specifically assesses the presence of factors VII, V, and X, prothrombin and fibrinogen. A prothrombin time within the interval of 11-15 seconds (depending on the source of thromboplastin used) indicates that the patient has normal amounts of upper coagulation factors [2]. With aPTT ratios of 1.5 to 2.5, in the therapeutic range used by many laboratories, variable levels of the

anti-Xa heparin factor have been achieved. According to this process it turns out that the defect is related to the negative reversible bond which will lead to a decrease in heparin and an increase in thromboplastin. These three cases with hemophilia have blood group A, Rh negative and according to the analysis hemophilia has a correlation with rhesus negative factor with genotype dd. We base this on the similarity of the rhesus negative dd genotype with the dd genotype in hemophiliacs. We therefore think that these diseases are caused by a correlation between genes on sex chromosome X and the gene on autosomal chromosome number 1.

F-VIII mutations in cases with HA were identified using a combination of conformational and DNA-sensitive gel electrophoresis or simply direct DNA sequencing. DNA Analysis The coding region, intron/exon boundaries, and F8 untranslated regions were amplified in multiplex polymerase chain reactions using specific primers [3]. All cases showed a consistent ratio of FVIII:C and FVIII: Ag activities. Blood group "A" showed a lower standard deviation indicating the expected value, while blood group "O" showed a higher standard deviation. This is due to the many discordant levels of CRM found in patients with this blood type.). RH alleles can be grouped according to their molecular structure. For the most part, these groups show point mutations (SNPs, single nucleotide polymorphisms) which cause missense, nonsense, frameshift or junction site mutations.

Table 1. Cytogenetic molecular analysis

Three cases with haemophilia	First person (first brother)	The second individual (second brother)	The third person, the cousin of the two brothers
Type of hemophilia	Hemophilia type A	Hemophilia type A	Hemophilia type A
Blood group	A, Rh negative	A, Rh negative	A, Rh negative
Factor VIII level	1.7% (preferably 50-150%)	1.5% (preferably 50-150%)	2% (preferably 50-150%)
Factor I level	338,5%	338%	339%
Factor IX level	91,5% (66)	91%	91%
Factor XI level	82.8% (117)	83%	83%
Fibrinogen	4.01% (130-300 mg/dL)	4% (130-300 mg/dL)	4% (130-300 mg/dL)
Genotype	ccdde	ccdde	ccdde
Intron 22	Region Xq28	Region Xq28	Region Xq28
aPTT(Partial Thromboplastin Time)	71.7s (preferably 25-35s)	68s (preferably 25-35s)	75s (preferably 25-35s)
Leukocytes	16.5	17	17.1
Erythrocytes	4.20	4.18	4.10
Hemoglobin	10.4 (12-15-g/dL)	10.6	10.7
vWF- Factors Von Willebrandov	46%	46%	45%

Analyzing the average values in Table 2 it is clear that factor VIII has a low level compared to the reference value. Therefore we say that the type of hemophilia in these 3 cases investigated is the average type because the average for these is; 1.37% reference is = 1-5% (F VIII C > 5 iu dL).

Table 2. Laboratory status based on the median values investigated

Test	Average	Reference range	Units
Factor VIII level	1.37%	50-150%	%
Heavy	<1%(F VIII<1 iu dL)	50-150%	iu- international unit
Average	1-5%(F VIII< 5 iu dL)	50-150%	iu- international unit
Easy	> 5% (F VIII>iu dL)	50.150%	iu- international unit
Genotype	ccddee	CCDDEE	Dominant
aPTT(Partial Thromboplastin Time)	71.56-s	25-36-s	Seconds -s
Hemoglobin	10.6	12-15 (120-150g/L)	g/dL or g/L
vWF- Factors Von Willebrandov	46%	50-160%	%

We can therefore conclude that the inheritance of these factors has been done with a high stability because gene expression has given the same results in these cases. Therefore we think that the intervention of factor VIII which results from the mutation in intron 22 in the Xq28 region of the X chromosome has had a similar penetration. According to genetic analysis-PCR it is clear that the genotype of three cases with hemophilia A is ccddee. The locus of the gene on the chromosome has the region on the Xq-28 arm. CRM-reduced hemophilia A patients have reduced plasma FVIII antigen levels, regardless of activity levels. Some patients with reduced CRM also exhibit lower activity values compared to plasma antigen levels.

Conclusions

From the genealogical analysis, we have established that we have not had cases of sick women, even though they have the same dose of the X chromosome as men. This means that the "inactive" chromosome of the Barr body differs morphologically only from the other sex chromosome that has the form X (X), but it is functional and with its genetic radiation contributes to enzymatic processes and thus prevents enzymopathies in women. This is confirmed by the fact that if in the female sex the hemophilic gene occurs on both sex X chromosomes the female will be ill. Therefore, we conclude that the Bar corpuscle (heterochromatin) is active in molecular processes, only in shape it changes and takes the appearance of a spot in the nucleus. Thromboplastin activation for coagulation is approximately the same values as shown: aPTT- 71.7s (25-35s), 68s (25-35s) and 75s (25-35s). According to the contact protein analysis E-cadherin has a filamentous deformation which slows down the activity of thromboplastin. Also the level of factor VIII in three cases has a very low penetration therefore the expressiveness is lower and thus does not stop the flow of blood. Vitamin K has a very low level in the cases mentioned. The values of factor VIII are as follows: Case 1. Brother 1.7% however

(Preferred 50-150%); Case 2. brother 1.5% (Preferred 50-150%) and case 3. Cousin 2% (Preferred 50-150%). Based on these results doctors can determine the doses around these cases of hemophilia. In this case it is suggested that the dose of heparin should be in correlation with the activity of aPTT to avoid thrombin.

At the end of the first week of pregnancy, before the implantation of the embryo in the uterus occurs morphological change, inactivation of the X chromosome originating from the sperm and this confirms that their father was not hemophilic and the Grass Body is not a carrier of hemophilia in these three cases, but the mother was a carrier but not ill. It is clear that the Grass Tree is not inactive because we have no hemophiliac females in the genealogical trunk of the family.

- a) From other studies it has been observed that the sensitivity of an aPTT reagent to heparin depends both on its phospholipid content and on the nature of the activator present. Therefore we say that in this case we have an average between these two factors that because the penetration and expressiveness of genes is such.
- b) We can say that rhesus negative factor with dd genotype on autosomal chromosome 1 has an epigenetic influence on sex X chromosome (ccddee). This means that hemophilia is an inherited disease of the correlative type but also epistasis because the phenotype is not the result only of the sex X chromosome gene.
- c) A deletion that occurs in the RHD gene during the evolution of hominids, so many modern humans completely lack the RHD gene. This haplotype is the leading cause of dd negative phenotype worldwide and is marked as nonsense mutation.
- d) According to genetic-biochemical analysis it has been found that factor VIII results from mutation in intron 22 in the Xq28 region of the X chromosome and their genotype is: ccddee. Through the genealogical analysis of cases with hemophilia A in three families, it is seen that the source of this disease is a female of generation III-8.
- e) Based on the analysis of laboratory results, it is concluded that it is about Hemophilia type A. From the obtained laboratory and genetic results it is seen that the genetic penetration is very similar to both factor VIII and aPTT factor. They belong to the medium hemophilia because they have a value with an average <1.37.
- f) As a solution to this problem, evolution has decided that women should always modify an X chromosome, due to space in the nucleus but not completely inactive. And this is accomplished precisely by methylizing DNA into histones on an X chromosome of the father which undergoes the epigenetic factor of the egg cell because it finds no adaptation to another cell that has a completely different medium from where it came from. And because of the

action of the cytoplasm and egg cell enzymes the X chromosome from the father undergoes a condensation in the body of Bar [4].

Therefore appeal to international associations of hemophiliacs to have a genetic care and counseling of populations endangered by these hemophilic factors.

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- Naser Kamberi¹, Hyzer RIZANI²** - ¹Direction Dentistry-Prishtina, University of Business and Technology (UBT), Calabria, 10000 Prishtina, Kosovo.* **e-mail: naser.kamberi@ubt-uni.net- mobile number: +38162423484;** ²University of Business and Technology (UBT) - Food Sciences and Biotechnology- Calabria, 10000 Prishtina. R. of Kosovo- mn.- +38345111044 **Corresponding Author:** Assistant professore-mail: hyzer.rizani@ubt-uni.net <https://kalaharijournals.com/ijme-vol7-issue-jan2022part2.php>- ISSN: 0974-5823 Vol. 7 No. 1 Januar-Part 2- 29.01.2022.
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