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Genealogical, Genetic, Biochemical Analysis of Bar's Body in Hemophilia type a

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Abstract
This study was included in a sample of 6000 individuals of the population of Presevo and villages. During family interviews we encountered cases, in women carriers with hemophilia while boys hemophiliac. Assuming that the sex X chromosome is inactive, we did not have the opportunity for women to be hemophilic. By doing the genealogical analysis of these families we will present the thoughts about the role of Bar's body when it comes to type A hemophilia. For these cases of hemophiliacs laboratory, biochemical and genetic analyzes have been done to observe the level of penetration and expressiveness 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 analysis using PCR, we will present the level of factor I, IX, XI, aTTP, vWF- Von Willebrandov factor, locus Xq28, genotype ccddee, fibrinogen, blood group, rhesus factor in hemophiliacs included in this study.

Keywords: Bar's body, hemophilia, genealogical tree

Introduction
Barry and Bertram in 1949 observed 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 condenses in the form of a spot (black dot) and for which it is thought that the genes in it are inactive. The research was conducted in the population of Presevo, which lies in the east of Kosovo, in the south-east of Serbia and the border of Northern Macedonia on the E75 road line. 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. Therefore 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).

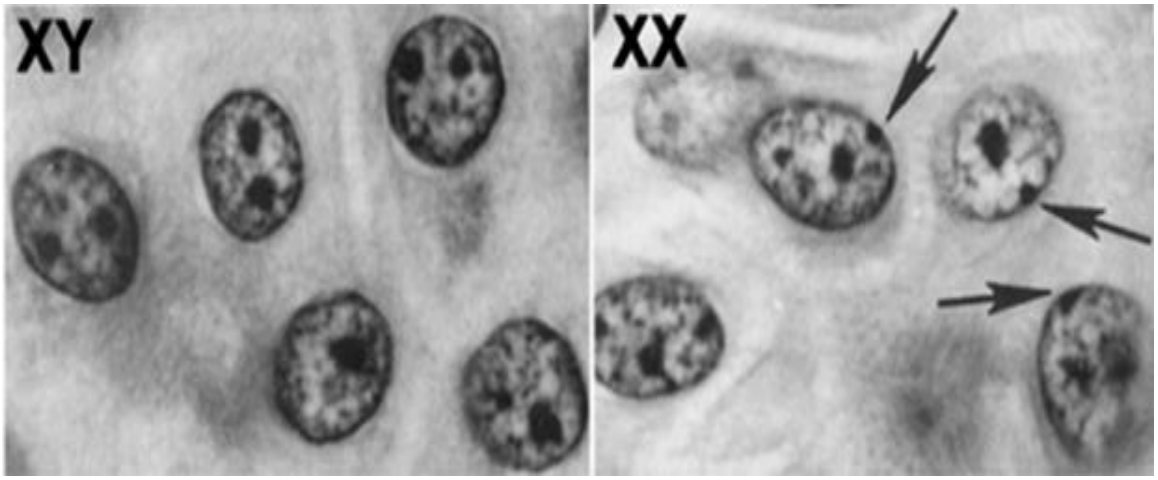

Fig.1.Heterochromatin-Body of Bar

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Material and methods

The material for this paper was collected in the form of a questionnaire in families in Presevo and in villages. During this questionnaire we encountered cases of hemophilia type A where out of 6000 inhabitants we identified 4 cases of hemophilia A male but one of them died. From the detailed analysis we learned that these are the first three cousins from the maternal line who are genetically close in the 4th degree of kinship. For this study we did the genealogical research of the family that were 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 performed at the Nis Institute. For molecular analysis we have the results from PCR by which the genotype for hemophilia ccddee was determined, the location of the hemophilic gene locus on the sex chromosome X in the Xq28 region. Analyzes of aTTP coagulation factors, factor VIII level, vWF factor-Von Willebrandov factor and other analyzes presented in Table 1 were performed.

Analysis and discussion of results

a) Genealogical analysis of haemophilus A

Genealogical analysis of cases with hemophilia A in three families shows that the source of this disease is from the female of generation III-8. This is ascertained by the analysis of previous generations where we have no cases with this disease. Making the analysis clearly shows that we have a dead hemophilia that is in the IV-generation and the ordinal number 10 (IV-10). Of the three cases with hemophilia we have also the son with hemophilic disease in the V band and with ordinal number 9 (V-9) and two hemophiliacs in the V band with ordinal number 11 (V-11) who is dead and the Vlach of the same generation V with ordinal number 12 (V-12) alive being haemophilic.

From genealogical analysis (Fig.2) it is clear that factor VIII factor for type A hemophilus is the individual in generation III with ordinal number 8 (III-8) which is inherited from her mother of stock exchange II with ordinal number 3 (II -3). The person of the generation IV-10 presented as a target with the arrows is a dead man.

According to genealogical cases, this dead brother IV-10 has two sisters carrying type A hemophiliacs that are underlined with the arrows in IV band with ordinal numbers 14 and 15. Both married sister (14 and 15) in different families there are three dead hemophilic boys in V band with ordinal number 11 (V-11). While two other hemophiliacs are alive of the V-9 and V-12 generation. These are in the 4th stage of kinship 1/16. If they make a marriage in this family with IV degree relatives every 16th born will be haemophilia.

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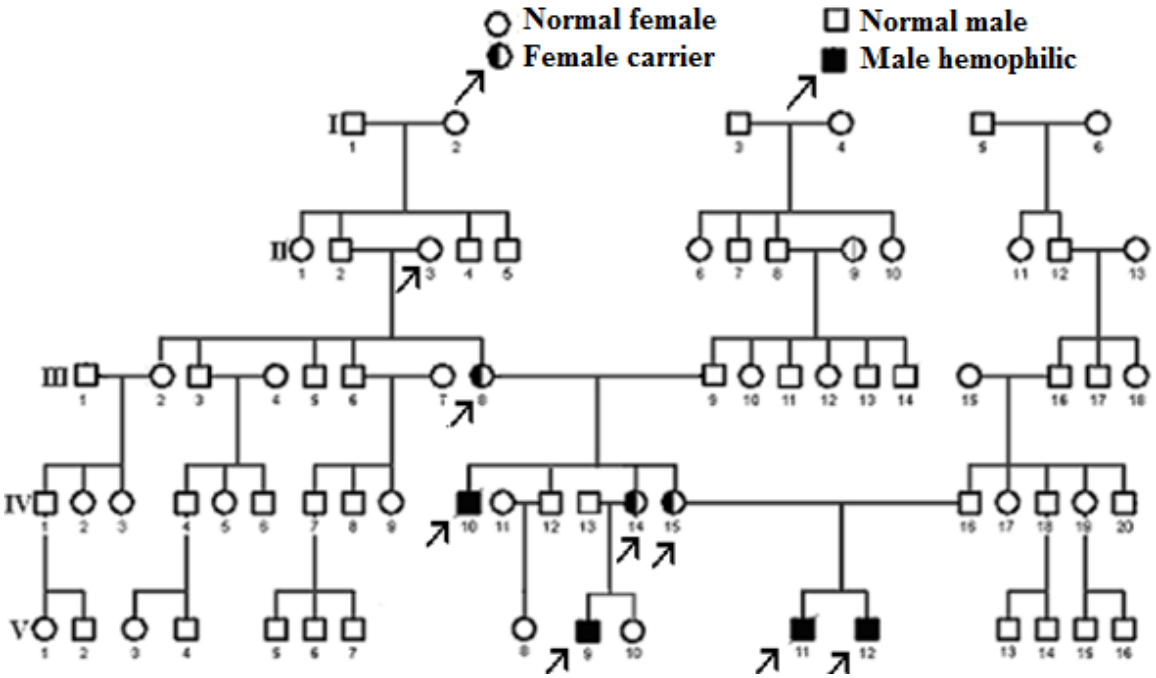

Fig. 2. The genetic tree of hemophilia's disease in cats type A-VIII

b) Biochemical and genetic analysis of hemophiliacs

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%.

The hemoglobin level in type A-VIII 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.

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.

Table 1. Laboratory, biochemical and genetic results of haemophilists

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**

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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	**ccddee**	**ccddee**	**ccddee**
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 investigated cases is the average type because the average for these is; 1.37% reference is = 1-5% (F VIIIC> 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 VIIIC(1 iu dL)**	**50-150%**	**iu-** international unit
Average	**1-5%(F VIIIC> 5 iu dL)**	**50-150%**	**iu-** international unit
Easy	**> 5% (F VIIIC>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

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intervention of factor VIII which results from the mutation in intron 22 in the Xq28 region of the X chromosome had the same 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.

Conclusion

From this study we can emphasize that women although based on scientific opinions have a sex X chromosome while the other is inactive non-functional, are not hemophilic like men and this leads us to a doubt about the activity of the body of Barr.

However, analyzing the cases, we did not have any sick women, even though they have the same dose of X chromosome as men.

This means that the "inactive" chromosome Heterochromatin or Barr body differs morphologically only from the other sex chromosome that has the shape X (X), but it is functional and with its genetic radiation contributes to enzymatic processes and thus prevents enzymopathies in the female sex. This is confirmed by the fact that if in the female sex the hemophiliac gene occurs on both sex X chromosomes the female will be ill. Therefore there is no dilemma that heterochromatin or Barr body is partially active if only in shape it changes and takes on the appearance of a stain on the nucleus.

Activation of thromboplastin 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.

Resume

a) In the early stage, 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 Body of Bar 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 hemophilic females in the genealogical trunk of the family.

b) Other research has shown that the sensitivity of an aPTT reagent to heparin depends on both its phospholipid content and 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.

c) 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 but also epistatic type because the phenotype is not the result only of the sex X chromosome gene.

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 from females 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 the same of 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, because of the 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.

Appeal to the international associations of hemophiliacs to have a genetic care and counseling of populations endangered by these hemophiliac factors.

Gratitude: In particular I thank the families who have enabled me to interview and gather all the material for this study.

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