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Possible transmission risks and genotype distribution of hepatitis C virus infection in Western Turkey

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Background/aims: *The aim of this study was to investigate the risk factors which may be involved in the transmission of hepatitis C virus and to determine the recent distribution of various genotypes in Western Turkey.* ***Materials and Methods:*** *The risk determination study consisted of 215 patients whose serum samples were sent to the Medical Microbiology Laboratory at Ege University Hospital between 2005 and 2010 and were anti-hepatitis C virus positive. For the determination of recent genotype distribution, genotyping results of all 535 patients sent to the same laboratory from 2007 to 2011 were analyzed. Information on possible risk factors for the transmission of hepatitis C virus was obtained by a telephone questionnaire. Hepatitis C virus typing was performed by restriction fragment length polymorphism analysis.* ***Results:*** *The most frequently reported risk factors were history of dental procedures in 171 (79,5%) patients and surgical operations in 137 (63,7%) patients. Genotype 1 was observed in 499 of the 535 patients (93,3%) with chronic hepatitis C virus infection. Of these, 69 patients showed infection with subtype 1a (12.9.%) and 430 - with subtype 1b (80.4%). Genotype 3 was determined in 20 patients (3,7%), genotype 2 - in 8 patients (1,5), and genotype 4 - in 8 patients (1,5%).* ***Conclusions:*** *Even though there is an increase in non-1 genotypes, Turkish patients with chronic hepatitis C still represent a rather homogenous group with genotypic diversity encountered rarely. The risk factors detected in the patients admitted to our hospital are mainly medical procedures which can be prevented by the use of simple infection control practices and implementation of an education program.*

Key words: Genotype, hepatitis C, risk factors

Türkiye'nin batısında hepatit C virüs infeksiyonunun olası bulaşım yolları ve genotip dağılımı

Giriş ve Amaç: *Bu çalışmanın amacı, bölgemizde, hepatit C virüsü infeksiyonunun olası bulaşım yollarının ve yakın dönemde hepatit C virüsü genotiplerinin dağılımının belirlenmesidir.* ***Gereç ve Yöntem:*** *Hepatit C virüsü risklerinin belirlenmesi amacı ile Ege Üniversitesi Hastanesi Tıbbi Mikrobiyoloji Laboratuvarına, 2005-2010 tarihleri arasında kan örnekleri yollanarak anti-hepatit C virüs pozitif olarak bulunan toplam 215 hasta çalışmaya alınmıştır. Hepatit C virüsü genotip belirleme çalışması için Mart 2007-Mart 2011 tarihleri arasında aynı laboratuvara başvuran 535 hasta örneği çalışmaya alınmıştır. Hepatit C virüsü bulaşında olası risk faktörleri hakkında bilgi, telefon anketi ile toplanmıştır. Hepatit C virüsü genotiplemesi için restriksiyon parça uzunluğu polimorfizmi analizi yapılmıştır.* ***Bulgular:*** *Çalışma grubunda en sık görülen risk faktörlerinin dental girişimler (171; %79,5) ve ameliyat öyküsü (137; %63,7) olduğu belirlendi. 535 hastanın 499'unda (%93.3) genotip 1 saptandı. Genotip 1 saptanan olguların 69'unun genotip 1a (%12,9), 430'unun (80,4%) 1b subtipi olduğu belirlendi. Genotip 3, 20 hastada (%3,7), genotip 2, sekiz hastada (%1,5) ve genotip 4, sekiz hastada (%1,5) saptandı.* ***Sonuç:*** *Genotip 1 dışı olgularda bir artış olmakla beraber bölgemizde genotipik çeşitlilik nadir olarak izlenmektedir. Hastanemize başvuran hastalarda hepatit C virüs infeksiyonu açısından risk faktörlerinin başında dental girişimler ve ameliyat öyküsü bulunmaktadır. Bu risk faktörleri basit infeksiyon kontrol uygulamaları ve eğitim programları ile önlenebilir niteliktedir.*

Anahtar kelimeler: Genotip, hepatit C, risk faktörleri

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INTRODUCTION

Chronic hepatitis C is a global health problem, and according to recent World Health Organization data, the overall prevalence of hepatitis C virus (HCV) infection is estimated to be 2%, with over 123 million people infected worldwide (1). HCV is transmitted by direct percutaneous inoculation or transfusion of blood/blood products, transplantation of tissues or organs from infected donors, or by administration of drugs with a contaminated injector. HCV is less efficiently transmitted by single small dose percutaneous exposures like accidental needlesticks or by mucosal exposures to blood or serum-derived fluids, for example birth to an infected mother, sex with an infected partner (2-5). However, there are regional differences in the extent to which the risk factors contribute to HCV transmission.

HCV shows a considerable genetic heterogeneity among HCV isolates from all over the world. The commonly used classification system proposed by Simmonds et al. is based on heterogeneity and classifies different genotypes with multiple subtypes on the amount of nucleotide variation. Presently, at least six main groups of sequence variants have been characterized, each group containing a number of more closely related subtypes (a, b, c, etc.) (6). Besides its epidemiological use, HCV genotype is important as a predictor of response to therapy and for the decision making on the length of time required for antiviral treatment (7).

Identifying the transmission routes of HCV infection and determining the genotypes in a population are crucial for the development and implementation of effective preventive measures as well as for the establishment of priorities for the development of health strategies. The primary aim of this study was to investigate the risk factors which may be involved in the transmission of HCV in our population. The additional objective of the study was to determine the recent distribution of various genotypes of HCV in patients with chronic HCV infection in Western Turkey.

MATERIALS and METHODS

Subjects

This study included 215 patients whose serum samples were sent to the Clinical Microbiology Laboratory of Ege University Medical Faculty between 2005 and 2010 and were found to be positive for antibodies to hepatitis C (Anti-HCV) (Architect, Abbott, USA) during hospitalization or during follow-up as outpatients. The study population consisted of 109 females and 106 males with a mean age±SD of 52.8±12.6 (range: 22-75) years.

For the determination of recent distribution of various genotypes of HCV in patients, HCV genotyping results of all 535 patients sent to the same laboratory during the period March 2007-March 2011 were analyzed. This study was carried out at the Ege University Hospital, a 2000-bed hospital serving as a reference center (viral confirmation, genotyping) for most of the Western part of Turkey.

Methods

Information on possible risk factors for the transmission of HCV was obtained by a telephone questionnaire. The questionnaire consisted of questions on demographic variables and on various potential parenteral exposures to blood or blood products (such as past hospitalization, surgical operation, injuries requiring hospital interventions, blood or blood product transfusion), hemodialysis, abortion, history of intravenous drug usage (IVDU), intrafamilial transmission, multi-partner sex, manicure/pedicure, common circumcision ritual outside a health facility, history of blood brotherhood ritual, perinatal risk factors as well as dental treatment, tattooing, acupuncture, ear piercing, and having a HCV-infected partner.

HCV Typing

Extraction of RNA was carried out using a commercial system (High Pure Viral Nucleic Acid Kit, Roche Molecular Systems, Branchburg, NJ, USA) and the RNA pellet was reverse-transcribed to complementary DNA (cDNA) using random hexanucleotide mix and avian myeloblastosis virus reverse transcriptase. Nested polymerase chain reaction (PCR) was carried out as described previously by using primers matching conserved regions in the 5'UTR (50 untranslated region). The expected 256-bp length was confirmed by agarose gel electrophoresis (8). PCR-positive samples were typed by restriction fragment length polymorphism (RFLP) analysis using BsuR1-Rsa1, Mva1-Hinf1, and Bsh 12361 (Fermentas International Inc., Canada). After amplification, two aliquots of product DNA were cleaved with BsuR1-Rsa1 and Mva1-Hinf1 as previously described (9). Subtypes 1a/b were distinguished by incubating the mixture using the restriction enzyme Bsh 12361. The DNA fragments were electrophoresed on agarose gel. Different cleavage patterns of the 5' UTR were evaluated according to the scale of McOmish et al. The nomenclature for bands produced by BsuR1-Rsa1 and Mva1-Hinf1 follows that described previously (10).

To confirm the samples with band patterns other than genotype 1, the 5' UTR was amplified and sequenced bidirectionally with internal PCR primers using the Big Dye Terminator DNA Sequencing Kit (Applied Biosystems, CA, USA) and ABI Prism 310 genetic analyzer (Perkin Elmer, USA).

Statistical analysis was performed with the SPSS 18.0 software package (Chicago, IL, USA). Results are expressed as means±SD or as percentages. The chi-square test and Student's t-test were used for data analysis. The significance level was set at a p-value of <0.05. Ethical approval was provided by the Ege University Research Ethics Committee.

RESULTS

The population included in the risk determination study consisted of 102 males and 113 females with a mean age±SD of 52,8±12,6 (range: 22-76) years. The following risk factors were reported: a history of dental procedures in 171 (79,5%) patients; surgical operations (minor or major) in 137 (63.7%) patients, procedures like endoscopy, bronchoscopy etc. in 96 (44,7%) patients, common circumcision ritual in 90 (41,9%) male patients, transfusion of blood and blood products in 76 patients (35,3%), hospitalization in 64 (29,8%) patients, curettage in 63 (29,3%) of the female patients, use of non-disposable sharps like razors in 52 (24,2%) patients, hemodialysis in 22 (10,2%) patients, organ transplantation in 20 (9,3%) patients, HCV-positive household contact in 19 (8,8%) patients, suspected sexual contact in 17 (7,9%) patients, manicure/pedicure in 15 (7%) patients, blood brotherhood ritual in 12 (5,6%) patients, tattoo/acupuncture in 8 (3,7%) patients, and IVDU 3 (1,4%) patients. Eight patients (3,7%) were employed in the healthcare unit. Reported risk factors in HCV transmission is summarized in Table 1.

The number of probable risk factors in the transmission of HCV was as follows: no risk factors could be found in one patient (0,5%), 1 risk factor was determined in 8 patients (3,7%), 2 risk factors were determined in 30 (14%) patients, 3 risk factors were determined in 45 (20,9%), and 4 or more risk factors were determined in 131 (60,9%) patients.

Statistical analysis showed that risk factors such as history of surgical operation and manicure/pedicure were significantly higher in female patients, while risk factors such as use of non-dispo-

Table 1. Reported risk factors in HCV transmission

Risk factor	Number (%)
Dental procedures	171 (79.5%)
Surgical operation	137 (63.7%)
Endoscopy	96 (44.7%)
Common circumcision ritual (men)	90 (41.9%)
Transfusion of blood and blood products	76 (35.3%)
Hospitalization	64(29.8%)
Curettage (women)	63 (29.3%)
Use of non-disposable sharps like razors	52 (24.2%)
Hemodialysis	22 (10.2%)
Transplantation	20 (9.3%)
HCV-positive household contact	19 (8.8%)
Suspected sexual contact	17 (7.9%)
Manicure/pedicure	15 (7%)
Blood brotherhood ritual	12 (5.6%)
Tattoo/acupuncture	8 (3.7%)
Healthcare worker	8 (3.7%)
IVDU	3 (1.4)

IVDU: Intravenous drug usage

sable sharps like razors and having multiple sexual contacts were significantly higher in male patients (p<0,05). The other risk factors were independent of gender.

Analysis of the possible risk factors according to age showed that factors such as history of dental procedures and procedures like endoscopy and bronchoscopy were more frequent among the patients older than 40 years, while organ transplantation and hemodialysis were recorded more frequently in the younger age group (less than 40 years of age).

The population included in the genotype distribution study consisted of 535 patients with a mean age±SD of 51,46±14,5 (range: 9-85) years. Genotype 1 was observed in 499 of the 535 patients (93,3%) with chronic HCV infection. Of these, 69 patients showed infection with subtype 1a (12,9.%) and 430 with subtype 1b (80,4%). Genotype 3 was determined in 20 patients (3,7% of all cases), genotype 2 - in 8 patients (1,5% of all cases), and genotype - 4 in 8 patients (1,5% of all cases). Genotypes 5 and 6 were not found in the study population. The distribution of HCV genotypes by gender and age of the studied population is shown in Table 2.

In 59 patients with determined risk factors, the genotype results were available and the genotype distribution was as follows: genotype 1b was determined in 39 (66,1%) patients, genotype 1a - in 10 patients (16,9%), genotype 2 - in two patients (3,4%), genotype 3 - in seven patients (11,9%), and genotype 4 - in one (1,7%) patient.

DISCUSSION

Turkey is located between Europe and the Middle Eastern countries which differ from each other in the distribution of the major HCV genotypes. Studies on HCV genotypes by different groups in Turkey have found 1b to be the predominant genotype (11-13). In the previous study from our laboratory, the results have indicated that in Western Turkey, the genotype distribution is similar (14). We aimed to gather recent data in our hospital on the predominant genotypes in order to determine if genotype distribution has changed and whether there is any association of genotype with gender and age.

An earlier report from our department performed on 170 samples collected from 1997 to 1999 revealed genotype distribution results as follows: 82% - genotype 1b, 10% - genotype 1a, 2,4% - genotype 2, 0,6% - genotype 3, and 1,2% - genotype 4 (12). In the previous report from our department performed on 345 samples collected between the dates February 2003-February 2007 using the same typing method, the genotype distribution was as follows: 9,9% for genotype 1a, 87,2% for genotype 1b, 0,9% for genotype 2, 1,4% for genotype 3, and 0,6% for genotype 4 (14). In this study, which aimed to gather recent data on the genotypes in order to determine if genotype distribution has changed, genotype 1 was observed in 93,3% of patients with chronic HCV infection. Of these, 12,9% of subjects showed infection with subtype 1a and 80.4% with subtype 1b. Genotype 3 was determined in 3,7%, genotype 2 in 1,5%, and genotype 4 in 1,5% of patients. When the genotype distribution results obtained in this study were compared to those of the previous one individually, a slight difference was observed, which was not statistically significant (p=0,063). On the other hand, when the results were grouped and analyzed as genotype 1 and non-1 types, it was noted that non-1 types significantly increased from 2,9% to 6,7% (p=0,013) for

Table 2. Distribution of HCV genotypes by gender and age in the studied population (n = 535)

	Genotype 1a	Genotype 1b	Genotype 2	Genotype 3	Genotype 4
Number (%)	69 (12.9%)	430 (80.4%)	8 (1.5%)	20 (3.7%)	8 (1.5%)
Gender (%)					
Females (n=294)	33 (11.2%)	245 (83.3%)	5 (1.7%)	6 (2%)	5 (1.7%)
Males (n=241)	36 (14.9%)	185 (76.8%)	3 (1.2%)	14 (5.8%)	3 (1.2%)
Age (years)					
Mean age (SD)	44.7(13.1)	53.2(13.9)	44.3(15.9)	38.1(15.7)	56(16.3)
Female (SD)	45.6(13.6)	53.7(14.0)	43.2(20.8)	27.8(14.5)	52.1(14.7)
Male (SD)	43.7(12.8)	52.6(13.9)	46.0(3.61)	42.5(14.4)	50.7(14.2)

the past four years; however, it is clear that the relatively small number of patients with genotype 2, 3 and 4 makes the statistical analysis difficult to interpret.

In some studies, it was determined that patients infected with genotypes 1b or 2 were older than patients infected with genotypes 1a, genotype 3, or genotype 4 (15,16). Analysis of the genotype distribution according to age showed that patients infected with genotype 1 were significantly older than patients infected with non-1 genotypes (mean age±SD: 52,0±14,1 years vs. 43,4±17,0 years; p=0,001). When genotype distribution (genotype 1 and non-1 genotypes) was investigated in relation to gender, no significant difference was found (chi-square=0,333, p=0,189). Although the calculated p-values indicate statistically significant difference, the relatively small number of patients with non-1 genotypes is again the limitation of this study.

Studies assessing the risk factors in relation to hepatitis C in Turkey are limited. There are temporal and geographical differences in the extent to which these risk factors have contributed to HCV transmission. Identifying the transmission routes of HCV infection in a population is important to develop and implement effective preventive measures as well as to determine the priorities for the establishment of health strategies. The primary aim of this study was to investigate the risk factors which may be involved in the transmission of HCV in our population. In a previous study from Turkey, the obtained results displayed a significantly high ratio regarding surgical operation, blood transfusion, multi-partner sex, dental therapy, and dental extraction when compared to the control group. The frequency of possible risk factors such as IVDU, tattooing, and acupuncture therapies was found quite low (17). In another study from Turkey, the most common risk factor in the study group was history of surgical operation (98%), the second most common risk factor was blood transfusion (40%), followed by dental procedure (27,5%), abortion (21%), and long-term hospitalization (11%) (18). In a case-control study from the Northern part of Turkey, blood transfusion, hospitalization, medical procedures like dental care, curettage, and delivery in a hospital were found to be risk factors for hepatitis C infection (19). When the risk factors which may be involved in the transmission of HCV infection were investigated in the study group, at least one risk factor was found in 99,5% of the patients. In this study, the most common possible risk factors were history of dental procedures in 171 (79,5%) patients, surgical operations (minor or major) in 137 (63,7%) patients, and procedures like endoscopy, bronchoscopy, etc. in 96 (44,7%) patients.

IVDU has been the predominant mode of transmission during the past 40 years in the United States and Australia, and now accounts for most newly acquired infections in many other countries, including those in Western, Northern, and Southern Europe (20,21). In this study, history of intravenous drug abuse was detected less frequently, in only 1,4% of the patients with HCV infection, since IVDU is relatively low compared to the other European countries (22).

Transfusion-associated HCV infection was a worldwide risk before HCV testing became available. It decreased in those countries which had implemented routine HCV testing of donors (23). In this study, history of transfusion of blood products was recorded in 35.3% of the patients which was consistent with the results of the other studies from Turkey (17,18). Most of these cases may be acquired before the implementation date of anti-HCV testing for blood banks, 1996, or there may be other means of transmission since most of the patients had multiple number of risk factors.

Because of the wide variety of human activities that involve the potential for percutaneous exposure to blood or body fluids, there are numerous other transmission modes besides those with clearly-demonstrated epidemiologic associations with infection. These are procedures like tattooing, cultural practices such as ritual scarification, circumcision, acupuncture. In most regions, there are insufficient data to determine whether these risk factors make any important contribution to overall HCV transmission. In those countries where adequate studies have been done, none of these activities have been consistently associated with HCV transmission (24-26). In the present study, the majority of the male patients, 90 (88,2%) of 102, had a history of common circumcision ritual which may also be the transmission route in these patients. On the other hand, another cultural practice, blood brotherhood ritual, was recorded in the minority of the cases (5,6%).

Statistical analysis revealed that risk factors such as history of surgical operation and manicure/pedicure were significantly higher in female patients, while risk factors such as use of non-disposable sharps like razors and having multiple sexual contacts were significantly higher in male patients ($p<0.05$). The other risk factors are independent of gender. Analysis of the possible risk factors according to age showed that risk factors such as history of dental procedures and procedures like endoscopy and bronchoscopy were more frequent among patients older than 40 years of age, while organ transplantation and hemodialysis were recorded more frequently in the younger age group (<40 years). Analysis of the possible risk factors according to genotype showed that genotype 1 was detected in patients with the history of endoscopy, bronchoscopy, etc. ($p=0.01$). No relation was detected with the other risk factors and genotype distribution. Due to the small number of patients infected with non-1 HCV genotypes caution must be used in interpreting these data.

There are some limitations of this study. First, it may be difficult to self-report the inherent like injecting drug use, having multiple partners, etc. so these risk factors may be undervalued. The second limitation is that it was unable to determine the biologic plausibility between HCV and identified factors since the cases were not acute HCV infections.

The focus of primary prevention efforts of HCV infection should be identification and removal of the risk factors. The risk factors detected in the patients admitted to our hospital are mainly medical procedures which can be prevented by the use of simple infection control practices. Following the rules of asepsis and antisepsis during surgical operations can prevent the transmission of HCV infection. Implementation of an education program based on the identified risk factors may reduce the spread of HCV in our area. There is a massive need to educate the general population as well as health professionals including dentists and allied healthcare workers about the preventive measures against HCV infection.

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