Viral hepatitis, HIV, human herpes virus and Treponema pallidum infection in haemodialysis patients from Kosovo, 2005

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The serological status of hepatitis viruses and other infectious diseases in the 66 dialysed patients of one haemodialysis unit in Kosovo were studied, comparing the data with a large group of blood donors and out-patients. All dialysed patients were hepatitis A virus (HAV) positive. Prevalence of hepatitis B surface antigen (HBsAg), hepatitis B surface antibodies (anti-HBs), and hepatitis B core antibodies (anti-HBc) was 14 of 66, 21% (95% confidence interval (CI): 12-33%), 5 of 66, 8% (95%CI: 5-22%), and 50 of 66, 76% (95%CI: 64-85%), respectively. Antibodies to hepatitis C virus (anti-HCV) prevalence was 57 of 66, 86% (95%CI: 76-94%). No human immunodeficiency virus (HIV) positive case was found. Prevalence of past herpes simplex virus type 2 (HSV-2) infection was 29% (95%CI: 18-41%). Two patients (3%, 95%CI: 0-10%) were positive for *Treponema pallidum* and 18% (95%CI: 10-30%) were human herpesvirus 8 (HHV-8) antibody positive. Four hundred and fifty-two subjects were recruited for comparison. Markers of past HAV infection was associated with haemodialysis (Fisher's exact test p-value=0.037). Dialysed patients were at a higher risk of being HBsAg positive than others: the sex- and age-adjusted odds ratio (OR) was 5.18 (95%CI: 1.87-14.32). Anti-HBc positivity was strongly associated with haemodialysis: the sex- and age-adjusted OR was 6.43 (95%CI: 3.22-12-85). Anti-HCV positivity was 86% and 1% in presence and absence of haemodialysis, respectively. The Fisher's exact test for association proved a strong association between haemodialysis and HCV (p-value<0.0001). The OR for association between haemodialysis and HSV-2 positivity was 3.20 (95%CI: 1.46-7.00). Significant associations were also observed between haemodialysis status and antibodies to *Treponema pallidum* (Fisher's exact test p-value=0.044). In Kosovo, the prevalence of viral hepatitis infection and other viral infections and *Treponema pallidum* among dialysed patients is high, indicating major ongoing nosocomial transmission.

Introduction

The population of Kosovo has suffered substantially after the break-up of the former Yugoslavia in the early 1990ies and the consequent armed conflict in 1999. Recently, the region has acquired a national autonomy, with some limitations of sovereignty and with the support of the European Union [1]. In 2006, the population was estimated at 1.9 million and was one of the youngest in Europe. About 37% lived in poverty; unemployment was estimated at around 40%, with a gross domestic product per capita of 834 EUR in 2006 (468 EUR in 2000) [2]. Health indicators remained among the most unfavourable in the Balkan region. The annual per capita government expenditure on health care was 35 EUR, the lowest in Europe. Kosovo had one of the highest perinatal mortality rates (23 per 1,000 live births) in Europe and the number of physicians per 1,000 inhabitants was 0.94 [3]. The transition to more modern concepts of health care management presented a challenge to health personnel and the population after the war. Currently, the healthcare system consists of primary centers located in each municipality, secondary health care facilities at the regional level (five hospitals), and tertiary health care centers (University of Pristine and a few other specialised institutions).

After the conflict, the number of end-stage renal disease (ESRD) patients progressively increased in Kosovo: from 190 in 1999 to approximately 600 in 2007. The rate of patients in DC treatment in Kosovo is 286 per million, lower than in other Central and Eastern European countries [4]. At the time of our study, patients were treated in six different dialysis centres (DC), with standard twice or three times a week five hour dialysis sessions (10% and 90%, respectively).We examined patients at the DC in Peja hospital which had no special areas dedicated to patients with positive history of hepatitis.

A number of reports have shown that viral hepatitis B (HBV) and viral hepatitis C (HCV) are common among ESRD patients [5-7]. In the dialysis centres of Kosovo and of other Eastern European countries, the prevalence of such infections has been poorly investigated. The few existing studies suggest that the prevalences are higher in patients dialysed in this part of Europe compared with other European countries [8-11]. The aim of this study was to

analyse the prevalence of viral hepatitis and other infections such as HIV, HVS-2, HHV-8 and syphilis in the ESRD patients of the hospital in the Peja region. Furthermore, we wanted to investigate whether the haemodialysis was associated with an elevated risk of infections. Our study was part of a survey carried out in the period 2004-2007 during a training project for healthcare workers at the hospital in the Peja region, supported by the Veneto Regional Health Authority and the Italian Co-operation Agency [12].

Table 1

General characteristics of the 66 haemodialysis patients, compared to 452 non-haemodialysed patients (n=518)

Characteristics of patients		Haemodialysis				Chi-square homogeneity test
		Yes		No		
		N	%	N	%	p-value
Sex	Females	27	41	296	65	<0.01
	Males	39	59	156	35	
Age	18-30	3	4	185	41	<0.01
	30-50	21	32	220	49	
	50+	42	64	47	10	
Domicile	Urban	21	32	173	38	0.31
	Rural	45	68	279	62	
Education	≤8	52	79	105	23	<0.01
	>8	14	21	347	77	
Married	No	8	12	165	63	<0.01
	Yes	58	88	287	37	
Employed	No	42	64	109	24	<0.01
	Yes	24	36	343	76	
Blood transfusion	No	2	3	442	98	<0.01
	Yes	64	97	10	2	
Dialysis (months)	0-24	28	42	-	-	-
	24-48	13	20	-	-	-
	48+	25	38	-	-	-
Pts. always in the same unit	No	5	7	-	-	-
	Yes	61	93	-	-	-
Total		**66**	**100**	**452**	**100**	

Table 2

Seroprevalence of viral hepatitis, HIV, HSV-2, *Treponema pallidum* and HHV-8 of patients in haemodialysis, compared to non-haemodialysed patients

Serology	Haemodialysis				Fisher's exact test p-value	Crude OR (95%CI)	Sex- and age-adjusted OR (95%CI)
	yes		no				
	N	%	N	%			
HAV[1]	66	100	424	94	0.037	NE	NE
HBsAg[2]	14	21	16	3	**<0.0001**	**7.34 (3.39,15.89)**	**5.18 (1.87,14.32)**
HBsAb[3]	5	8	69	15	0.13	0.45 (0.18,1.17)	**0.27 (0.09,0.79)**
HBcAb[4]	50	76	107	24	**<0.0001**	**10.08 (5.51,18.42)**	**6.43 (3.22,12.85)**
HBV vax[5]	2	3	0	0	**0.016**	NE	NE
HDV[6]	1	1	0	0	0.127	NE	NE
HCV[7]	57	86	3	1	**<0.0001**	947.89 (249.39,3602.83)	NE
HIV[8]	0	0	0	0	1	NE	NE
HSV-2[9]	19	29	45	10	**<0.0001**	**3.66 (1.98,6.77)**	**3.2 (1.46,7)**
T. pallidum[10]	2	3	1	0.2	**0.044**	**14.09 (1.26,157.66)**	NE
HHV-8[11]	12	18	-	-	-	-	-

In bold: results significant at an alpha ≤ 0.05. Abbreviations used: OR: odds ratio; NE: not estimable; 1: hepatitis A virus; 2: hepatitis B surface antigen; 3: hepatitis B surface antibody; 4: hepatitis B core antigen: 5: HBV vaccinated subjects; 6: hepatitis delta virus; 7: hepatitis C virus; 8: human immunodeficiency virus: 9: human herpes virus 2; 10: Treponema pallidum; 11: human herpes virus 8

Methods

Field work for this cross-sectional study was conducted from 1 January 2005 to 30 March 2005. The association between the prevalence of viral hepatitis and other infections and the haemodialysis status was assessed by comparing the ESRD patients with a group of blood donors and subjects who had been examined for routine laboratory testing. In addition, the scientific literature was reviewed to compare the HBV and HCV prevalence of patients in DC of different Eastern and Western European countries.

Study population

All 66 ESRD patients treated at the DC of Peja regional hospital were enrolled in the study. Candidate blood donors being screened for donation suitability and individuals (18 years of age and older) who had undergone routine check-ups in two clinics in Peja and whose serum was sent for routine testing, were included in the study as a comparison group. In order to approximately randomise the group, patients screened on Monday, Wednesday and Friday were selected. In the three months of the study period, 285 blood donors and 187 subjects examined in clinics were potentially eligible for comparison. Out of the total number of 472 subjects, 20 refused to be tested or to respond to the questionnaire. The final number of 452 subjects was recruited. Approval from the Kosovo Institute of Public Health, the Regional Health Authorities and the Ethical Committee of the Peja region was obtained and a signed informed consent form from each participant was requested before entering the study.

Table 3

HBsAg prevalence in haemodialysis centres in Western and Eastern European countries. Data on the general population is reported for comparison

Country	General population	Year	Reference	Haemodialysis Centres	Year	Reference
North European countries						
Germany	0.60%	1998	Thierfelder	4.60%	2001	Burdick
UK	< 0.5%	2001	Eurohep	<0.5%	2001	Burdick
South European countries						
Italy	1%	2001	Eurohep	4.30%	2001	Burdick
Spain	1.70%	2001	Solà	3.10%	2001	Burdick
Eastern European countries						
Moldovia	9%	2004	Emiroglu	17%	1999	Covic
Romania	6%	2001	Eurohep	22%	1998	Vladutiu
Bulgaria	5%	2001	Eurohep	-	-	-
Serbia	-	-	-	15%	1999	Djukanovic

Table 4

HCV prevalence in haemodialysis centres in Western and Eastern European countries 1997-2001. Data on the general population is reported for comparison

Country	General population	Year	Reference	Haemodialysis centres	Year	Reference
North European countries						
Germany	0.60%	1999	Esteban	3.80%	2003	Fissell
UK	1%	2001	Bird	2.60%	2003	Fissell
South European countries						
Italy	3.50%	1997	Esteban	20.50%	2003	Fissell
Spain	2.50%	2001	Dominguez	22.90%	2003	Fissell
Eastern European countries						
Moldavia	5%	1997	Covic	75%	1999	Covic
Romania	6%	2001	Esteban	73%	1998	Vladutiu
Bulgaria	3%	2001	Esteban	48%	2008	Atanasova
Poland	2%	2001	Esteban	44%	1999	Jadoul
Hungary	0.50%	2001	Müller	15%	1999	Jadoul
Serbia	-	-	-	23%	1999	Djukanovic

Questionnaire

For all study participants information on socio-demographic characteristics and information related to haemodialysis treatment were collected by local physicians and nurses, interviewing patients using a structured questionnaire. The questionnaire included queries on age, sex, occupation, education, area of residence, partner status, length of dialysis treatment, number of transfusion received and if the patient remained always in the same unit of treatment. The serum was collected for laboratory investigations.

Laboratory investigations

The collected serum was tested for the following hepatitis markers: total anti-HAV (IgG and IgM), HBsAg, anti-HBs, total anti-HBc (IgG and IgM), and anti-HCV using AxSYM microparticle enzyme immunoassay (MEIA) (Abbott Diagnostics, North Chicago IL). HBsAg-positive subjects were tested for antibodies to hepatitis delta virus (anti-HDV IgG) using a commercial enzyme-linked immunosorbent assay test (ELISA) (DiaSorin, Saluggia, Italy). A line immunoassay (LIA) (INNO-LIA HIV I/II Score, Innogenetics N.V., Gent, Belgium) was used for detecting antibodies to HIV type 1 and 2, and samples that were reactive were confirmed with Western blot. To detect anti-HSV-2 antibodies, a commercial HSV-2 specific IgG enzyme immunoassay (EIA) (HSV 2 IgG EIA WELL, Radim, Pomezia, Italy) was used. IgG and IgM antibodies to *Treponema pallidum* were detected by a *Treponema pallidum* recombinant EIA (Syphilis Screening Recombinant EIA WELL, Radim, Pomezia, Italy). HHV-8 serum antibodies were detected by a commercially available ELISA assay (HHV-8 IgG Elisa, Advanced Biotechnologies Incorporated, Columbia, MD, Unites States). All tests were performed according to the manufacturer's instructions at the Istituto Superiore di Sanità Laboratory, Rome, Italy, and partner institutions.

Statistical analysis

Prevalence of viral hepatitis and other infectious diseases in haemodialysis patients was estimated and 95% confidence intervals (CI) calculated. We tested whether viral hepatitis and other infectious diseases were associated with haemodialysis by comparing seroprevalence in dialysis patients to seroprevalence in two comparison groups: blood donors and subjects who had been examined in clinics. At a first stage, association was tested separately in dialysis patients vs. blood donors, and in dialysis patients vs. patients who had been examined in clinics. Provided that the estimates were homogeneous in the two analyses, the two groups were pooled together to form a unique comparison group. To account for data sparseness, association was tested by means of Fisher's exact test. Odds ratios (OR) and 95% CI were calculated using logistic regression models. All statistical analyses were performed using R 2.8.0 [13].

Results

Sixty-six haemodialysis patients were recruited (males: 59%, mean age: 55±14 years). The patient characteristics are reported in Table 1. The duration of haemodialysis treatment ranged from 12 to 264 months (median time 48 months). Concerning the aetiology of ESRD, glomerulonephritis was the first cause (20 cases, 30%), followed by diabetes mellitus (12 cases, 18%), pyelonephritis (9 cases, 14%), hypertension (7 cases, 10%), polycystic kidney diseases (4 cases, 6%), and systemic diseases (2 case, 3%). Aetiology was unknown for 12 cases (19%) of haemodialysis patients.

When comparing the distribution of hepatitis status in ESRD patients with subjects not undergoing haemodialysis, we found consistent results. Here we present results to the comparison between haemodialysis patients and the pooled group of comparison subjects. In total, 452 individuals (males: 35%, mean age: 34±11 years) were recruited for comparison. Participants' characteristics were all heterogeneous between haemodialysis and non-haemodialysis patients, except for the domicile (p-value=0.31) (Table 1).

Serological status of dialysed patients

All ESRD patients were HAV positive indicating previous infection (Table 2). Prevalence of HBsAg, HBsAb, and HBcAb was 14 of 66, 21% (95%CI: 12-33%), 5 of 66, 8% (95%CI: 5-22%), and 50 of 66, 76% (95%CI: 64-85%), respectively. Two patients had been vaccinated for HBV. One male patient in his late forties was the only patient positive for HDV: he was also positive for HAV, HBV (HBcAb) and anti-HCV. HCV prevalence was 57 of 66, 86% (95%CI: 76-94%). Concerning the co-occurrence of HBV and HCV in haemodialysis patients, we observed that 45 (70%, 95%CI: 58-81%) were both HBV (HBcAb) and HCV, 10 (16%, 95%CI: 8-27%) had HCV but no HBV, five (8%, 95%CI: 3-17%) had HBV but no HCV, and four (6%, 95%CI: 2-15%) had none (Fisher's exact test p-value=0.096).

No HIV positive case was found. Prevalence of HSV-2 was 19 of 66, 29% (95%CI: 18-41%). Two patients (3%, 95%CI: 0-10%) were positive for *Treponema pallidum* and 12, 18% (95%CI: 10-30%) were HHV-8 positive.

HAV was associated with the haemodialysis status (Fisher's exact test p-value=0.037). Given that all dialysed patients were HAV positive, the estimation of OR was not possible. ESRD patients were at a higher risk of being HBsAg positive than others: sex- and age-adjusted OR was 5.18 (95%CI: 1.87-14.32). When additionally adjusting for the level of education, employment, marital status, and domicile, the OR increased up to 7.92 (95%CI: 2.31-27-12). HBcAb positivity was strongly associated with haemodialysis: the sex- and age-adjusted OR was 6.43 (95%CI: 3.22-12-85); it increased slightly when further adjusting for education, employment, marital status, and domicile as well to OR 6.9 (95%CI: 3.17-15.03). HCV prevalence was 86% and 1% in presence and absence of haemodialysis treatment, respectively. For ESRD patients and the comparison group an OR could not be calculated. However, the Fisher's exact test for association proved a strong association between haemodialysis and HCV (p-value<0.0001). The OR for association between haemodialysis and HSV-2 positivity was 3.20 (95%CI: 1.46-7.00) when adjusting for sex and age, and rising up to 6.44 (95%CI: 2.40-17.27) when further adjusting for education, employment, marital status, and domicile. Significant associations were also observed between haemodialysis status and *Treponema pallidum* status (Fisher's exact test p-value=0.044). Results of the association study are reported in Table 2.

Prevalence of HBV and HCV in DC of Eastern and Western European countries

Table 3 shows the prevalence of serological markers for HBV in DC of Northwestern European countries, Southwestern European countries and Eastern European countries [8,10,14,15]. Table 4 shows the difference for HCV prevalence among DC in Northwestern European countries (UK, Germany) Southwestern European countries (Italy, Spain) and different Eastern European countries [8-11,14,16].

In the majority of the Eastern countries, the prevalence is over 40%, with more than 70% in Romania and Moldavia. Unlike HBV the HCV prevalence in the general population of Eastern countries is in some cases lower than in Western countries (Table 4) [8, 22-25].

Discussion

The prevalence of viral hepatitis and other agents among ESRD patients in the current study indicates a very high level of endemicity. Twenty-one per cent of patients were found to be HBsAg carriers and more than 78% had been exposed to the virus (anti-HBc positive), with a sex- and age-adjusted six-fold risk when compared to non-haemodialysis patients. In analysing the data in the literature, it was found that approximately 20% of dialysis patients are chronic carriers of HBV in Eastern Europe, compared to approximately 4 % in Western countries. On the other hand, the general population carriage is at least three times higher than in Western Europe (Table 3) [17-20].

Kosovo is a country with a low prevalence of HCV infection [12,21]. This was reflected in the group of non-hemodialysis patients, where the prevalence of HCV was as low as 1%. Nevertheless, the prevalence of HCV in dialysed patients was strikingly high (86%). It was not possible to calculate the OR with the observed numbers. However, the great difference should suggest that, even taking into account potential differences between the two groups compared in this study, hemodialysis should be considered a strong risk factor for HCV infection, in line with the results of other studies carried out in Eastern European countries [8-10,15]. The HCV prevalence in DC in Western versus Eastern countries differs: from around 2% in Germany and the United Kingdom (UK) to 20% in Spain and Italy and up to 50-70% in Eastern European countries [8,15]. In the Dialysis Outcomes and Practice Patterns Study (DOPPS), the mean prevalence of HCV infection in five Western European countries (France, Germany, Italy, Spain and the UK), Japan and the United States (US) was 13% [16].

In Europe, the overall prevalence of HBV and HCV in ESRD patients has been decreasing over the years as a result of HBV vaccination, routine screening of blood products, and the use of recombinant human erythropoietin [6,11,16]. Guidelines for universal precautions - 'Recommendations for preventing transmission of infections among chronic hemodialysis patients' - had been initially recommended by the US Centers for Disease Control and Prevention (US, CDC) in 1985 and successively updated [26]. In Kosovo, erythropoietin started to be used in 2004 but with marked differences between centres. The percentage of haemodialysis patients receiving erythropoietin in Kosovo is, to date, less than 50%. The situation appears to be improving slightly, but precise figures are not available. Screening of blood-donors for blood-borne viruses has only been implemented regularly since 2001. No immunisation policy for hepatitis vaccination existed in general in Kosovo before the war. In Kosovo there is the policy for HBV vaccination of haemodialysis patients and medical staff. The lack of available vaccines hampers its implementation; for example, in 2005 the percentage of vaccinated individuals among the 253 health care workers of the Peja hospital was 16.6% [12]. An important measure for the control of hepatitis infection is the segregation of positive patients and their haemodialysis equipment [27]. Until recently, the lack of resources prevented this practice in Kosovo.

In our study the syphilis prevalence (anti-*Treponema pallidum* IgG) among dialysed patients was 3%, much higher than the 0.2% of non-dialysed subjects. There is little data on syphilis seroprevalence in DC patients. Sexual contact is the primary mode of transmission of syphilis, but blood transfusion, blood contact and accidental inoculations are other modes of infection that place ESRD patients at risk. A report from Taiwan showed a prevalence of syphilis among dialysed patients of 5.6% [28]. In a more recent study, the syphilis seroprevalence in 167 ESRD patients was 6.7%, more than two times higher than the overall prevalence reported in the general population [29].

HHV-8 is a gamma-herpes virus, closely related to the Epstein-Barr virus. We do not have data to compare our study population with the Kosovar general population. Nevertheless, in nearby Albania, HHV-8 seroprevalence in the general population is reported to be 20% [30]. Transmission of HHV-8 infection through blood, although suggested, is controversial. A case-control study performed in 97 dialysed patients from Northern Italy found a prevalence of 9.2% (in this geographic area the prevalence of HHV-8 in blood donors was 12.7%) [31]. In Greece, HHV-8 prevalence in 485 dialysed patients was 7.2% [32]. In Southern Italy, the seroprevalence of HHV-8 among ESRD patients was 27% (comparable to 25% as observed in the general population) [33].

In Kosovo, the prevalence of infection from viral hepatitis,HHV-8, HSV-2 and Treponema pallidum among ESRD patients is high, indicating major ongoing nosocomial transmission. Even though this may be a consequence of limited resources available, targeted recommendations could be implemented to improve the current situation:

- rigorous attention should be paid to infection control procedures such as changing gloves between patients and the decontamination of equipment and surfaces after each patient treatment episode;
- all single-use injectable medications and solutions should used on a single patient, and all parenteral medications should be prepared in a clean area separate from potentially contaminated items and surfaces;
- hepatitis B vaccination should be given to all patients and staff [34];
- HBsAg and HCV positive patients and their dialysis equipment should be segregated;
- periodic diagnostic testing of patients and healthcare workers needs to be carried out;
- dialysis providers should be aware of their responsibility to report clusters of infections to the local health authorities, as the failure to report illness clusters can result in delays in the recognition of disease outbreaks; and
- training for health care workers should be implemented periodically.

Our study has several limitations that have to be emphasised. As the data were restricted to one DC, the results presented here cannot be considered indicative for Kosovo as a whole and figures on serological status of the health personnel are not available. Furthermore data on the incidence of infectious diseases after the regular screening of blood transfusion for blood-borne viruses were implemented (2001) are not available; and information on possible risk factors is also missing. In Kosovo further studies on the prevalence and incidence of blood borne viruses among ESRD patients are needed, involving more than one DC, and exploring possible risk factors in these patients and settings.

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