



Coinfection of Hepatitis B and C with Human Immunodeficiency Virus in Hemophilia: A Cross Sectional Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TSE, TMMP and LOWR designed the study, performed, data analysis/ data collection for research/ preparation of the manuscript, wrote the first draft of manuscript managed the analyses, the literature searchers and contributed in preparation of the manuscript. Authors ACAS, RDMA, OFS, RLM and JASL managed the analyses, the literature searchers and contributed in preparation of the manuscript. Author DOWR elaborated, designed, coordinated and supervised all stages of research and study. Participated in the final review of the manuscript, statistical and epidemiological analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To identify coinfection by Hepatitis B (HBV), Hepatitis C (HCV) and Human immunodeficiency virus (HIV) in Hemophiliacs.

Methodology: We included 107 hemophiliacs. For the current analysis, age, type, severity of Hemophilia and serum profile for Hepatitis B, C and HIV were included. The serological tests performed for Hepatitis and HIV were carried out using the enzyme-linked immunosorbent assay method (ELISA) and the confirmatory tests for HIV and HCV were done by the Western Blot and Polymerase Chain Reaction (PCR). The results of positive and negative serology were compared using the Mann-Whitney test using the Fundação Hemominas reproducibility map of serological reactions, through the CUT-off of each test.

Study Design: A cross-sectional study was carried out on hemophiliacs to assess the prevalence of infectious diseases transmitted by transfusion.

Place and Duration of Study: Department of Hematology, public service on Coagulopathies at Fundação Hemominas Juiz de Fora Brazil, between January 2008 to December 2018.

Results: The average age was 32.77 years with standard deviation (SD)= 16.8 years. In regard to classification, 57.65% of patients with Hemophilia A were severe, and 57% of patients with Hemophilia B were moderate. Laboratory results demonstrated that 24.3% of the patients were HBV positive, and 40.2% were HCV positive, with 21.42% positive by PCR. The prevalence of HIV positive was 11.2%. In general analysis, 44.82% had at least one type of viral infection and 23.4% presented coinfections. HIV positive patients were all positive for HCV (11.2%) and 7.5% of patients were infected by HIV, HCV and HBV. The coexistence of hepatitis C and HIV was statistically relevant with ($P = .001$), considering the year of birth, most patients with HCV and HIV were born before 1980s ($P = .001$).

Conclusions: Infections by HBV, HCV and HIV are late complications in patients that received blood products before 1990s. Infection by a viral agent and the year of birth has a direct association, due to the standardization and implantation of tests for HIV and HCV in the 80s and 90s.

Keywords: Hemophilia; hepatitis; HIV infection; transfusion; co-infection; treatment.

1. INTRODUCTION

Hemophilia is the most common severe hereditary hemorrhagic disease. There are mainly two types of Hemophilia, A and B, both of which are the result of factor VIII and factor IX protein deficiency or dysfunction, respectively, and are characterized by prolonged and excessive bleeding after minor trauma or sometimes even spontaneously [1,2]. The deficiency of the coagulation factor XI is called Hemophilia C, however, it is considerably rarer, with a higher incidence among the Jewish community [3].

In the 1950s, fresh frozen plasma was first used as a replacement factor for Hemophilia patients, followed by cryoprecipitates in the 1960s. In the 1970s, lyophilized factor VIII was derived from plasma and has brought a huge change in the treatment of Hemophilia patients, it allowed them to get home infused therapy [2,4,5]. However, in the 1980s, many Hemophiliac patients were affected by contaminated factor concentrates, as 60 to 70% got infected with Human

immunodeficiency virus (HIV) and almost 100% got infected with Hepatitis C. This tragedy prompted more research aiming to make the plasma-derived factor concentrate safer. Cloning of the gene for factor VIII occurred in 1984 and recombinant factor VIII concentrate became available in 1992. Even though, plasma factor concentrates are available, about 75% of patients with Hemophilia worldwide receive recombinant factor VIII products, as they are much safer of contamination by Hepatitis B and C, HIV and HTLV. The recombinant factor VIII, together with viral inactivation and the best screening technology, made factor products safer and revolutionized the Hemophilia treatment [6,7].

About 80% of adult hemophiliacs develop antibodies against surface antigen of hepatitis B virus (HBsAg). In 77% of aged patients with Hemophilia A and 42% with Hemophilia B 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, compared to 14% of patients treated with cryoprecipitate. 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. 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% [6-8].

New treatments not derived from human plasma, such as Emicizumab, a bispecific monoclonal antibody that bridges activated factor IX and factor X to replace the function of the not activated factor VIII, restoring hemostasis, were recently developed and launched by the pharmaceutical market for the treatment of Hemophilia comorbidities [9,10]. Another therapeutic option is Fitusiran, which targets antithrombin (AT) in the liver and interferes with its translation by binding and degrading mRNA-AT, which silences the expression of the gene. Thus, providing hemostasis and reduced annual bleeding rate in patients with Hemophilia A and B, with and without inhibitors [11].

The importance of studying blood-borne infections in hemophilia is due to the improvement in treatment with increased survival and, consequently, an increase in the chronic complications determined by the use of clotting factors before the 1980s.

2. METHODOLOGY

A cross-sectional study was made, in which a group of 120 patients with hemophilia were observed at Fundação Hemoninas between 2008 and 2018. All patients registered in the period mentioned with severe or moderate Hemophilia A or B were included in the study. Exclusion criteria were: three patients with mild hemophilia (factor VIII or IX dosage above 30%), considering that these patients rarely present bleeding and use a coagulation factor, three with other coagulopathies, one registered outside the study period, and six Hemophiliacs without completed serologies. The analyzed variables were: gender, age, type of Hemophilia, clinical severity and the

serologic screening through the annual results according to the Coagulopathy Attending Protocol. The serological analysis were: Hepatitis B surface antigen (HbsAg); Hepatitis B core antibody (Anti-HBV); Hepatitis C antibody (Anti-HCV); Human immunodeficiency virus antibody (Anti-HIV 1+2), these assays used the of enzyme-linked immunosorbent assay method (ELISA), an immunoenzymatic test that allows the detection of criteria in the blood plasma, using an antigen fixed on a plate in which it is incubated or serum in which it is desired to test for the presence of it, afterwards, it comes in contact with a second enzyme-labelled anti-immunoglobulin antibody. The patients with positivity to anti-HCV were submitted to the *Polymerase Chain Reaction* – PCR. The results of positive and negative serology were compared using the Mann-Whitney test using the Fundação Hemoninas reproducibility map of serological reactions, through the CUT-off of each test and the differences between proportions were recognized by Chi-Square test and *P-value* < 0.05 was considered significant. The statistic program was the Statistical Package for Social Sciences version 15.0 (SPSS Incorporation®).

3. RESULTS AND DISCUSSION

Regarding the type of Hemophilia, Fig. 1 shows that, of the 107 selected patients, 83 have Hemophilia A (77.6%) and 24 have Hemophilia B (22.4%). The Fig. 2 shows the correlation between the severity of the disease and the type of Hemophilia, the severe form is prevalent in Hemophilia A (57.65%) and the moderate form was predominant in Hemophilia B (57%). The final population was composed of 107 Hemophiliacs.

Epidemiological data with the final sample after inclusion and exclusion criteria were described in Table 1. The absolute majority of patients were male (99.1%) and the average age was 32,77 years (with the mode of 40-59 years old, and median of 40-59 years old).

Table 1. Epidemiological characters of hemophilic patients attending at FH

Age	N	%
10-21 years	25	23.3
21-39 years	27	25.2
40-59 years	33	30.8
60-69 years	17	15.9
70 years or more	5	4.7

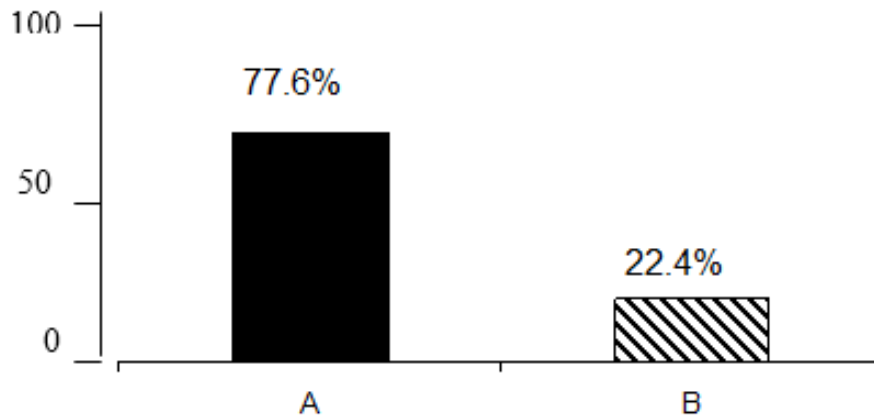


Fig 1. Stratification of patients registered in the FH by type of hemophilia

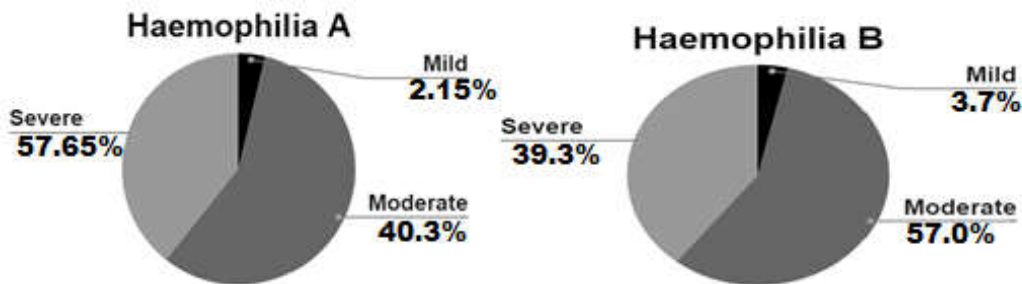


Fig 2. Severity of hemophilia according to type

The serological analysis depicted in Table 2 shows positivity in 24.3% of the patients for anti-HBV, 40.2% for anti-HCV and 11.2% for anti-HIV 1+2. In patients with Hemophilia A, 28.9% were positive for anti-HBV, 48.2% for anti-HCV and 13.3% for anti-HIV 1+2, while in patients with Hemophilia B, 8.3% were positive for anti-HBV, 12.5% for anti-HCV and 4.2% for anti-HIV 1+2. Among the patients with anti-HCV reagents, 20.9% have polymerase chain reaction positive.

Table 2. Serological profile in hemophiliacs registered in FH

Exams	Result			
	Positive		Negative	
	n	%	N	%
HbsAg	-	-	107	100.0
Anti-HBV	26	24.3	81	75.7
Anti-HCV	43	40.2	64	59.8
Anti-HIV 1+2	12	11.2	95	88.8

Legends: Hepatitis B surface antigen (HbsAg); Hepatitis B core antibody (Anti-HBV); Hepatitis C antibody (Anti-HCV); Human immunodeficiency virus antibody (Anti-HIV)

The Venn's diagram below (Fig. 3) indicates that, of the 107 patients we analyzed, 44.82% had at least some type of blood-borne viral infection. The percentage of co-infected patients is 23.4%, and all patients who have anti-HIV 1 + 2 positive are co-infected, 11.2% due to the hepatitis C virus and 7.5% due to the virus hepatitis B. We emphasize that the latter group also corresponds to those that are reactive to the three serological markers obtained. Patients who show reactivity for both anti-HCV and anti-HBV make up 19.6% of all patients.

The analysis shows that individuals with anti-HCV positivity higher risk of coinfection by the Hepatitis B Virus, as well as Human Immunodeficiency Virus, with a prevalence of 46.51% and 25.58%, respectively (Table 3). The positive and negative group were compared with the Mann-Whitney test and the association of HIV and HCV were evaluated by Chi-Square test. The association force was 5.96 (P value =.001). Table 4 shows that time of birth was considered a risk factor to contamination. The majority of patients with serological positivity were born before 1980, with an association force

of 12.0 and P value of 0.001 with Chi-Square test.

Analysis of the treatment received among hemophiliacs with co-infection with HCV and HIV showed that more than 90% of patients received blood components before 1993 (Table 5). The main hemorrhagic manifestation observed in patients was hematoma in 78.5% of them, followed by hemarthrosis (77.4%).

Historically, people with Hemophilia have higher percentages of transfusion-transmitted infections than the general population [12]. In 2007, a study carried out at the University Hospital Ladoke Akintola in Osogbo, with 75 Hemophiliacs, showed that infection by the hepatitis C virus was found in a large portion of the patients, 29 patients or in 38.7% of cases. Infection by hepatitis B virus and the HIV virus was found only in a small number of cases, 2.7% and 1.4%, respectively [12]. The prevalence found in 2007 is similar to the pattern found in this study, which supports the claim that the Hepatitis C virus shows predominance in Hemophiliac patients.

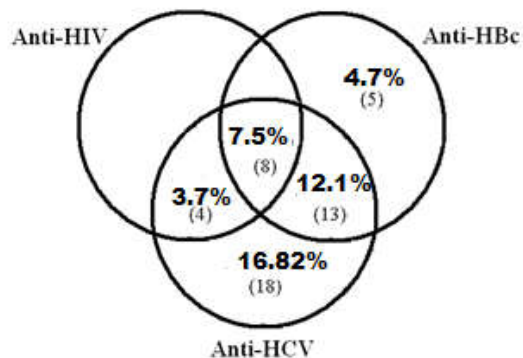


Fig. 3. Diagram correlating co-infections in patients with hemophilia

Legends: Hepatitis B core antibody (Anti-HBV); Hepatitis C antibody (Anti-HCV); Human Immunodeficiency virus antibody (Anti-HIV)

Another study in agreement, carried out at the Fundação Hemominas, Juiz de Fora, Brazil, showed an evaluation made with 39 patients, 33 had Hemophilia A and 6 had Hemophilia B. In eight cases (20.5%) Hemophilia was classified as mild, in 16 (41%) as moderate and in 15 cases (38.5%) as severe [13].

This study revealed a predominance of patients with Hemophilia A (77.5%), with ages ranging from 10 to 77 years old, with the main age group between 40 to 59 years old. There is a majority of severe forms (48.47%) and males (99.06%),

there was only one woman with Hemophilia B. In the work of Martins et al. (2004), carried out in Goiás, Brazil, the sample size was similar, with the following profile: a population of 102 patients, in which 97.1% of the cases analyzed of patients with hemophilia A the ages varied from 2 to 53 and all were male. As for severity, 36.3% of them presented a severe form of Hemophilia [14]. Carapeba (2006), in the other hand, analyzed 113 patients registered in the Hemomat at State of Mato Grosso, Brazil, with ages varying from 0 to 63 (mean of 23 years). In this study, in discrepancy from what was found in FH, the predominant age group was from 5 to 14 years [15]. In the work by Fontes et al. (2003), 664 patients from the entire state of Rio de Janeiro, Brazil, 85.48% have hemophilia A, while 14.51% have Hemophilia B [15,16,17].

Ege University, Turkey, conducted a study with 156 patients with Hemophilia from 2016 to 2018 in Kosovo. The proportion of patients with Hemophilia A is 79.4% and Hemophilia B is 20.6%. None had HIV, Hepatitis B was detected in 3 (1.92%), for which 2 were treated with entecavir, Hepatitis C in 17 (10.8%) patients for which with sofosbuvir. Two patients had liver cirrhosis due to Hepatitis C [18].

The infections in the Hemophilic population caused by transfusion are expensive for the public service and brings harm to the quality of life of these patients. In Canada, it was found that the presence of Hepatitis B or C or HIV can affect negatively the quality of life [19]. The average cost of treatment per year in the US is from \$300,000 to \$1,500,000, while in Europe it is from 77,000 to 112,000 euros, patients suffering from inhibitors may have to pay 3.3 times more than patients who have no inhibitors [20].

Several experiments have been testing various ways to obtain the protein Factor VIII, however, there are technical obstacles (such as the size of the gene, their low level of mRNA expression, a possible secretion is not enough of the product of this gene) and it is bureaucratic. Picanço et al. (2008) has a project, conducted by the University of São Paulo in Ribeirão Preto Blood Center, Brazil, which used a system of plasmid DNA followed by promoters to stimulate liver production of coagulation factor VIII. The success of this research will enable a considerable reduction of the risks of multiple transfusions [21].

Table 3. Comparison between the existing co-infections in hemophiliacs registered in FH

	Anti HCV				P value
	Positive n=43		Negative n=64		
	n	%	n	%	
Anti-HBV positive	20	46.5	5	7.8	P < .001
Anti-HIV positive	11	25.5	0	0	P < .001

Legends: Hepatitis B core antibody (Anti-HBV); Hepatitis C antibody (Anti-HCV); Human immunodeficiency virus antibody (Anti-HIV)

Table 4. Comparative data of infected and uninfected hemophiliacs, recorded in the FH in relation to the year of birth

Year of Birth	Infected		No infected		P-value
	n	%	n	%	
Before 1980	39	81.2	4	6.7	P < .001
Between 1980 e 1993	9	18.7	13	22.0	P = .67
After 1993	-	-	42	71.2	P < .001
Total	48	100.0	59	100.0	

Another factor that brought countless benefits to the population with hereditary hemorrhagic diseases was the implantation of the CoagulopatíaWeb platform by the Ministry of Health of Brazil (www.coagulopatiaweb.datasus.gov.br). It allows the management of clinical incidents with medication dispensing and provides a better interface for information and for updating medical records. These tools are useful in the planning and strategy of public policies for coagulopathies [22].

Table 5. Treatment received by hemophiliac patients registered before 1993 at the FH

Treatment	n	%
Plasma-derived factor	97	90,7
Cryoprecipitate	8	8,0
Activated prothrombin complex	6	5,6

To ensure patient safety during blood transfusion, serologic evolution is essential. For this to happen, a development and application of more sensitive tests for detecting infectious agents, such as NAT (nucleic acid test), is necessary. The NAT was adopted in Brazil in September of 2010 across the public network, since then, lab Bio-Manguinhos, Fundação Oswaldo Cruz, Brazil filled for the record of the NAT test at the Agência Nacional de Vigilância Sanitária (ANVISA, Brazil) [23].

The Fundação Hemominas implemented in 2019 the Hepatitis zero project. Through it, rapid tests for Hepatitis B and C are performed and patients

with positive serology have the treatment available free of charge [24]. The authors identified a difficulty in patients' adherence in relation to care and follow-up with the multidisciplinary team considering that the population studied, especially those born before 1980, attributed the health institutions to HIV contamination.

4. CONCLUSION

Infections by HBV, HCV and HIV are late complications in patients that received blood products before 1990s. Infection by a viral agent and the year of birth has a direct association, due to the standardization and implantation of tests for HIV and HCV in the 80s and 90s.

CONSENT AND ETHICAL APPROVAL

The authors requested the release of the signature of a free and informed consent term and assent to the ethics committee in research, considering the use of anonymized institutional data that guaranteed safety, secrecy and protection to all research participants. respondents' written consent has been collected and preserved by the author(s). We clarify that all patients received alphanumeric code guaranteeing the anonymity of the individuals during the research. We clarify that the identification of patients would imply an increased risk of breach of confidentiality. The principal investigator managed all the data and the doctor was responsible for the coagulopathy

clinic. The research ethics committee authorized the waiver of signing the term.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The present study is approved and registered with the research ethics committee of the Fundação Hemominas under the number 231.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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