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ORIGINAL RESEARCH

Prevalence of Hepatitis C Virus Infection, Genotypes and Subtypes in Migrants from Pakistan in Barcelona, Spain

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Background: Hepatitis C virus (HCV) is a major cause of chronic liver infection with 71 million people infected worldwide. Pakistan has the second highest prevalence of HCV infection and more than half (52%) of Pakistani living in Spain reside in Barcelona. The aim of this study was to analyse the seroprevalence and viraemic rate and determine the genotypes and subtypes of HCV among Pakistanis living in the southern metropolitan area of Barcelona.

Methods: We included all Pakistani patients seeking primary healthcare in the southern metropolitan area of Barcelona from August 2011 to July 2014. Serum samples were screened for HCV antibodies. HCV viral load was determined by reverse transcription polymerase chain reaction and genotypes and subtypes were performed using Versant HCV Genotype and/or deep-sequencing. Screening for hepatitis B virus (HBV) was also carried out.

Results: Among 5877 Pakistani patients, 565 (9.61%) were screened for anti-HCV antibodies, with 68 (12.04%) being positive. The viral load was determined in 65, with 31 presenting active infection and the viraemic rate was 47.69% (95% confidence interval 36.02–59.62). HCV genotyping and subtyping were performed in 24 individuals. Most infections corresponded to HCV genotype 3 (91.67%), and high resolution HCV subtyping was performed in 18 samples, 16 of which presented subtype 3a. One subject presented HBV coinfection with undetectable HBV DNA. During the study period, we identified a possible case of HCV vertical transmission followed by spontaneous viraemia clearance in a chronically infected mother with a C/T IL28B genetic polymorphism.

Conclusion: These results suggest that general HCV screening protocols in patients from high prevalence countries, such as Pakistan, would be helpful to identify and treat active HCV infections. This could avoid further transmission and contribute to building targeted health policies for micro-elimination of HCV infection in specific communities.

Keywords: hepatitis C virus, migrants, HCV genotype, HCV subtype

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver infection, causing almost 400,000 deaths annually.[1] Chronic HCV infections (CHC) are usually asymptomatic for several decades but can lead to cirrhosis and hepatocellular carcinoma (HCC) over time.[7] In 2015, the global prevalence of HCV infection was estimated to be 1% (0.5–2.3%), corresponding to

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71M chronically infected people.[8,9] The highest prevalence rates of HCV infections are found in Egypt (6.3%), followed by Pakistan (3.8%) and Russia (3.3%).[9] In Spain, the prevalence of viraemic HCV infection in 2015 was estimated to be 0.8% (0.3% and 1.2%) decreasing to 0.17% in 2019 and mainly affects males over 40 years old.[9–11] Migrants born in countries with a high prevalence of HCV infection, such as Pakistan, may have a high prevalence of CHC.[12,13] According to the Spanish National Institute of Statistics, between 2011 and 2014, of the 77,000 Pakistanis living in Spain, 52% were residing in the metropolitan area of Barcelona.[14]

HCV is an RNA virus with eight confirmed HCV genotypes and each genotype is subdivided into 86 confirmed subtypes differing by at least 15%–25%.[15,16]

The distribution of HCV genotypes and subtypes around the world is highly variable, being HCV genotype 1 the most globally prevalent (46%), followed by genotype 3 (30%),[15,17,18] which is the most prevalent in Pakistan.[19–21] A novel genotype 7 was identified in the Democratic Republic of Congo[22] and genotype 8 has been detected in Punjab, India.[16]

In 2016, the World Health Organization (WHO) proposed a global plan to reduce the incidence of HCV infection by 80%, mortality by HCV by 65% and to test 90% of targeted populations by 2030[2,3]. The huge scale, complexity and cost of implementation programmes to eliminate HCV could jeopardize the global strategy objectives. However, targeted interventions, such as "micro-elimination" could be successfully adopted[4,5] even in prisons with a test-and-treat strategy.[6]

To develop strategies to control HCV infection and build health policies for micro-elimination of HCV infection,[5,23] updated estimates of the seroprevalence, viraemia, genotypes and subtypes of HCV are highly recommended.[18] Moreover, direct-acting antiviral (DAAs) treatment against HCV infection, its duration, cure rates, and the need for ribavirin, remain partially dependent on the HCV genotype and subtype, at least in high income countries.[7,24]

More than half of Pakistanis living in Spain reside in the metropolitan area of Barcelona and Pakistan has the second highest prevalence of HCV worldwide.[25,26] Thus, Pakistanis in Barcelona could likely contribute to the high number of HCV infections among migrants in our geographical area. Taking this into account, the aim of this study was to determine the seroprevalence, viraemic rate and percentage of HCV genotypes and subtypes among Pakistanis living in the southern metropolitan area of Barcelona.

Materials and Methods

Study Design and Population

A cross-sectional prospective study was performed including Pakistanis attending primary healthcare centres of the Catalan Institute of Health in the southern metropolitan area of Barcelona from August 2011 to July 2014. This area has a population of about 1,200,000 people of which 14.7% are migrants and 4.17% are from Pakistan. All Pakistani patients seeking healthcare and who were asked to undergo HCV testing (anti-HCV, RNA HCV and genotyping) under medical criteria (elevated alanine aminotransferase [ALT], HCV-infected family members or coming from a high prevalence country), were included in the study. High-resolution HCV subtyping was also performed when samples were available. All samples were also tested for hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) status was determined if requested by physicians.

The study was approved by the Ethical Committee of Bellvitge University Hospital (IDIBELL) PR 123/17. Informed consent was obtained from all the subjects included in the study. All methods were performed in accordance with relevant guidelines and regulations outlined in the Declaration of Helsinki.

HCV Screening

Screening of HCV antibodies (HCVAb) was performed using the chemiluminescence Vitros Immunodiagnostic Anti-HCV assay (Vitros® Johnson & Johnson, USA) following the manufacturer's instructions. Samples with a signal/cutoff (S/CO) ratio >1.00 were considered positive.

HCV Viral Load, Genotyping, and High-Resolution HCV Subtyping

The HCV viral load was determined by the Abbott RealTime HCV assay (Abbott Molecular, Des Plaines, IL, USA), and an in vitro reverse transcription polymerase chain reaction (RT-PCR) assay.

Genotyping was performed using the VERSANT HCV Genotype 2.0 assay (LiPA HCV v.2.0; Siemens Healthcare Diagnostics, Eragny, France), and reverse-hybridization technology and subtyping was performed using a high-resolution HCV subtyping method to conclusively identify the HCV subtype and to determine whether there was simultaneously more than one subtype.[27] RT-PCR and nested PCR were performed using the One-Step RT-PCR Transcriptor kit (Roche Applied Science, Basel, Switzerland) and Fast-Start High Fidelity PCR system, dNTPack (Roche Applied Science, Basel, Switzerland), respectively. Purification of amplified products, quality and quantity measurements and final library construction for MiSeq sequencing with the MiSeq reagent kit v3 (Illumina, San Diego, CA) has been reported elsewhere.[28] The raw data generated by MiSeq (fastq) in 2×300 mode were analysed using in-house R scripts, after FLASH software[29] to obtain the final RAS report. The main R[30] libraries used were Biostrings, ShortRead, ape, and stringr.[31–34] Of these alignments, all variants by site or haplotype at 1% or above were included in the analysis. The dominant haplotype is then selected, and all sequences with a pairwise identity above 90% compared to this haplotype are clustered with reference sequences downloaded from Genbank.[15] The dominant haplotype is then used as the representative sequence of the cluster (the centroid) and the centroid frequency as representation of the frequencies' total addition of all clustered haplotypes. This process is done ensuring no haplotypes are left. The result is a set of representative haplotypes (centroids) with their observed population frequencies. The threshold of 90% identity was selected on the basis of the minimum observed distance between pairs of reference sequences of different subtypes.

Hepatitis B Virus Testing

The presence of HBV infection was determined using the VITROS® HBsAg assay (Vitros® Johnson & Johnson, USA) following the manufacturer's instructions. In patients with HBsAg positive results, HBV-DNA PCR was performed, and the viral load was quantified with the Abbott Real Time HBV DNA Assay®.

HIV 1+2 Antibody Testing

IgG antibodies against HIV were measured using the Vitros® Anti-HIV 1+2 Assay according to the manufacturer's instructions.

IL28B Genotyping

DNA was obtained using a Flexigene DNA kit (Qiagen, Hilden, Germany). Second, LightMix Kit (Berlin, Germany) IL28B (TIB MolBio) to define the genotype of the bi-allelic polymorphism rs12979860 (CC, CT, TT). This commercial kit has primers to amplify the 139 bp region and specific probes for the 3176C allele. Since each allele has a specific melting temperature (Tm of 51°C for the T and Tm of 59°C for C alleles), the DNA polymorphism was identified by melting curves using the LightCycler 2.0 (Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

Calculated frequencies were used to describe qualitative variables and the median and interquartile range (IQR) to describe quantitative variables. Estimation of percentages with their respective 95% confidence intervals (CIs) were obtained. The summary statistic was carried out with the statistical package StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: Stata Corp LLC. The HCV viraemic rate was defined as the proportion of anti-HCV positive and HCV RNA positive individuals to total anti-HCV positive individuals.

Results

Characteristics of the Study Population

A total 5877 patients from Pakistan were attended in primary healthcare centres during the study period. Of these, 565 (9.61%) were screened for anti-VHC following medical advice and 68 tested positive. The HCV seropositive population had a median age of 36 years (interquartile range (IQR): 32–44). Women represented 29.4% (n=20) of the population and had a median age of 35 years (IQR 31.25–42) whereas the median age of the men was 37 years (IQR 32–36.5).

HCV RNA detection was performed in 65 samples and 31 individuals were positive, resulting in a viraemic rate of 47.69% (95% CI 36.02–59.62) (Figure 1).

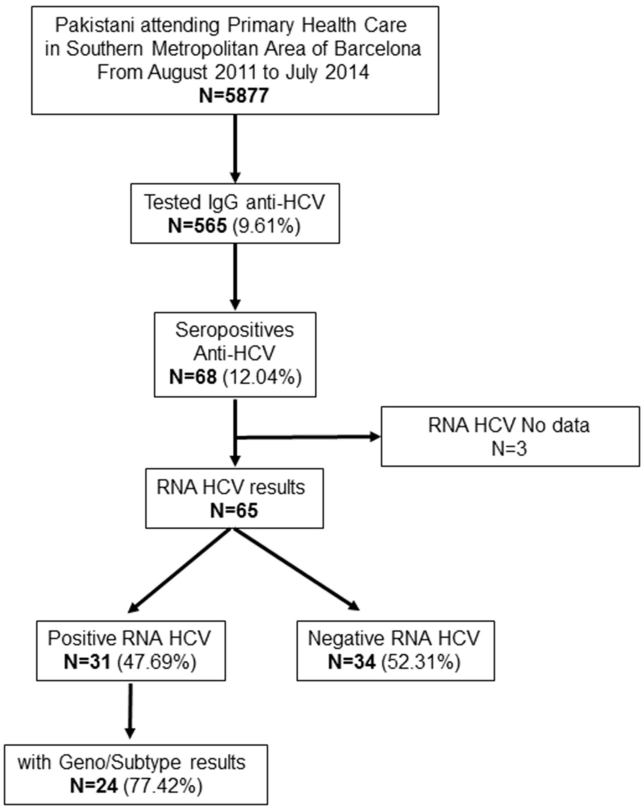

Figure 1 Flowcharts defining the enrolled and tested study population after exclusions, and indicating the number of patients.

HBsAg testing was carried out in all HCV seropositive individuals, with one subject presenting HBV coinfection with undetectable HBV DNA. HIV antibody testing was performed as requested by physicians in 9 patients, and all were negative.

HCV Genotype and Subtyping

HCV genotyping and subtyping was performed in 24 individuals using either Versant HCV Genotype 2.0 HCV and/or deep sequencing. Of these, 16 were classified as subtype 3a, 1 as subtype 3k, 3 as genotype 3, 2 as subtype 4c and 2 as subtype 1a (Figure 2). Three discordant results were obtained when comparing both techniques. In two individuals, Versant HCV Genotype 2.0 classified HCV infection as genotype 4 and were identified as subtypes 3a and 3k and one

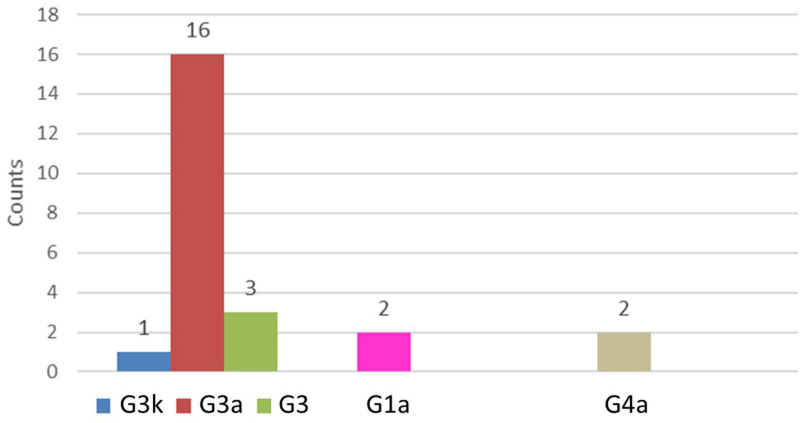

Figure 2 Geno/Subtype analysis of the Pakistani population (n=24) over the 3-year period analysed by Versant HCV Genotype 2.0 and/or next-generation sequencing.

genotype 1a was determined to be 3a. As final results we considered those obtained from deep sequencing. No mixed infections were found.

HCV in Pregnant Women

During the outpatients visits, a possible case of vertical transmission was detected. A mother with chronic HCV infection with a C/T IL28B genetic polymorphism and her newborn were HCV-RNA positive. Both were infected by HCV genotype 3a. After delivery, the mother spontaneously cleared serum HCV RNA without treatment. Unfortunately, we were unable to obtain an HCV-RNA test from the mother to perform a phylogenetic analysis. A third HCV RNA test was carried out showing spontaneous clearance of HCV.

Discussion

This study describes the seroprevalence, viraemic rate, genotypes and subtypes of HCV among the Pakistani population living in Barcelona. According to data from 2018, 39,809 Pakistanis were living in the Barcelona area.[35] Pakistan has the second highest global burden of HCV infection, with 3.8% of the population infected.[36] Hence, the Pakistani population living in Barcelona could likely contribute to the burden of CHC. During the study period, screening of HCV was advised to around 10% of Pakistanis seeking healthcare at the Catalan Institute of Health in the southern metropolitan area of Barcelona.

A recent meta-analysis estimated the prevalence of anti-HCV antibodies among the general population in Pakistan at 6.2%, being 12.8% among populations at intermediate risk (healthcare workers, household contacts of HCV-infected patients, patients with diabetes and prisoners, etc.) and 34.5% among high-risk clinical populations (populations exposed to frequent medical injections and/or blood transfusions, such as haemodialysis, thalassemia, haemophilia and multi-transfused patients, etc.).[12] The prevalence of anti-HCV antibodies found in our population was 12.04%, which is higher than that reported in a recent study among Pakistanis (5.5%) or Pakistani migrants (2.8%) in European Union/European Economic Area countries[37] and still higher than global prevalence of HCV of 6.7% described in a study conducted in 2014.[18]. It is possible that the majority of the Pakistani population living in Barcelona is from an area with a high prevalence of HCV in Pakistan, such as Punjab region, in which the prevalence of anti-HCV antibodies (14.63%) and viraemic rates (49.50%) are similar to those obtained in our study population.[38]

Only one patient presented HBsAg, resulting in a prevalence of HBsAg of 3.2% in viraemic HCV patients. Although the results of only one patient should be considered with caution, our results agree with previous data in Pakistan, reporting a prevalence of HBsAg of 2.5% in the general population according to estimates from 2010[39] and 3.8% (range 1.4—11.0%) in the non-blood donor population and 2.3% (range 1.4—8.4%) in the blood donor population based on estimates from 2008.[40] In addition, knowledge of HBV coinfection before initiating treatment with DAA is important as well as monitoring HBV DNA during treatment as reactivations have been described.[41,42]

In Pakistan, the HCV 3 genotype comprises 79.0% of all HCV infections, with around 5% of all infections being genotype 1a, and 1.6% genotype 4.[9,18,43] Although limited by the sample size, we found that around 92% of all infections were genotype 3. This higher prevalence of genotype 3 compared with the Versant HCV Genotype 2.0 assay might be explained by the usage of a high-resolution HCV subtyping methodology in our study.[27]

A previous study reported that the Versant HCV Genotype 2.0 assay was unable to identify the HCV subtype in 7.7% of HCV genotype 3[44] and was unable to identify the HCV genotype and/or subtype in more than half of the non-genotype 1 samples. Taking into account these findings, discordant results were classified according to deep sequencing subtype analysis. No mixed infection was found, probably because of the reduced sample size.

One possible case of vertical transmission was observed in an HIV negative pregnant woman, who presented spontaneous clearance of HCV viraemia. This phenomenon has previously been described in pregnant women in Egypt,[45] where 26.9% of HCV-infected women spontaneously cleared infection postpartum. Despite the information described in the Egyptian study, which reported that the IL28B-C/C genetic polymorphism was the best predictor for viral clearance, the pregnant woman presenting spontaneous clearance in our study had an IL28B-C/T polymorphism. In Pakistan, spontaneous clearance has recently been described, especially in association with the IL B28 C/T genotype, the same as in our patient.[46]

The WHO global health sector strategy on viral hepatitis encourages the use of strategic information systems sensitive enough to identify specific groups at high risk. It also recommends the gathering of strategic information on affected populations, risk factors and locations to better implement hepatitis control measures in specific populations, country settings and contexts.[47] Depending on the specific context and country epidemiology, priority might be given to certain age groups or high prevalence groups, such migrants or haemodialysis patients. In addition, as vertical transmission is possible, screening of women before pregnancy has been recommended.[48,49]

Additionally, at the 69th World Health Assembly in 2016, the need for better data on migrants health, refugees and displaced persons was highlighted.[50] Effective priority-setting to achieve the 2030 goal of reducing the incidence of HCV by 90% and decreasing in liver-related deaths by 65% will require countries to have adequate health information systems with robust epidemiological data on migration and HCV infection.[51] However, a lack of data on the prevalence of HCV in the European region limits the ability of health systems to monitor disease burden and provide quality services to those most affected. National HCV surveillance systems have not been fully implemented in most of the European region and HCV-specific data on migrant populations are particularly lacking.[51,52]

On one hand, health information systems at the Spanish national and subnational level have unequal migration categorisations, use different variables as proxies for migration status (such as nationality, ethnicity and origin), and frequently lack data on country of birth, making it difficult to compare migrant health data across different communities.[51] In contrast, however, universal access to DAA therapy for all HCV-infected patients, regardless of liver damage, was approved in Spain in June 2017[4] and this measure could reduce the overall prevalence of active HCV infection and the incidence of new infections, similar to what has been reported in other countries.[53,54] Nonetheless, once universal treatment is available, the overall target population for elimination should be screened for HCV. Although recent studies indicate that universal screening should be considered a cost-effective strategy to increase diagnostic rates, it could be more difficult to carry out in the short term.[55] HCV screening programmes can be addressed to specific groups, such as risk groups, generational cohorts, and geographic areas, etc. "Micro-elimination" strategies for which treatment and prevention are easier to implement might be successfully achieved in specific sub-populations,[5] in which it is easier to adapt diagnosis, treatment and surveillance. The populations to target for "micro-elimination" would differ depending on the epidemiology and health context of the country. In Spain, people who use drugs, prison inmates, HIV/HCV co-infected subjects and migrants from countries with a high prevalence of HCV are candidates to micro-elimination target groups.[4–6,49]

The present study has some limitations. Patients seeking healthcare might have different characteristics compared with the general population and the sample size could limit the inference of our conclusions to the general Pakistani migrant population.

Conclusion

The prevalence of anti-HCV positive patients amongst the Pakistani immigrant population in Barcelona is higher (12.04%) than that reported in previous studies probably because of their origin from the Punjab area which has a very high prevalence of HCV. The viraemia rate of almost 50% in the present study was also similar to that described in Punjab. Most individuals assayed, carried the G3a viral subtype. The present results suggest that performing HCV screening in all Pakistanis seeking primary healthcare followed by positive HCV RNA testing would be helpful to identify and treat actively infected patients and avoid further transmission. Here we also describe a putative case of HCV vertical transmission followed by spontaneous viremia clearance after delivery. Our results will contribute to building targeted health policies for micro-elimination of HCV infection in specific communities.

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Disclosure

The authors report no conflicts of interest in this work.

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