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VIRAL HEPATITIS

Seroprevalence and genotyping of hepatitis B, hepatitis C and HIV among healthy population and Turkish soldiers in Northern Cyprus

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Abstract

AIM: To compare the prevalence of hepatitis viral markers among soldiers from Turkey, blood donors from Northern Cyprus, and soldiers from Northern Cyprus.

METHODS: Hepatitis B surface antigen (HBsAg), anti-HCV and anti-human immunodeficiency virus (HIV) seroprevalence, HBV DNA, HCV RNA and HCV genotyping among soldiers from Turkey (group Ⅰ), civil blood donors from Northern Cyprus (group Ⅱ), and soldier candidates from Northern Cyprus (group Ⅲ) were studied and compared to one another. In total, 17545 cases (13546 males and 3999 females with a mean age of 34.5 ± 10.3 year, group Ⅰ = 11234, group Ⅱ = 5057, and group Ⅲ = 1254) were included into the study.

RESULTS: Among all cases, HBsAg positivity rates were 2.46%, anti-HCV was 0.46% and anti-HIV was 0.00%. HBV DNA was 2.25%, HCV RNA was 0.33% in all groups. HBsAg positivity rates were 2.16% in group Ⅰ, 3.00% in group Ⅱ and 2.71% in group Ⅲ. There was a significant difference between group Ⅰ and group Ⅱ (χ^2 = 6.11, P = 0.047 < 0.05). Anti-HCV positivity rates were 0.45% in group Ⅰ, 0.45% in group Ⅱ, and 0.56% in group Ⅲ. Genotypes of HCV were 1b and 1a in group Ⅰ, 1b, 1a and 2 in group Ⅱ, and 1b, 1a in group Ⅲ. HBsAg carrier rates were 2.20% in females and 2.53% in males. Anti-HCV prevalence was 0.38% in females and 0.48% in males. HBsAg positivity rates were 2.53% in individuals younger than 50, and 1.47% in older than 50. There was a significant difference between the two groups (χ^2 = 23.48, P = 0.001).

CONCLUSION: Prevalences of HBsAg, HCV and HIV infections in Northern Cyprus population are similar to those of Turkey.

Key words: Cyprus; Donor; Healthy adults; Hepatitis B; Hepatitis C; Human immunodeficiency virus

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INTRODUCTION

Worldwide, two billion people have been infected with hepatitis B virus (HBV), 360 million have chronic infection, and 600 000 die each year from HBV-related liver disease or hepatocellular carcinoma[1]. Meanwhile, hepatitis C virus (HCV) infection is the most common chronic blood borne infection in the World. An estimated 3.9 million persons are currently infected and it is responsible for approximately 8000 to 10000 deaths each year in the United States[2]. Both diseases cause serious mortality, morbidity and financial costs, continuing thus to be a major health problem[3]. In addition, prevalence of human immunodeficiency virus (HIV) is increasing everyday and it has become a disaster for humankind in some areas. Prevalence studies of these blood-borne diseases show certain risky groups and risky attitudes that should be studied further. Northern Cyprus population shares many characteristics with Turkey's population. Turkey is among the moderate endemic countries and rates of HBV carriers are 4.4%-12.5% and of HCV positivity 0.3%-4.0%[4,5]. Acquired immune deficiency syndrome (AIDS) is one of the most important mortality causes in the world although it is not endemic in Turkey and Cyprus[6,7]. There is an increasing risk in recent years in this area of this disease. Seroepidemiological studies about blood-borne diseases could have provided us with the opportunity of learning basic knowledge about

epidemiology of these diseases in the community. The aim of this study was to determine prevalence of HBV, HCV and HIV infections among normal population and Turkish soldiers in Northern Cyprus.

MATERIALS AND METHODS

Study population

Population of Northern Cyprus is about 175 000 and it has close relation with Turkey. Ethical permission was obtained from Afyon Kocatepe University Human Ethics Committee.

Study samples

A cross-sectional study was performed using the data of blood centers of two hospitals (Nalbantoglu General Hospital, Nicosa and Akcicek Military Hospital, Kyrenia) in Northern Cyprus between January 2000 and January 2001. There were three different groups in this study: group Ⅰ, Turkish soldiers who came from Turkey and their results represent Turkish population; Group Ⅱ, civilian blood donors who applied to the hospitals from Northern Cyprus and who were inhabitants there, and donors with any history of jaundice in the past were eliminated from the study; Group Ⅲ, Northern Cyprus inhabitants who applied for soldier recruitment and were screened for these diseases.

Serology

The blood samples were screened for hepatitis serology [Hepatitis B surface antigen (HBsAg), anti-HCV] and anti-HIV. HBsAg, anti-HCV and anti-HIV positivity were investigated by microparticle ELISA (Axsym, Abbott) in blood samples.

HBV and HCV molecular diagnosis

We screened HBV DNA and HCV RNA markers by reverse transcription polymerase chain reaction (RT-PCR) (7700 Sequence Detection System, AB).

HCV genotyping

HCV-RNA sequences were amplified by RT-PCR that targeted the 5' non-coding region of the viral genome and were genotyped by line probe assay using INNO-LIPA HCV Ⅱ kit.

Collection of serum samples

Blood samples were obtained from different groups and stored at -20℃ until use. Prior to RNA extraction, sera were tested on INNO LIA HCV Ab Ⅲ (Innogenetics NV, Antrewp, Belgium) to confirm the presence of anti-HCV antibodies.

HCV RNA extraction

HCV RNA was extracted from serum or EDTA-plasma. To avoid carryover contamination, the steps of the protocol were carried out at four separate locations. Cotton-plugged tips were generally used, and all buffers, primers, and dNTPs were divided into single-use aliquots. Fifty microliters of serum were mixed with 150 μL of Trizol LS Reagent (Life Technologies Gent, Belgium) at room temperature. After lysis and denaturation, 40 μL of $CHCl_3$ was added. The mixture was vigorously shaken, incubated for 15 min at room temperature, and centrifuged, and the minute amounts of viral RNA were precipitated from the colorless aqueous phase with 20 μL of 1 μg Dextran T500 (Pharmacia, Brussels, Belgium) per μL and 100 μL of isopropanol. The RNA pellet was washed with 200 μL of ethanol and collected by centrifugation. Finally, the RNA pellet was briefly air dried. If the procedure was interrupted at this stage, the RNA was stored at -20℃ as a pellet instead of being dissolved in water.

cDNA synthesis and PCR amplification

The RNA pellet was dissolved in 15.1 μL random primers (20 mg/L, pdN_6; Pharmacia). After denaturation at 70℃ for 10 min, 4.9 μL of a cDNA mixture was added which was composed of 4 μL of 5 × avian myeloblastosis virus reverse transcriptase buffer (250 mmol/L Tris HCl [pH 8.5], 100 mmol/L KCl, $MgCl_2$, 25 mmol/L dithiothreitol), 0.4 μL of 25 mmol/L dNTPs, 0.2 μL or 25 U of RNase inhibitor (HPRI; Amersham, Gent, Belgium), and 0.3 μL or 8 U of avian myeloblastosis virus reverse transcriptase (Stratagene, La Jolla, Calif.). cDNA was synthesized at 42℃ for 90 min. PCR was performed in a volume of 50 μL. Other PCR amplified the cDNA over 40 cycles (1 min at 94℃, 1 min at 50℃, and 1 min at 72℃). One microliter of product was amplified with nested PCR primers for another 40 cycles with the same thermal profile. All primers were tagged with a biotin group at the 5' end.

Preparation of LIPA strips

A poly (dT) tail was enzymatically added to the 3' end of each oligonucleotide as previously described[8]. The tailed probes were precipitated and washed with ice-cold ethanol. Probes were dissolved at their respective specific concentrations and applied as a positive control (LIPA line 1). The oligonucleotides were fixed to the membrane by baking at 80℃ for 12 h. The membranes were then sliced into 4-mm-wide strips.

LIPA

Equal volumes (10 μL each) of the biotinylated PCR fragment and the denaturation solution (400 mmol/L NaOH, 10 mmol/L EDTA) were mixed in test troughs by pipetting and incubated at room temperature for 5 min, after which 2 mL of the prewarmed (37℃) hybridization solution (3 × SSC [1 × SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate], 0.1% sodium dodecyl sulfate) was added, followed by the addition of one strip per trough. Hybridization occurred for 1 h at 50℃ ± 0.5℃ in a closed water bath with back-and-forth shaking. The strips were washed twice with 2 mL of wash solution (3 x SSC, 0.1% sodium dodecyl sulfate) at room temperature for 20 s and once at 50℃ for 30 min. Following this stringent washing, strips were rinsed twice with 2 mL of a standard rinse solution. Strips were incubated on a rotating platform with an alkaline phosphatase-labeled streptavidin conjugate diluted in a standard conjugate solution for 30 min at 20℃ to 25℃. Strips were then washed twice with 2 mL of rinse solution and once with standard substrate buffer, and color development was initiated by addition

Table 1 Distribution of HBV and HCV markers in various population

Group	*n*	HBsAg	HBV DNA	Anti-HCV	HCV RNA
		n (%)	*n* (%)	*n* (%)	*n* (%)
Turkish soldiers	11234	243 (2.16)[a]	223 (1.98)	50 (0.45)	35 (0.31)
Civil donors	5057	154 (3.00)	150 (2.90)	23 (0.45)	13 (0.25)
Northern cyprus soldiers	1254	34 (2.71)	23 (1.83)	7 (0.56)	5 (0.39)
Total	17545	431 (2.46)	396 (2.25)	80 (0.46)	53 (0.33)

[a]$P = 0.047$ *vs* the group of civil donors (group Ⅱ).

Table 2 Distribution of HCV genotyping in various population

Group	HCV RNA	Genotype 1b	Genotype 1a	Genotype 2
		n (%)	*n* (%)	*n* (%)
Turkish soldiers	35	34 (97.1)	1 (2.8)	
Civil donors	13	11 (84.6)	1 (7.7)	1 (7.7)
Northern Cyprus soldiers	5	4 (80.0)	1 (20.0)	
Total	53	49 (92.4)	3 (5.7)	1 (1.9)

Table 3 Distribution of HBV and HCV markers by gender

Group	*n*	HBsAg	Anti-HCV	Anti-HIV
		n (%)	*n* (%)	*n* (%)
Female	3999	88 (2.20)	15 (0.38)	0 (0)
Male	13546	343 (2.53)	65 (0.48)	0 (0)
Total	17545	431 (2.46)	80 (0.46)	0 (0)

Table 4 Distribution of HBV, HCV and HIV markers according to age group

Age group (yr)	Anti-HIV	HBsAg	Anti-HCV
	n (%)	*n* (%)	*n* (%)
20-29	10352 (59.0)	222 (2.14)[b]	50 (0.48)
30-39	4532 (25.8)	142 (3.13)[b]	21 (0.46)
40-49	1432 (8.2)	49 (3.42)[b]	5 (0.35)
> 50	1229 (7.0)	18 (1.47)	4 (0.33)
Total	17545 (100.0)	431 (2.46)	80 (0.46)

[b]$P < 0.001$ *vs* the group of older individuals (> 50 yr).

of 5-bromo-4-chloro-3-indolylphosphate (BCIP) and nitroblue tetrazolium to 2 mL of substrate buffer. After 30 min at room temperature, the color reaction was stopped by aspiration of the substrate buffer and addition of distilled water. Immediately after drying, the strips were interpreted[8].

Statistical analysis

The differences between groups were analyzed by the Chi-square test. $P < 0.05$ was taken as significant.

RESULTS

Totally 17 545 individuals, aging 20-56 (mean ± SD, 34.5 ± 10.3) years, of whom 13 546 were men and 3999 were women were included into the study. HBsAg positivity rate was 2.46%, anti-HCV was 0.46%, anti-HIV was 0.00%, HBV DNA was 2.25% and HCV RNA was 0.33% in all groups. HBsAg positivity was the lowest in group I (2.16%) among all groups. There was a significant difference between groups I and Ⅱ for HBsAg positivity rates ($\chi^2 = 6.11$, $P = 0.047$). There were no significant differences between groups for anti-HCV prevalence ($\chi^2 = 0.32$, $P = 0.852$) (Table 1).

Three different genotypes were determined such as 1b, 1a, and 2 genotypes. Number and ratios of 1b, 1a, and 2 types were 49 (92.4%), 3 (5.7%), 1 (1.9%), respectively (Table 2). There was no significant difference in the distribution of HCV genotypes with respect to age, sex and transfusion history ($P > 0.05$).

HBsAg prevalence was higher in males (2.53% *vs* 2.20%). There was no significant difference between the groups for HBsAg prevalence ($\chi^2 = 1.42$, $P = 0.234$). Anti-HCV positivity was higher in males (0.48% *vs* 0.38%). There was no significant difference between two groups for anti-HCV prevalence ($\chi^2 = 0.75$, $P = 0.387$) (Table 3).

Positivity of HBsAg had a close relation with age. The HBsAg carrier rate was highest in the 40-49 year old group (3.42%) and there was a significant difference between older and younger than 50 years groups (2.53% in younger than 50 years, and 1.47% in older; $\chi^2 = 23.48$, $P = 0.001$). Anti-HCV prevalence was higher in younger age groups than in the older age groups, however, there was no significant difference among different age groups for anti-HCV prevalence ($\chi^2 = 0.99$, $P = 0.803$) (Table 4).

DISCUSSION

Epidemiological studies about blood-borne diseases such as hepatitis B, C and HIV are important for revealing the risk groups and risk factors for these infections. Evaluation of the prevalence among blood donors and army recruits is a common and easy method to obtain the epidemiology of these infections in a community. There are some differences between normal population and blood donors or soldiers, however, this approach is very common for screening studies. Screening these groups helps us to solve difficulties in collecting information among healthy populations[9,10].

Northern part of Cyprus has very close relationship with Turkey and both peoples share similar demographic features. At the same time, there is similar close relationship between Southern Cyprus and Greece. Prevalence of HBsAg in Greece has low endemicity level and seropositivity is lower than 1% among volunteer blood donors and military recruits[11,12]. In contrast, HBsAg positivity is reported between 4% and 10% among civil blood donors according to a study including over 10 000 individuals in Turkey[9,10]. Many studies showed that the incidence of HBsAg has a moderate level in Turkey. These data suggest that epidemiology of HBV in Northern

Cyprus population is similar to Turkey. On the other hand, Papaevangelou[13] showed that the carrier rate of HBsAg in the blood donor and army recruit samples ranged between 0.77% and 1.01% in Southern Cyprus. Therefore, Southern Cyprus results are similar to Greece and other south European countries.

At the beginning, our expectation was that the results of Northern Cyprus might have been between Turkey and Southern Cyprus. However, the result has suggested that prevalence of hepatitis B in native population of Cyprus is not lower than in Turkish soldiers. In the last ten years, the intensive efforts on vaccination and prevention precautions might have been effective on prevalence of the disease in Turkey. Our results could be attributed to good infection control and vaccination program against hepatitis B in Turkey. In contrast, there is no effective prevention program against hepatitis B in Northern Cyprus[14].

The incidence of HCV is about 0.01% in North Europe and 1%-1.5% in South Europe[15]. In Turkey, HCV prevalence is between 0.1% and 1.5% in blood donors[9,10,16]. It could be seen easily that HCV prevalence in Turkey and Greece is similar to that of other South European countries. In this study, the incidence of anti-HCV in Northern Cyprus is similar to Turkey and Greece results. In Turkey, prevalence of HCV is increasing in older age.

On the basis of its extensive genetic heterogeneity, HCV has been divided into six major genotypes and at least 100 subtypes. Genotypes 1, 2 and 3 are found throughout the world; but the other genotypes are common in particular geographic regions (genotype 4 is common in North Africa and the Middle East, genotype 5 is common in South Africa, and genotype 6 is common in Southeast Asia)[17]. The predominant genotype in patients with chronic HCV infection in Turkey is genotype 1, followed by genotype 2 and 3[18]. Predominant molecular techniques used (hybridization and direct DNA sequencing) are based on nucleotide differences in the highly conserved 5' UTR among genotypes[17]. The widely used INNO-LIPA HCV Ⅱ assay (Innogenetics, Ghent, Belgium) uses PCR products from the 5' UTR that hybridize to type-specific probes embedded on a nitrocellulose strip[17].

Abacioglu's study showed that the predominant genotype was 1b (75.3%), followed by 1a (19.1%), 2 (3.4%) and 4 (2.2%). In that study, HCV RNA sequences were amplified in the 5' non-coding region and were typed by restriction fragment length polymorphism analysis[19]. These results, together with the results of two previous studies, indicate that HCV genotypes 1, 2, 3 and 4 are prevalent at different frequencies in the Turkish population. In the present study, three different genotypes were determined such as 1b, 1a, and 2 genotypes. There was no significant difference in the distribution of HCV genotypes with respect to groups, age, sex, transfusion history ($P > 0.05$) (Table 2).

In this study, male gender had a higher rate for HBsAg carrier status but the difference was not significant (2.20% in females *vs* 2.54% in males). Koulentaki's[20] study from Greece showed that a greater number of males than females were HBsAg positive (0.41% *vs* 0.28%, respectively). In many studies, males are more frequently exposed to HBV and become carriers more often than females. For HCV, an opposite gender trend was reported that females being infected more frequently than males (0.49% in females *vs* 0.37% in males). In our study, prevalence of HCV was higher in males.

Another agent, which has similar transmission ways to HBV, is HIV. There is a potential hazard for everybody in the world because of the characteristics of the diseases. Although we did not detect any HIV positive case in this study, Cyprus is a sensitive area because of visitors from other countries. There is a young population and many sea workers, and individuals are not well informed about the subject, and drug-users may increase.

In conclusion, seroprevalence of blood-borne disease in Northern Cyprus is similar to Turkey. This study suggests that it is necessary to investigate risk factors and risk groups for these infections in Northern Cyprus. In the light of this result, an effective control and training program for soldiers and civilians should be implemented.

REFERENCES

1 **Shepard CW**, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev* 2006; **28**: 112-125

2 **Gasiorowicz M**, Hurie M, Russell A, Hoxie N, Vergeront J. Epidemiologic trends in infection, mortality, and transplants related to hepatitis C in Wisconsin. *WMJ* 2006; **105**: 34-39

3 **Kane M**. Global programme for control of hepatitis B infection. *Vaccine* 1995; **13** Suppl 1: S47-S49

4 **Demirel Y**, Duran B, Toktamis A, Erden O, Cetin M. Seroprevalence of syphilis, hepatitis B and C, and human immunodeficiency virus infections among women. *Saudi Med J* 2004; **25**: 2037-2038

5 **Ozsoy MF**, Oncul O, Cavuslu S, Erdemoglu A, Emekdas G, Pahsa A. Seroprevalences of hepatitis B and C among health care workers in Turkey. *J Viral Hepat* 2003; **10**: 150-156

6 **Altuglu I**, Sayiner AA, Erensoy S, Zeytinoglu A, Bilgiç A. Screening for human immunodeficiency virus type 1 and 2 in a Turkish blood donor population. *Int J Infect Dis* 1998; **2**: 202-204

7 **Kyriakides T**, Eleftheriou A, Michaelides N, Papantoniou L. Surveillance of HIV in the army of the Republic of Cyprus (SHARC); rationale, design, and implementation of an inexpensive system. *Sex Transm Infect* 2002; **78**: 50-52

8 **Mitchell PS**, Sloan LM, Majewski DW, Rys PN, Heimgartner PJ, Rosenblatt JE, Cockerill FR 3rd, Smith TF, Patel R. Comparison of line probe assay and DNA sequencing of 5′ untranslated region for genotyping hepatitis C virus: description of novel line probe patterns. *Diagn Microbiol Infect Dis* 2002; **42**: 175-179

9 **Gurol E**, Saban C, Oral O, Cigdem A, Armagan A. Trends in hepatitis B and hepatitis C virus among blood donors over 16 years in Turkey. *Eur J Epidemiol* 2006; **21**: 299-305

10 **Gunduz T**, Mumcuoglu I, Güray M. Evaluation of hepatitis B surface antigen, anti-hepatitis C virus and anti-human immunodeficiency virus antibodies and syphilis seropositivity in blood donors: six years' seropositivity. *Int J Clin Pharmacol Res* 2005; **25**: 155-158

11 **Zervou EK**, Dalekos GN, Boumba DS, Tsianos EV. Value of anti-HBc screening of blood donors for prevention of HBV infection: results of a 3-year prospective study in Northwestern Greece. *Transfusion* 2001; **41**: 652-658

12 **Kyriakis KP**, Foudoulaki LE, Papoulia EI, Sofroniadou KE. Seroprevalence of hepatitis B surface antigen (HBsAg) among first-time and sporadic blood donors in Greece: 1991-1996. *Transfus Med* 2000; **10**: 175-180

13 **Papaevangelou G**, Róumeliotou A, Chatziminas M,

Kotsianopoulou M, Ioannou P, Trichopoulou E, Nestoridou A. Epidemiological characteristics of hepatitis B virus infection in Cyprus. *Eur J Epidemiol* 1988; **4**: 150-153

14 **Karaoglu L**, Pehlivan E, Gunes G, Genc M, Tekerekoglu SM, Ercan C, Egri M, Yologlu S. Evaluation of the immune response to hepatitis B vaccination in children aged 1-3 years in Malatya, Turkey. *New Microbiol* 2003; **26**: 311-319

15 **Janot C**, Couroucé AM, Maniez M. Antibodies to hepatitis C virus in French blood donors. *Lancet* 1989; **2**: 796-797

16 **Mutlu B**, Meriç M, Willke A. [Seroprevalence of hepatitis B and C virus, human immunodeficiency virus and syphilis in the blood donors]. *Mikrobiyol Bul* 2004; **38**: 445-448

17 **Richter SS**. Laboratory assays for diagnosis and management of hepatitis C virus infection. *J Clin Microbiol* 2002; **40**: 4407-4412

18 **Selcuk H**, Kanbay M, Korkmaz M, Gur G, Akcay A, Arslan H, Ozdemir N, Yilmaz U, Boyacioglu S. Distribution of HCV genotypes in patients with end-stage renal disease according to type of dialysis treatment. *Dig Dis Sci* 2006; **51**: 1420-1425

19 **Abacioglu YH**, Davidson F, Tuncer S, Yap PL, Ustacelebi S, Yulug N, Simmonds P. The distribution of hepatitis C virus genotypes in Turkish patients. *J Viral Hepat* 1995; **2**: 297-301

20 **Koulentaki M**, Spanoudakis S, Kantidaki E, Drandakis P, Tzagarakis N, Biziagos E, Moschandrea J, Kouroumalis EA. Prevalence of hepatitis B and C markers in volunteer blood donors in Crete. A 5-year study. *J Viral Hepat* 1999; **6**: 243-248

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