TRANSFUSION-TRANSMITTED INFECTIONS IN HAEMOPHILIA PATIENTS

Bukurije Zhubi[1*], Ymer Mekaj[1], Zana Baruti[2], Ilirijane Bunjaku[3], Mazllum Belegu[1]

[1] National Blood Transfusion Centre of Kosovo Prishtina (NBTCK). Mother Theresa str., 10000 Prishtina, Kosovo
[2] Institute of Biochemistry, University of Prishtina, Mother Theresa str., 10 000 Prishtina, Kosovo
[3] Department of Pulmology, Regional Hospital "Isa Grezda", Dëshmorët e Lirise str. PN, 50000 Gjakova, Kosovo

* Corresponding author

Abstract

One of the largest therapeutic problem during the continuous treatment of the patients with Hemophilia A and B, are viral infections as Hepatitis B and C, and HIV, and the other infective diseases, which can be transmitted by the transfusion of blood products.
The aim of this study is to analyze the complications of the hemophiliacs in Kosovo which have been treated with fresh frozen plasma, cryoprecipitate and concentrated products of FVIII and FIX. We have tested 75 patients with hemophilia A or B and there were used enzyme immunoassay test-Elisa method for the following: anti-HCV, HBsAg, HIV and TPHA.
The serological data showed that HCV infection was positive in 29 cases or 38,7%, whereas infection with HBV and HIV were present in a smaller percentage of the patients (2,7% HBV and 1,4% for HIV). HCV infection was present only in 9,5% of the cases of the age group under 18 years. Infected hemophiliacs with one or two infective agents were found in 34,7%, respectively 4%. Infection with *T. pallidum* was present at none of the examined patients with hemophilia. HCV infection was higher in severe forms of hemophilia B (44,4%), compared with severe form of hemophilia A (30%).
Based on our results, despite the infrequent application of FVIII and FIX concentrates, and other anti hemophilic preparations used in treating hemophilia patients, the number of infected hemophiliacs with blood-transmittable infectious agents was substantially high, especially with hepatitis C virus.

KEY WORDS: hepatitis C Virus (HCV), hepatitis virus B (HBV), Human immune deficiency Virus (HIV), Hemophilia A and Hemophilia B.

Introduction

Transfusion-transmitted infections (TTI) are serious complications at Hemophilia patients treated by factor VIII and IX concentrates (1, 2). Multitransfused hemophiliacs with antihemophilic products are endangered of acquiring viral hepatitis (3). These infections occurred more often before 1985 (4). From these infections we must mention viral hepatitis A (HAV) (5), viral hepatitis B (HBV) (6), viral hepatitis C (HCV) (7), viral hepatitis G (HGV) (8) etc. The cause of manifestation of these infections is on the fact that concentrating coagulation factors are prepared of plasma from thousand of blood donors that didn't undergo viral inactivation (9, 10, 11). Although the majority of infected patients do not suffer acute symptoms and clear the infection spontaneously, the remaining portion (<50%) become chronic carriers of virus (12).

The latter may develop into chronic active hepatitis and finally progress to liver cirrhosis; especially in HBV and HCV infections (13, 14, 15). About 80% of adult hemophiliacs develop antibodies against surface antigen of hepatitis B virus (HBsAg), while 10% among this group become chronic carriers (6). HBsAg positive hemophiliacs are very often accompanied with delta hepatitis infection, which causes severe active hepatitis, cirrhosis and hepatocellular carcinoma that appears six times more often in haemophiliacs than in other population (16, 17, 18). In 77% of aged patients with Hemophilia A and 42% with Hemophilia B (19) were found HIV antibodies as well as with middle aged patients, but with lower incidence that is in correlation with the used amount of FVIII concentrates. Some data suggesting that 80% of patients treated with Factor VIII concentrates have HIV antibodies (20), compared to 14% of patients treated with cryoprecipitate (12). After 1985, the risk of acquiring HIV infection through antihemophilic products is increasingly rare because of the new used methods of factor VIII concentrates preparation and laboratory tests for blood donors' examination (21, 22). At many HIV positive hemophiliacs AIDS is developed. According to literature data, during period 1976-1991 HIV infection was the main death cause of hemophiliacs, compared to bleeding with only 5% (23, 24). FVIII concentrates available on the market are considered safe in the sense of not transmitting infectious agents, especially to recombinant factor concentrates (25, 26, 27). Due to the fact that haemophilia patients received multiple transfusions of FFP, cryoprecipitate, the factor VIII and IX concentrates prepared from pool plasma of thousands of donations, there exists a risk of acquiring TTI. So, this paper is focused on the above mentioned complications of the patients with hemophilia associated with transfusion of blood components or derivatives.

Material and Methods

Study Subjects

Our cohort comprises 75 patients with Hemophilia A and B, with average age 24,7 years, with SD 15,3 (age range 3 to 61 years); 48 (64%) with haemophilia A and average age 23,5 years, and 27 (36%) with hemophilia B and average age 23,5 years. From the total number of individuals studied, only 30 (40%) are below age 18 years.

Anamnesis

The study group have included all hemophiliacs in Kosovo, diagnosed at NBTCK. They are all males due to X-linked recessive inheritance pattern for hemophilia A and B. Anamnesis data suggest that most of patients were treated occasionally during bleeding episodes (or prophylactically prior to dental extraction or surgery.

Analysis in detail

All the patients with hemophilia A and B were tested for known blood-transmittable disease agents. The tests were performed on blood samples drawn with special sealed tubes (VACUETTE) containing Sodium citricum (3, 8 %) at 1:9 ratios. After centrifugation, plasma has been separated and stored at -30°C until the tests were performed. The tests were carried using ELISA immunoassay method using the sophisticated automated platform "TECAN" (Mini Swift). The test and companies were as follows: HIV1/2: Ag/Ab Combination (Abbot-Murex-HIV), this reagent detected antigens of virus tip 1 (HIV-1, HIV-1 group O) and also anti–HIV-2. HBV Surface antigen: HBsAg (ver.3, Abbot-Murex) with this reagent was detected superficial antigen of hepatitis B virus. HCV: anti-HCV (ver.4 Abbot-Murex) this reagent detected the antibodies against hepatitis C virus.. *T. pallidum*: ICE*Syphilis (Abbot-Murex), very sensitive reagents which detected antibodies against *T. pallidum*.

Diagnosis

Diagnosis was determinated on the following infectious agents: HIV, HBV, HCV and *T. pallidum*. Most of the patients showed no clinical picture of blood-transmittable disease.

Infection with viruses	H C V			H B V			H I V		
Hemophiliacs A and B	Pos	Neg	Total	Pos	Neg	Total	Pos	Neg	Total
Nº	29	46	75	2	73	75	1	74	75
%	38,7	61,3	100	2,7	97,3	100	1,4	98,6	100
Average age XB	31,7	20,4	24,8	47	24,2	24,8	28	24,7	24,8
Standard Deviation SD	11,1	16,6	15,3	14,4	14,9	15,3	0	15,4	15,3
Note: All the patients are TPH negative									

TABLE 1. Viral infectious complications at patients with Hemophilia A and B

Therapy

Generally, hemophiliacs were treated with fresh frozen plasma (FFP) and cryoprecipitate, and only occasionally with DDAVP, FVIII and FIX concentrates. None of them had received recombinant products, like rFVIIa. Patients with clinical picture of infection, after being diagnosed were referred to the clinic of infectious diseases for further treatment.

Statistical analysis

Study results were stored in Instat 2 statistical software. Arithmetic average, standard deviation, minimal and maximal values, and P value (student t-test) of ages in both groups of Haemophilia A and B were used for statistical data procesing. All the results are presented in data tables.

RESULTS

In Table 1. are presented data for the TTI in patients with hemophilia A and B, which shows that infection with HCV is found in a large group of patients, in 29 patients or in 38, 7% of cases. But, infection with hepatitis virus B (HBV) and HIV virus (HIV) is found only in small number of cases, in 2,7% (HBV) respectively 1,4% (HIV). There were no patients with *T. pallidum* infection. In Table 2, the major percentage of the infected patients (45%) with HCV is in the mild form of hemophilia A. In the moderate and severe form of hemophilia A infection with HCV was lower (30% respectively 25%). From the total number of patients with hemophilia A (48), 41,7% are infected with hepatitis C virus. At the mild form of hemophilia A it is found a case infected with hepatitis B virus and another case with HIV virus.

From the total number of the patients (Table 3.) with hemophilia B (27), 33,3% were infected with HCV. From the infected patients, the majority of them (44,4%) have the severe form of hemophilia B, 22,2 % moderate form and 33,4% the mild form. One patient with moderate form of hemophilia B was diagnosed with HBV infection, but patients with hemophilia B were not infected with HIV virus.

Patients with hemophilia A, 60,4% of them were not infected, while infected with one or two infective agents were found 35,4%, respectively 4,2% (Table 4.). An approximate number of infected patients (66,6%) was found and in cases of hemophilia B, while infected with one or two infective agents were 29,6%, respectively 3,7%.

Virus Infections		H C V			H B V			H I V		
Hemophilia A		**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**
Severe	**Nº**	5	10	15	0	15	15	0	15	15
	%	25	75	100	0	100	100	0	100	100
(<1%)	**Average age**	26,6	17,9	20,8	0	20,8	20,8	0	20,8	20,8
	SD	5,4	16,3	13,9	0	13,9	13,9	0	13,9	13,9
Moderate	**Nº**	6	6	12	0	12	12	0	12	12
	%	30	70	100	0	100	100	0	100	100
(1-5%)	**Average age**	38,7	15	26,8	0	26,8	26,8	0	26,8	26,8
	SD	11,3	12,7	16,8	0	16,8	16,8	0	16,8	16,8
Mild	**Nº**	9	12	21	1	20	21	1	20	21
	%	45	55	100	4,8	95,3	100	4,8	95,3	100
(>5%)	**Average age**	29,8	19	23,6	57	21,9	43,7	28	23,4	23,6
	SD	4,9	14,9	15,4	0	13,7	23,6	0	15,8	15,4
Total	**Nº**	20	28	48	1	47	48	1	47	48
	%	41,7	58,3	100	2,1	97,9	100	2,1	97,9	100
(No=48)	**Average age**	31,6	17,8	23,6	57	22,8	23,6	28	23,5	23,6
	SD	12,4	14,5	15,2	0	14,5	15,2	0	15,3	15,2

TABLE 2. Viral infectious complications in patients with different forms of hemophilia A

Virus infections		H C V			H B V			H I V		
Hemophilia B		**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**	**Pos**	**Neg**	**Total**
Severe	**Nº**	4	6	10	0	10	10	0	10	10
	%	44,4	66,6	100	0	100	100	0	100	100
(<1%)	**Average age**	31	23,7	26,6	0	26,6	26,6	0	26,6	26,6
	SD	8,04	19,4	15,7	0	15,6	15,6	0	15,6	15,6
Moderate	**Nº**	2	9	11	1	10	11	0	11	11
	%	22,2	78,8	100	9,1	90,9	100	0	100	100
(1-5%)	**Average age**	38,5	23,9	26,6	37	25,5	26,6	0	26,6	26,6
	SD	2,1	18	17,2	0	17,7	17,2	0	17,2	17,2
Mild	**Nº**	3	3	6	0	6	6	0	6	6
	%	33,4	66,6	100	0	100	100	0	100	100
(>5%)	**Average age**	28,7	27,7	28,6	0	28,2	28,2	0	28,2	28,2
	SD	9,6	22,8	15,7	0	15,6	15,6	0	15,6	15,6
Total	**Nº**	9	18	27	1	26	27	0	27	27
	%	33,3	66,7	100	3,7	96,3	100	0	100	100
(No=27)	**Average age**	31,9	24,4	26,9	37	26,6	26,9	0	26,9	26,9
	SD	7,9	18,1	15,7	0	15,8	15,7	0	15,7	15,7

TABLE 3. Viral infectious complications in patients with different forms of hemophilia B

In Table 5. it can be noticed that only 2 (9,5%) hemophilia A patients within this subgroup were infected with HCV. Other blood-transmittable infectious agents were not found within this subgroup. Age was not significantly different between hemophilia A (9,7 years) and B(11,3 years) (P=0,4425).

Discussion

After the application of factor VIII concentrates there was a significant reduction in the morbidity and mortality from bleeding in hemophiliacs. However, the use of replacement therapy wasn't without significant complications especially the development of antibodies against factor VIII and TTI (19). The use of clotting factor concentrates manufactured from a large pool of plasma that didn't undergo viral attenuation increased the risk of TTI (14) such as HBV, HCV, and HIV with subsequent rise in morbidity and mortality rate up to five fold (8). While before 1960 bleeding was the main lethal complication in haemophilia patients, during the 1980s the leading cause of mortality were infectious complications with HBV, HCV and HIV (28). The aim of this study was to investigate the infectious complications at hemophilia patients in Kosovo, who have been enrolled for a long-term treatment with anti hemophilic preparations such as FFP, cryoprecipitate, and occasionally FVIII and FIX concentrates in small doses. The administration of these preparations was mostly carried during spontaneous or traumatic episodes of bleeding, or on patients undergoing surgery. Our study results revealed that out of 75 patients with Hemophilia A or B, 38,7% were seropositive for HCV, while conversely, seropositivity for HBV and HIV was significantly lower; 2,7% and 1,4%, respectively. Other publications (18, 29, 30, 31, 32, 33, 34, 35, 36, 37) have given higher HIV infection percentage (1,7~44%). Higher prevalence of HIV infection was noted especially in

Forms of hemophilia	Uninfected		Infected			
			1 agent		2 agents	
Hemophilia A	**Nº**	**%**	**Nº**	**%**	**Nº**	**%**
<1%	10	35,7	5	27,8	0	
1-5%	6	21,4	6	33,3	0	
>5%	12	42,9	7	38,9	2	
Total (48)	28	100	18	100	2	
100%		58,3		37,5		4,2
Hemophilia B	**Nº**	**%**	**Nº**	**%**	**Nº**	**0**
<1%	6	33,3	4	50	0	
1-5%	9	50	1	12,5	1	3,7
>5%	3	16,7	3	37,5	0	
Total (27)	18	100	8	100	1	3,7
100%		**66,6**		**29,6**		**3,7**
Total Hemophilia patients (75)	**46**	**61,3**	**26**	**34,7**	**3**	**4**

TABLE 4. Presentation of uninfected and infected hemophiliacs with one or two infective agents.

Viral infections		H C V			HBV	HIV	TPH
		Pos	**Neg**	**Total**	**Neg**	**Neg**	**Neg**
Hemophilia A No (21) (70%)	**Nº**	2	19	21	calc	21	21
	%	9,5	90,5	100	100	100	100
	XB age	14,5	9,21	9,7	9,7	9,7	9,7
	SD	4,95	4,7	4,9	4,9	4,9	4,9
Hemophilia B No (9) (30%)	**Nº**	0	9	9	0	0	0
	%	0	100	100	0	0	0
	XB age	0	11,3	11,3	0	0	0
	SD	0	4,7	4,7	0	0	0
Total No. of hemophiliacs 30							
p = 0,4425							

TABLE 5. Viral infectious complications at boys with hemophilia < or = 18 years of age.

hemophilia patients treated before 1985. HIV seropositivity prevalence in such patients was up to 80% (4). The results for hepatitis C were similar to results published by Goedert et al., whose studies conducted in a dozen of Hemophilia centres during the period 2001-2003 found HCV seroprevalence in 30% of hemophiliacs (38). Other studies suggest higher prevalence ranging 43,9%~84% (33, 35, 39, 40, 41, 42). The prevalence at patients treated before 1985 when blood screening for HCV first began is the highest, ranging from 92% to 100% (13, 43). The prevalence of hemophilia patients infected with HBV (2,7%) is at the lower level of the range published by other authors (0,3%-8,86%) (18, 30). None of the subjects in this study resulted seropositive for *T. pallidum*, while the other authors reported an incidence of 4,9% (20).

Hepatitis C prevalence is closely associated with the frequency of exogenous FVIII and FIX concentrates administered. Therefore, an increase in the number of doses directly increases the residual risk of acquiring an infective agent (34). Such statement was confirmed by Makris et al. on a study done in England; 154 hemophilia patients were taking different amounts of FVIII and FIX concentrates; they confirmed that anti-HCV prevalence was dependent on the number of doses. For example, HCV was found to be in 76% of patients treated with above 10.000 UI of factor concentrates, while HCV infection at patients who took less than 10 000 UI was 46%. Non-virus-inactivated Clotting Factor Concentrates had the probability of containing HCV up to 80%, while preparations undergoing such procedure have the probability of 20%. The presence of viral agents in plasma antihemophilic products depends on whether donations are from volunteer or paid donors. The presence of HCV in plasma products prepared from paid donations may be up to 64% (3). Due to such infectious complications in hemophilia patients, alternative pathways through recombinant clotting factor manufacturing took place. The safety and efficacy of recombinant therapy was confirmed on a study involving 93 hemophiliacs, treated with recombinant clotting factors (26). TTI can be transmitted through cryoprecipitate despite donor screening due to the fact that cryoprecipitate didn't undergo viral inactivation. Studies from several authors confirmed that 23,3% of hemophiliacs contracted HIV, 58,9% HCV, and with lesser extend HBV counting 5,5% (44). In reference to incidence between hemophilia A and B patients, it was found that out of 48 individuals with hemophilia A, 41,7% were seropositive on HCV. Coinfection with HIV and HBV was found on a single case of hemophilia A patients. Other authors published data suggesting higher rates of HIV infection in hemophilia A patients (69,4%) (45). Within hemophilic boys < or =18 years of age, HCV infection was present on 9,5%. None of the individuals within this subgroup was seropositive for HIV or HBV, while other authors published higher rates of TTI; HBV 3,7%: HIV 11,1% and HCV 35,2% (9) Other studies found dissimilar HBV seroprevalence (6,4%) (6), or HCV (6%) (46). Our data corresponds with Kavakli et al. (47) which found no HIV infection in boys with Hemophilia. Although our results found co-infection only in 3 individuals (4%), other author's studies found a higher rate of HIV-HCV coinfection ranging 18,8%-19,2% (36, 48). Another group of authors suggest that the rate of coinfection with HIV and HCV among hemophiliacs ranges between 50-80 % (14, 41), although on specific occasions coinfection rate was up to 99% (23).

Conclusion

Based on our results, despite the insufficient application of FVIII and FIX concentrates, and other antihemophilic preparations used in the treatment of hemophilia patients, the number of infected hemophiliacs infected with blood-transmittable infectious agents is substantially high, especially with hepatitis C virus, found in 38,7% of cases. In spite of that, HBV and HIV infection was very low (2,7% and 1,4 %, respectively), while *T. pallidum* infection was not found.
Improvements in transfusion-transmitted disease risk reduction through nucleic acid amplification assays (PCR), implementation of efficient viral inactivation techniques and the use of recombinant factor concentrates would significantly reduce the infectious complications hemophiliacs face today.

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