Immigration and hepatitis B virus: epidemiological, clinical and therapeutic aspects

E. Palumbo,[1] G. Scotto,[1] D.C. Cibelli,[1] G. Faleo,[1] A. Saracin[1] and G. Angarano[1]

الهجرة والالتهاب الكبدي "بي": الجوانب الوبائية والسريرية والعلاجية

إميليو بالومبو، جيتانو سكوتو، دوناتيلا كونتشيتا تشيبيلّي، جوزيبينا فاليو، أناليزا ساراشينو، جواكشينو أنغارانو

الخلاصة: هدفت هذه الدراسة التي أجريت في إيطاليا إلى تقييم الجوانب الوبائية والسريرية والعلاجية للعدوى بفيروس الالتهاب الكبدي "بي" بين السكان الذين لم تمض على هجرتهم أكثر من ستة أشهر. وقد أجرى الباحثون في المدة ما بين شباط/فبراير 2003 وكانون الأول/ديسمبر 2004، اختبارات على 890 مهاجراً، تبيَّن أن 83 منهم (9.3%) إيجابيون للمستضد السطحي لالتهاب الكبد "بي". وقد كان جميعهم من الرجال، وقَدِمَ 62.6% منهم من أفريقيا، و21.6% منهم من آسيا و16.8% من أوروبا الشرقية. ولاحظ الباحثون ارتفاعاً في مستويات إنزيم ناقلة أمين الألانين ALT لدى نصف المفحوصين تقريباً (54.3% منهم)، إلى جانب مقدار يمكن كشفه من دنا فيروس الالتهاب الكبدي "بي". أما توزيع الأنماط الجينية فكان على الوجه التالي: E (20 حالة)، D (14 حالة) وA (11 حالة). وتوضح هذه الدراسة أهمية التدفق الذي قد يرافق الهجرة بإدخال نمط جيني غير النمط D من فيروسات الالتهاب الكبدي "بي" إلى إيطاليا.

ABSTRACT This study in Italy aimed to evaluate the epidemiological, clinical and therapeutic aspects of hepatitis B virus (HBV) infection in a population of recent (< 6 months) immigrants. Between February 2003 and December 2004, 83 (9.3%) out of 890 immigrants tested positive for hepatitis B surface antigen. All were men and 62.6% came from Africa, 21.6% from Asia and 16.8% from Eastern Europe. About half (54.3%) of the patients had elevated alanine aminotransferase levels and detectable serum HBV DNA. Genotype distribution was as follows: E (20 cases), D (14 cases) and A (11 cases). Our study underscores the potential of migratory flow to introduce genotype non-D hepatitis B virus into our country.

Immigration et virus de l'hépatite B : aspects épidémiologiques, cliniques et thérapeutiques

RÉSUMÉ Cette étude menée en Italie avait pour but d'évaluer les aspects épidémiologiques, cliniques et thérapeutiques de l'infection par le virus de l'hépatite B (VHB) dans une population d'immigrants de fraîche date (< 6 mois). De février 2003 à décembre 2004, 83 (9,3 %) des 890 immigrants ont été testés positifs pour l'antigène de surface de l'hépatite B. Tous étaient des hommes et 62,6 % d'entre eux venaient d'Afrique, 21,6 % d'Asie et 16,8 % d'Europe de l'Est. Chez environ la moitié des patients (54,3 %), le taux d'alanine aminotransférase était élevé et l'ADN du VHB sérique était détectable. La répartition du génotype était la suivante : E (20 cas), D (14 cas) et A (11 cas). Notre étude souligne la possibilité que les flux migratoires introduisent un génotype du virus de l'hépatite B autre que D dans notre pays.

[1]*Clinic of Infectious Diseases, University of Foggia, Foggia, Italy (Correspondence to E. Palumbo: emipalu2003@yahoo.it).*

Received: 20/11/05; accepted: 27/03/06

Introduction

Hepatitis B virus (HBV) infection is an important public health problem and more than 400 million of the world's population are chronic carriers of the virus [*1,2*]. Some regions are characterized by a prevalence of infection > 8%, including the major part of sub-Saharan Africa, South-East Asia, some regions in central South America and some European countries, such as Albania and Turkey. Italy is characterized by a low prevalence of HBV (< 2%) [*3*], but immigration could be leading to the introduction into our area of people infected by HBV, with a consequent increase in prevalence.

HBV is characterized by a genetic heterogeneity and 8 genotypes (A to H) can be classified based on comparison of complete HBV genomes and according to the criterion of ≥ 8% differences in the complete nucleotide sequence of the viral genome [*4–6*]. HBV genotypes have a characteristic geographic distribution. Genotype A is widely distributed in North West Europe, North America and Central Africa, while genotypes B and C are present in Asia only; genotype D has been found worldwide with its highest prevalence in the Mediterranean area, the Middle East and South Asia, particularly India. Genotype E is found in sub-Saharan Africa and genotype F in South and Central America. Genotype G has been found in France and in the United States of America, while the newly discovered genotype H seems so far to be restricted to the northern part of Latin America, including Central America and Mexico [*5*].

Some studies have recently suggested that the genotype affects the clinical features of HBV infection and the response to antiviral treatments. In fact, with regard to genotypes B and C, which are prevalent in Asia, genotype C has been shown to be more frequently found in severe liver disease and in hepatocellular carcinoma and it has a lower response rate to interferon alpha therapy, while genotype B is associated with faster hepatitis Be antigen (HbeAg) and antibody to HbeAg (anti-HBe) seroconversion [*7–12*]. During treatment with lamivudine, YMDD variants [(Y: tyrosine; M: methionine; D: aspartic acid; D: aspartic acid] seem to appear more frequently in genotype A patients than genotype D but appear earlier in genotype D [*13*].

In Italy the prevalent genotype is D; in fact about 95% of Italian patients with HBV infection present with this genotype [*3*]. The migratory flow towards our country has increased in the last 10 years, particularly from endemic areas for HBV (prevalence > 8%) such as sub-Saharan Africa, and one consequence is the possible introduction into our country of genotype non-D HBV infections. This is important both from the epidemiological and clinical point of view, since other genotypes may have a peculiar natural history and response to antiviral treatments.

The aims of the present study were to evaluate the epidemiological, clinical and therapeutic aspects of HBV infection in a population of recent (< 6 months) immigrants and in patients affected by active chronic hepatitis HBV-related infections to determine the HBV genotype.

Methods

Data collection

Between February 2003 to December 2004 a total of 890 immigrants were tested for HBsAg. All gave written informed consent and all participants tested were temporary guests in a camp for refugees, without contact with the indigenous population. The

criteria for inclusion were: age > 14 years and presence in Italy for a period < 6 months. All subjects were tested in the medical ambulatory clinic in the camp for refugees and the blood samples were transported to the department's virology laboratory where they were tested for HBsAg.

In HBsAg-positive patients the biochemical and virological activity of infection and the eventual presence of coinfections—with hepatitis C and D virus (HCV, HDV) and human immunodeficiency virus (HIV)—were evaluated. In patients with detectable serum HBV, DNA analysis determined the HBV genotype.

All patients positive for HBsAg were invited to fill in a questionnaire regarding risk factors for HBV infection (sexual risk-taking, family history of HBV infection, etc.).

Laboratory analysis

HBsAg was assayed by commercial immunoassay (Abbott-Auszyme Mc, Abbott Laboratories, North Chicago, Illinois). Hepatitis Be antigen (HbeAg) and antibody to HbeAg (anti-HBe) were detected by radioimmunoassay (HbeAg/antiHBe immunoradiometric DiaSorin, Vercelli, Italy). IgM and IgG anti-HDV were tested with commercially available enzyme-linked immunoassay (ELISA) kits (Abbott Diagnostica, Weisbaden-Delkenheim, Germany). The presence of antibodies to HCV (anti-HCV) was determined with the use of a 3rd-generation HCV-ELISA (Ortho Diagnostic System, Raritan, New Jersey, USA) and confirmed by a 3rd-generation recombinant immunoblot assay (RIBA) (Ortho Diagnostic Systems, Raritan, New Jersey, USA). Antibodies to HIV (anti-HIV) were determined by enzyme immunoassay (HIV1/HIV2 EIA, Abbott) and positive results were confirmed by western blot. Serum HBV DNA levels were measured by polymerase chain reaction (PCR-real time) with a detection limit of 100 copies/mL. Serum alanine aminotransferase (ALT) was quantified by ultraviolet enzymatic assay (normal range 0–40 IU/L).

Determination of HBV genotypes

The serum of all patients with detectable serum HBV DNA was stored at –80 ºC then thawed for determination of HBV genotypes. First, HBV DNA was extracted as described by Stuyver et al. [*6*]. The second step was the nucleic acid amplification of the pooled HBV gene domain B and C by means of the PCR, for obtaining sequence information about codons 180, 204 and 207 in the polymerase open reading frame. The extracted DNA was amplified over 2 rounds of PCR using biotinylated PCR primers. An exact copy of the template was produced after 1 cycle of denaturation, annealing and extension.

Because the amount of amplification product is generally not sufficient, a nested (2nd round) PCR was needed. After the 2nd PCR for amplifications, HBV-genotype were determined by a line-probe assay (INNO-LiPA HBV genotyping, Innogenetics NV, Gent, Belgium) as described by Stuyver et al. [*6*].

Statistical analysis

A two-tailed Pearson χ^2 test was used to compare categorical data. Statistical significance was taken as $P < 0.05$. The software used for the statistical analysis was *Epi-info*, version 6.

Results

Table 1 shows the demographic data of the 890 studied subjects. Among the subjects tested, 83 (9.3%) were HBsAg positive. All were men, with a mean age of 23 years

Table 1 **Demographic characteristics of all immigrants tested for hepatitis B surface antigen**

Variable	Tested	Positive
Total (No.)	890	83
Male/female (No.)	758/132	83/0
Mean time in Italy [days (Range)]	72 (4–192)	
Mean age [years (Range)]	24 (15–39)	23 (16–37)
Origin [No. (%)]		
Africa	625 (70.2)	52 (62.7)
Asia	162 (18.2)	18 (21.7)
Eastern Europe	77 (8.7)	13 (15.7)
South America	26 (2.9)	0 (0)

(range 16–37), and 52 (62.7%) came from Africa (20 from Liberia, 15 Sudan, 11 Eritrea and 6 Ethiopia), 18 (21.7%) from Asia (9 from Pakistan and 9 from Bangladesh) and 13 (15.7%) from Eastern Europe (all from Albania). All patients were anti-HBe positive, with IgM and IgG anti-HDV negative.

No subject presented coinfection with HCV and HIV. Serum bilirubin, albumin and prothrombin times were normal in all subjects. No patient was aware of his positivity for HBsAg before our screening. In 27% of cases (all African) the risk factor was sexual, while in the majority of cases the risk factor for HBV infection was unknown. A total of 38/83 (45.8%) patients had normal ALT levels (< 40 IU/L) and undetectable serum HBV DNA (< 100 copies/mL), while 45 (54.2%) patients had ALT levels elevated above the laboratory normal (mean level was 167 IU/L, range 74–387) and serum HBV DNA levels detectable by PCR-real time (mean 972 866 copies/mL, range 22 933–1 697 833).

Genotype distribution was determined in patients with detectable serum HBV DNA. Distribution of HBV genotypes in these patients and correlation with biochemical and virological activity of disease are shown in Table 2. All 45 patients had been in Italy for a mean period of 30 days (range 5–55) and certainly were infected in their country of origin.

A liver biopsy was proposed to all patients affected with active chronic HBV, but written informed consent was obtained

Table 2 **Distribution of hepatitis B virus (HBV) genotypes in 45 patients with detectable serum HBV-DNA and correlation with biochemical and virological activity of disease**

HBV genotype	Immigrants tested		Origin	No.	Mean ALT (IU/L)	Mean HBV DNA (copies/mL)
	No.	%				
E	20	44.4	Liberia	12	178	1 181 900
			Sudan	8		
D	14	31.1	Eritrea	4	167	878 831
			Sudan	4		
			Albania	6		
A	11	24.4	Eritrea	7	156	776 900
			Ethiopia	4		

Statistical analysis showed no significant correlation between biochemical and virological activity of the disease and the different HBV genotypes.
ALT = alanine aminotransferase.

only by 7 patients: 3 were affected by genotype E (all from Eritrea) and 4 affected by genotype D (3 from Albania and 1 from Sudan). At the histological diagnosis the 3 patients with genotype E presented a chronic HBV-related hepatitis (grade 2, stage 2), while among the 4 patients with genotype D, 3 patients were affected by a chronic HBV-related hepatitis (grade 1, stage 1) and 1 patient by cirrhosis (grade 3, stage 4). These 7 patients were treated with lamivudine (100 mg/day, orally): actually, 3 patients (2 affected by genotype D and 1 by genotype E) after 12 months of therapy had a complete response (ALT < 40 UI/L and undetectable serum HBV DNA). Among the other 4 patients, 2 (both of whom were affected by genotype D) had a complete response after the 2nd year of treatment, while 2 patients (affected by genotype D and E) developed YMDD mutants after 14 and 16 months of therapy respectively. It was not possible to treat the other patients affected by active chronic B hepatitis because they moved to other cities in north Italy.

Discussion

Some recent studies have demonstrated that the prevalent infectious diseases in immigrants are HIV, tuberculosis and chronic viral hepatitis, more frequently caused by HBV, particularly in African people coming from sub-Saharan areas [*14–17*]. The principal aim of this study was to evaluate the prevalence of HBV infection in a population of recent immigrants living in Italy for < 6 months. We tested 890 subjects. The majority were from sub-Saharan Africa which reflects the prevalent migratory flow to Italy, characterized by migrants from Africa and, less frequently, from Eastern Europe. Among this group, 83 subjects (9.3%) tes-ted positive for HBsAg; this rate is similar to observations presented in the Italian and international literature [*14,15*]. No cases of coinfection were observed our series.

Many of our cases (about 50%) had normal ALT and undetectable serum HBV DNA. This is not surprising because many studies have shown, particularly in sub-Saharan African populations, that HBV infection in these countries is highly prevalent (> 8%), but many infected subjects (40%–65% in different studies) do not have biochemical and virological symptoms of disease (healthy carriers) [*14,18,19*]. In the majority of our cases the risk factors for HBV infection remained unknown, but in 30%, the patients reported a sexual risk factor, which is frequent in African populations due to high levels of prostitution and to cultural refusal to use condoms. Therefore, our data showed a high prevalence of HBV infection in immigrants, particularly in Africans.

An important result concerned the sex of infected patients; all HBsAg positive subjects were men. This is probably a result of the difficulty in testing females (only 82/556 of tested subjects were females). In fact, in African communities females are afraid to know their eventual diseases, in particular infectious diseases that are considered a cause of social discrimination.

The second aim of the study was to determine the clinical and therapeutic aspects with particular attention to the HBV genotype. Recently, there have been several studies reporting the influence of HBV genotypes on the clinical features and on the response to antiviral treatment (interferon and lamivudine) of patients infected with HBV [*5,7–11*]. Therefore these different genotypes, probably characterized by a different natural history and a different response to therapy, could require a dif-

ferent clinical and therapeutic approach as compared to genotype D. In our study the prevalent genotype was E, evidenced exclusively in sub-Saharan patients. This coincides with the usual geographic distribution of this genotype, which is primarily found in sub-Saharan areas. The 11 patients affected by genotype A came from central Africa (Eritrea and Ethiopia), where this genotype is prevalent.

An indirect demonstration of the potential redistribution of HBV genotypes comes from the analysis of persons infected by genotype D in our series. In fact among the 14 patients infected with genotype D, 6 came from Albania, where this genotype is diffused, while 8 patients came from Central Africa, not usually characterized by the presence of this genotype. This could demonstrate that the global migratory flow in the world can effect a partial modification of the normal geographic distribution of HBV genotype with the distribution of some genotypes in areas where they are not normally found. No case of genotypes B and C was seen because the 10 Asiatic patients who were infected with HBV had undetectable serum HBV-DNA and it was therefore not possible to obtain an HBV genotype. Concerning the biochemical and virological activity of HBV infection, while the mean ALT level was similar among the 3 different genotypes, the patients with genotype E had a mean serum HBV DNA higher than subjects with genotypes A and D, but the statistical analysis did not show a significant difference.

An important factor concerned the difficulty in treating the immigrant patients affected with active chronic HBV; in fact only 7 patients initiated antiviral therapy with lamivudine. This difficulty is connected with the continual movements of these people; the majority of immigrants pass through southern Italian regions and after a short time move to north Italy or to other European countries were work opportunities are better, without the possibility to start or continue treatment. Another important aspect is concerned with the difficulty of treating subjects for a chronic and asymptomatic disease; for many populations, particularly African people, the disease is exclusively an acute and symptomatic event, characterized by symptoms such as fever. For this reason it is very difficult to treat patients who are asymptomatic.

In conclusion, our study shows a moderate prevalence of HBV infection in immigrants to Italy, particularly in people from sub-Saharan Africa, and underscores the potential of migratory flow for the introduction of genotype non-D hepatitis B virus, as well as the difficulty in treating immigrant patients affected by active chronic B hepatitis.

References

1. Chu CH, Liaw YF. Natural history of chronic B virus infection: an immunopathological study. *Journal of gastroenterology and hepatology*, 1997, 12, S218–22.
2. Purcell RH. The discovery of the hepatitis viruses. *Gastroenterology*, 104:955–63.
3. Fattovich G et al. Hepatitis B virus precore/core variation and interferon therapy. *Hepatology*, 1995, 22:1355–62.
4. Arauz-Ruiz P et al. Genotype H: a new Amerindian genotype of hepatitis B virus

revealed in Central America. *Journal of general virology*, 2002, 83:2059–73.

5. Yuen M et al. Epidemiological study of hepatitis B virus genotypes, core promoter and precore mutations of chronic hepatitis B infection in Hong Kong. *Journal of hepatology*, 2004, 41:119–25.
6. Stuyver L et al. A new genotype of hepatitis virus: complete genome and phylogenetic relatedness. *Journal of general virology*, 2000, 81:67–74.
7. Kao JH et al. Hepatitis B genotypes and the response to interferon therapy. Jo*urnal of hepatology*, 2000, 33:998–1002.
8. Sumi H et al. Influence of hepatitis B virus genotype on the progression of chronic type B liver disease. *Hepatology*, 2003, 37:19–26.
9. Yuen MF et al. Significance of hepatitis B genotype in acute exacerbation, HbeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. *Hepatology*, 2003, 37:562–7.
10. Chan HL et al. Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HbeAg-negative chronic hepatitis B virus infection. *American journal of gastroenterology*, 2002, 97:406–12.
11. Ding X et al. Different hepatitis B virus genotype distribution among asymptomatic carriers and patients with liver diseases in Nanning, southern China. *Hepatology research*, 2002, 22:37–44.
12. Tsubota A et al. Genotype may correlate with liver carcinogenesis and tumor characteristics in cirrhotic patients infected with hepatitis B virus subtype adw. *Journal of medical virology*, 2001, 65:257–65.
13. Valdes CA et al. The role of HBV genotype in the emergence of YMDD variants in chronic hepatitis B patients treated with lamivudine. *Journal of hepatology*, 2003, 38(Suppl.1):613.
14. Scotto G et al. Studio epidemiologico multicentrico sulla prevalenza di epatiti virali in immigrati ospedalizzati in Italia nel 2002 [Epidemiological multicentric study on prevalence of hepatitis in hospitalised immigrants in Italy during 2002]. *Annali di igiene*, 2005, 17:11–8.
15. Lopez-Velez R et al. Infectious diseases in immigrants from the perspective of a tropical medicine referral unit. *American journal of tropical medicine and hygiene*, 2003, 69:115–21.
16. Ramos JM et al. Examen de salud en la poblacion inmigrante: prevalencia de infeccion tuberculosa latente, hepatitis B, hepatitis C, infeccion por el VIH y sifilis [Health in the immigrant population: prevalence of latent tuberculosis, hepatitis B, hepatitis C, human immunodeficiency virus and syphilis infection]. *Enfermedades infecciosas y microbiologia clinica*, 2003, 21:540–2.
17. Roberts A et al. Infectious diseases of refugees and immigrants: hookworm. *Journal of the American Academy of Nurse Practitioners*, 2002, 14(5):194.
18. Schwanig M. Migration: public health issues (polio, hepatitis C, hepatitis B, tuberculosis, diphtheria). *Biologicals*, 1997, 25:187–93.
19. Paterson R. Screening immigrants for infectious diseases. Lancet *infectious diseases*, 2003, 3(11):681.