Hepatitis C virus prevalence and serotypes associated with HIV in The Gambia

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ABSTRACT

Hepatitis C virus (HCV) serotypes are important in the epidemiology and pathogenesis of HCV-related disease, but little is known of this connection in West Africa. Co-infection with human immunodeficiency virus (HIV) is associated with significant morbidity and mortality. This study aims to determine the prevalence of HCV and its serotypes associated with HIV in The Gambia. A total of 1500 individuals referred to the Royal Victoria Teaching Hospital for HIV serology between July and December, 2002 were screened for antibodies to HIV and subsequently for HCV, and seropositive samples were typed. This study shows HIV and HCV prevalence of 6.7% and 1.6 %, respectively, with a co-infection rate of 0.6%. Serotype 2 showed the highest prevalence (58.1 %), followed by serotype 1 (19.4%). Prevalence of HCV serotype 3 was 6.5 % and five samples were untypeable. Co-infection of HIV-1 with HCV serotype 1 showed a prevalence of 44.4%, and with HCV serotype 2 of 33.3%. The findings support the evidence to suggest the West African subregion as the origin of HCV serotype 2. It also demonstrates the need for routine HCV screening of HIV-infected persons and blood donations, and calls for further studies to elucidate the sources of the HCV virus.

KEY WORDS: Hepatitis C.
HIV.
Prevalence.
Serotyping.

Introduction

Hepatitis C virus (HCV) serotypes are important in the epidemiology and pathogenesis of HCV-related disease. Several studies have shown that they relate to the source of the infection,[1,2] while others have suggested disparity in the severity of liver disease[3] and response to therapy due to differences in serotype of the HCV infection.[4] This disparity has prompted suggestions that routine serotypic determination of HCV isolates is necessary for the effective management of HCV-infected patients.[5] Furthermore, variations in the epidemiological pattern of HCV transmission have been recognised globally, with suggestions that each requires a specific intervention programme. Thus, our understanding of the serotypic profile of HCV types in each community could facilitate patient management and appropriate intervention programmes. However, knowledge of serotypic characteristics of HCV in most developing countries, including The Gambia, is poor and, where such data are available, often lacks correlation with sources of the infection and pathology.

Unlike HCV, human immunodeficiency virus (HIV) infection has gained endemic status in almost all sub-Saharan African countries, including The Gambia, contributing significantly to the disease burden of the continent.[6] Some studies show that HCV/HIV co-infection are complementary, with significant morbidity and mortality.[4] In HIV/HCV co-infection, HCV serotype may influence the outcome of infection and response to therapy.[4]

This study aims to determine the prevalence of HIV and HCV, its serotypes and their association with HIV as a means of providing a framework for possible intervention, and to contribute to understanding of the natural history of HIV/HCV co-infection in the region.

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Materials and methods

Subjects and sample collection

A total of 1500 people (age range: 11 months to 76 years) consecutively referred to the serology unit of Royal Victoria Teaching Hospital, Banjul, between July and December 2002 for HIV serology were included in the study. All were interviewed and bled following informed consent and approval granted by the Department of State for Health. In the case of minors, the approval of parents or guardians was obtained. Informed consent included follow-up tests where necessary.

Blood samples (5–10 mL) were collected from each participant and linked by name and code number. Samples were separated to obtain serum within eight hours of collection and divided into two sample aliquots. One was stored refrigerated and the other at –70°C.

Serology

Preserved sera were screened for HIV antibodies using the Murex HIV–1, 2, 0 enzyme-linked immunosorbent assay

(ELISA) kit (Murex Biotech, UK) following the manufacturer's instructions. All positive samples were further tested using PEPTI-LAV 1-2 (Sanofi, France) for confirmation of the presence of antibodies to HIV and for differentiation into subtypes following the manufacturer's instructions. Samples reactive with Murex HIV 1, 2, 0 but unreactive with PEPTI-LAV 1-2 were considered non-reactive. Those reactive with Murex HIV 1,2,0 and with PEPTI-LAV 1-2 (either on the HIV-1 band, HIV-2 band or both) were confirmed as having HIV antibodies.

The Ortho HCV Version 3.0 enzyme immunoassay (EIA) test kit (Ortho Clinical Diagnostics, USA) was employed for the detection of HCV antibodies. Screening was carried out in batches on a weekly basis or within 10 days of collection using the refrigerated sample aliquots. The test procedure followed was that recommended by the manufacturer. The Ortho HCV Version 3.0 test is a direct solid-phase EIA and some reports have shown that it has improved sensitivity (99%) and specificity (99.9%) as it includes the core and the non-structural (NS) genome regions of HCV NS3, NS4 and NS5.[7] Positive and negative controls are supplied by the manufacturer. Reactions were read at 492 nm using a spectrophotometer and the absorbance of each well recorded. A summary of non-reactive samples is presented in Table 1.

Statistical analysis

Prevalence rates were determined by percentage while association between HIV and HCV prevalence, age and gender were calculated using χ^2 and Fisher's exact test and differences were considered significant at $P<0.05$.

Results

Demographic characteristics of participants

All participants in the study had no previous knowledge of their HIV or HCV status. Females accounted for 61.9% (928/1500) of participants, and 84.1% (780/928) were aged 13–40 years, while 1.7% (16/928) were 12 years or younger. Only 14.2% (132/928) of the females were aged over 40 years. Males accounted for 38.1% (572/1500) of participants but a comparatively higher percentage were aged over 40 years (25.2%). A significant proportion (72.9%, 417/572) were aged 13–40 years, while only 1.9% (11/572) were 12 years or younger.

Over 70% (693/928) of the female participants were attending antenatal clinic. Other participants included blood donors (30.7%, 460/1500), in-patients and out-patients (19.3%, 290/1500), those attending the family planning clinic (1.9%, 28/1500) or undergoing medical examination for employment and educational purposes (1.9%, 29/1500).

Distribution of HIV antibodies

A summary of the distribution of antibodies according to age and gender is presented in Figure 1. Overall prevalence of HIV was 6.7% (101/1500; 95% confidence interval [CI]: 5.6–8.2). Prevalence of HIV-1 was 4.3% (65/1500; 95% CI: 3.4–5.5), HIV-2 was 1.9% (28/1500; 95% CI: 1.2–2.7) and dual HIV-1/HIV-2 (HIV-D) was 0.5% (8/1500; 95% CI: 0.2–1.0). Mean age of HIV-1-infected females was 26.5 years and males was 35.1 years. For HIV-2, the figures were 28.6 years and 35.3 years, respectively.

Overall HIV prevalence in females was 8.4% (78/928; 95% CI: 6.7–10.4) and in males was 4.0% (23/572; 95% CI: 2.8–6.0). Females aged 13–40 years had an HIV prevalence rate of 9.9% (77/780) while in males the prevalence was 2.6 % (15/572). Among subjects aged over 40 years, HIV prevalence was 5.5% (8/144) and 0.7% (1/132) in the male and female subjects, respectively. Antibody to HIV was detected in only one child (11.1%) aged five years or younger.

HIV-1 prevalence among males was 2.4% (14/572; 95% CI: 1.3–4.1) and a progressive increase was seen with increasing age, which peaked in those aged over 55 years (6.9%; 95% CI: 0.8–22.8). Lower prevalence rates were found in males aged 19 years or younger (2.3 %) and in the 27–40 age group (2.1 %).

HIV-1 prevalence among females was 5.5% (95% CI: 4.1–7.2). This peaked in the 27–33 age group (12.4%; 95% CI: 8.3–17.7), with lower rates in those over 40 years (0.8%). There was a stronger association ($P<0.05$, odds ratio [OR] 2.32, 95% CI: 1.25–4.58) with HIV-1 in females than in males.

HIV-2 prevalence among males was 1.4% (95% CI: 0.6–2.7). This peaked in those aged 55 years and above (6.9%; 95% CI: 0.8–22.8). No HIV-2 infection was detected in those under 20 years or in the 34–54 age group. Among females, HIV-2 prevalence rates were higher (2.2%, 20/928; 95% CI: 1.3–3.3). This peaked in the 27–33 age group (5.7%, 12/209; 95% CI: 3.0–9.8). Risk of infection was higher in females than the males (OR: 1.55, 95% CI: 0.65–4.10).

Prevalence of HIV-D infection among all the subjects sampled was 0.5% (8/1500). Infection with HIV-D was found mainly in those aged 20 years and above, with the peak of infection in those 55 years and over (2.3%, 1/43; 95% CI: 0.1–12.3). Dual infection was only found in one 67-year-old

Table 1. Prevalence of HIV, HCV, HIV and HCV co-infection among study subjects in relation to clinical history or clinic attended.

Clinical history or clinic attended	Number (%) (n=1500)	HIV-positive (%) (n=101)	HCV-positive (%) (n=31)	HIV&HCV-positive (%) (n=9)
Antenatal	693(46.2)	28(4.0)	6(0.9)	2(0.2)
Blood donors	460(30.7)	11(2.4)	5(1.1)	2(0.4)
Family planning	28(1.9)	4(14.3)	0(0.0)	0(0.0)
HCC patients	13(0.9)	4(30.1)	1(7.7)	1(7.7)
Non-HCC cases*	277(18.4)	53(19.1)	18(6.5)	4(1.4)
Others†	29(1.9)	1(3.4)	1(3.4)	0(0.0)

*Includes in-patients and out-patients, and AIDS cases; HCC: hepatocellular carcinoma.

†Mainly persons seeking medical certificates for employment and visa purposes.

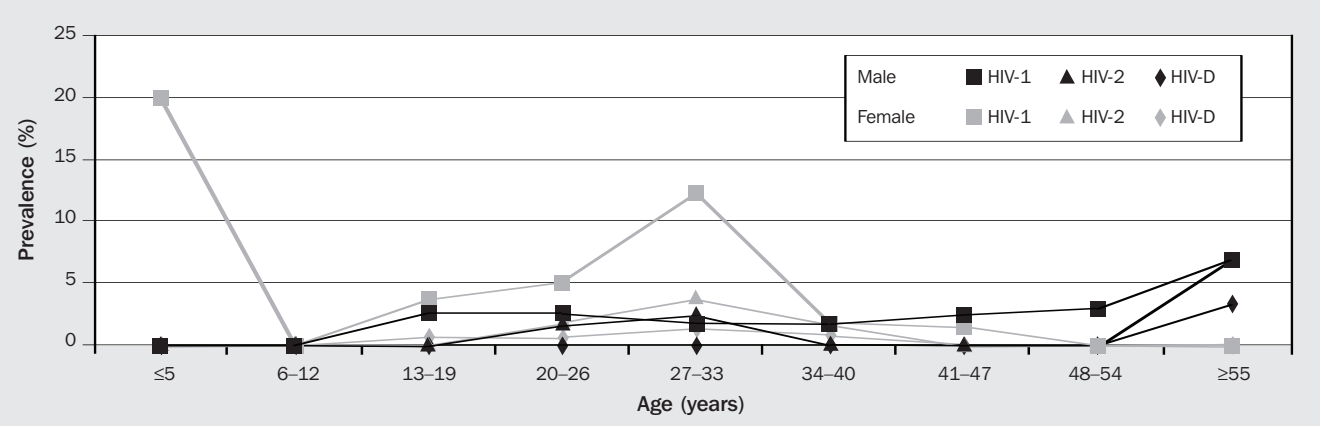

Fig. 1. Prevalence of HIV antibodies among study subjects by age and gender.

man (0.2%), while females accounted for seven of the eight cases of dual infection detected in this study. Infection was mainly found in the 13–40 age group (mean: 24.9 years), and with the peak of infection in the 27–33 age group (1.4%, 3/209; 95% CI: 0.3–4.1).

HCV distribution

Antibodies to HCV were detected in 31 of the individuals screened (2.1%, 95% CI: 1.4–2.9). This peaked in the 41–47 age group (males: 6.0%, 10/431; 95% CI: 2.8–11.1; females: 2.9%, 3/150; 95% CI: 0.4–10.2). Antibodies were detected mainly in those aged 20 years and above.

Males accounted for 71% (22/31) of the HCV infections detected, with a higher prevalence than in females (3.8%, 95% CI: 2.4–5.8 versus 1.0%, 95% CI: 0.4–1.8). No antibody to HCV was detected in females aged 48 years and above. A highly significant association (P=0.0001; OR: 4.08; 95% CI: 1.83–8.55) was found between HCV prevalence and male gender. A summary of the age and gender distribution is presented in Figure 2, while Table 1 shows a summary of the prevalence of HIV, HCV and HIV/HCV co-infection according to clinical condition or clinic attended.

Distribution of HCV serotypes

A summary of the distribution of HCV serotypes is presented in Figure 3. Twenty-six out of 31 (83.9%) samples were successfully typed. Sera from five patients (16.2%) were untypeable.

Serotypes 1 and 3 were found predominantly in those aged less than 40. Serotype 1 showed the highest prevalence (83.3%) in the 20–26 age group. Females in this age group had the highest prevalence of serotype 1 (75%). These differences in age and gender were not statistically significant (P>0.05).

Serotype 2 showed a broader distribution pattern across all age groups, with the highest prevalence in those over 41 years (38.9%, 7/18). Males accounted for 72% of the serotype 2 cases. The lowest prevalence was found in females in the 20–26 age group. Serotype 3 showed a different pattern and lowest prevalence, with distribution limited to males below the age of 40 years. These differences in age and gender were not significant (P>0.05).

Serotypes associated with HIV/HCV co-infection

Distribution of HIV/HCV co-infection according to HCV serotype showed that HCV serotype 1 had the highest prevalence (44.4 %), followed by serotype 2 (33.3%). No co-infection was seen with serotype 3. The four cases of HIV-1/HCV serotype 1 co-infection were found in three males (aged 24, 26 and 34 years) and a 27-year-old female. HIV-1/HCV serotype 2 co-infection was found in three males (aged 24, 41 and 48 years). HIV-2/HCV serotype 2 co-infection was found in a 36-year-old female, and one case of HIV-2/non-typeable HCV co-infection was detected in a 56-year-old male.

Discussion

In The Gambia, HIV prevalence has remained stable for a considerable number of years so the prevalence of 6.7% found in the present study may be considered to be high

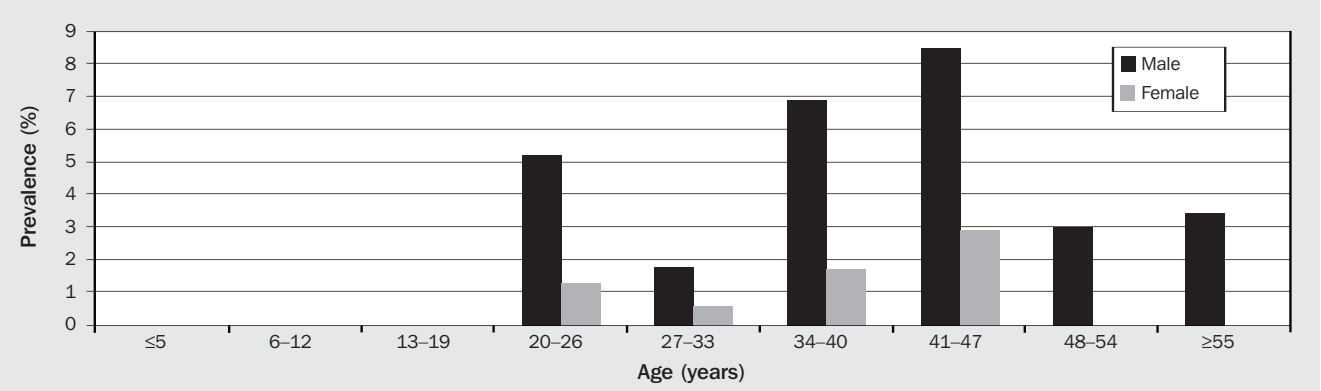

Fig. 2. Prevalence of HCV among study subjects by age and gender.

compared to previous estimates for the country.[6] This may be due to the fact that this study included patients and children. When these groups are excluded, the HIV prevalence drops to 3.6%. Although the latter rate is twice the previous estimate for the country, it may be in line with the changing trend in HIV prevalence or due to differences in the study subjects evaluated. Similarly, the lower prevalence rate of 2.4% among blood donors may not reflect the rate in the healthy population at large.

Few published data exist on the distribution of hepatitis C in The Gambia, partly due to the fact that facilities for its routine diagnosis did not exist in government hospitals at the time of this study. Hence, blood donations were not routinely screened for hepatitis viruses. This has made it difficult to obtain baseline information on the distribution of the virus in the community. The overall HCV prevalence reported in this study is lower than the estimated worldwide prevalence (3%) and lower than the estimated level for West Africa.[6] It is also lower than the prevalence (3%) found by Kirk *et al*.[8] among a total of 382 apparently normal Gambians recruited as a control group. This may be due to the smaller population size and also to geographical differences in the population sampled.

Other studies have reported higher HCV prevalence in West Africa.[9] Similarly, earlier studies by Coursaget *et al*.[10] reported an anti-HCV prevalence of 4.2% in apparently healthy adult populations in Senegal, Burundi, Tunisia and Madagascar, while lower rates were reported by Ka *et al*.[11] six years later. This disparity may be associated with differences in prevailing risk factors or the test kits employed,[12] or may be influenced by the increasing incidence of HIV infection.[13]

The finding that the peak age for HCV infection was in the 41–47 age group, with males accounting for more cases than females, reflects the global epidemiological pattern.[1,2] However, the finding of significant HCV infection in the 20–26 age group cannot be explained. Generally, owing to the long incubation period of HCV, it is diagnosed more frequently in those of middle age and above,[14] most especially in communities where intravenous drug use is not the principal route of its transmission. In addition, conclusive evidence has been provided for a progressive increase with age.[15] In The Gambia, intravenous substance abuse, the principal route of transmission of the virus in most developed countries,[1,2] is a rare event. Wasley and Alter[14] observed that high HCV distribution in those under the age of 49 years in the developed countries is generally suggestive of recent infection. It is not certain if this observation can be extrapolated to developing countries, but it is known that regional variation in age-specific HCV prevalence is influenced by prevailing contributory risk factors.

The relationship between HCV and age has been demonstrated in several studies.[2,14,15] However, in the present study, the detection of HCV antibodies in those in their sexually active years cannot be clearly explained. It may be consistent with a report showing that up to 50% of patients with hepatitis C have had no parenteral exposure,[16] or with the observations of Hyder *et al*.[17] of significant transmission of HCV through non-conventional methods. It may also be suggestive of sexual activity as a possible route of transmission of the virus, despite the fact that HCV is less efficiently transmitted sexually than HIV.[14] This may be in line with the work Fletcher,[18] which showed increasing

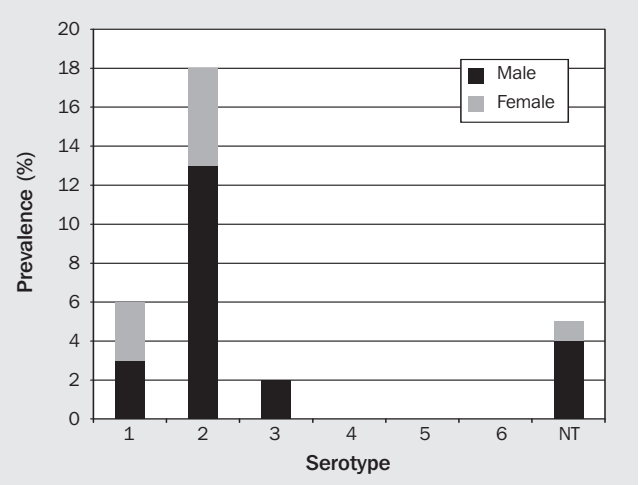

Fig. 3. Distribution of HCV serotypes by gender.

sexual transmission of HCV particularly among HIV-positive males.

Although very little is known about the natural history of HCV infection in children, it has been suggested that asymptomatic infection and complications resulting in liver failure may occur.[19] In this study, anti-HCV was detected mainly in those aged 20 years and above. However, in a study reported in China, Chen and Xia[20] found an HCV prevalence rate of 0.35% among 4055 healthy children of 14 years or younger in Beijing. The risk factor reported for this group was blood transfusion.

The finding in the present study of a high prevalence of HCV type 2 may support the assertion that HCV serotype 2 originated in West Africa.[21] It also corroborates the report of independent small-scale surveys conducted in West African countries including Burkina-Faso,[22] Benin and Guinea[23] that show predominance of HCV type 2 in the region. Furthermore, the finding of serotypes 1 and 3 in those under the age of 40 years could suggest that these are relative new to the area.

The five cases of untypeable serotype could be due to infection with variants of HCV not covered by the competing peptides used in the assays, or due to the non-specificity of the NS4 peptides used to coat the plates.[24] This highlights some of the limitations associated with HCV serotyping compared to molecular assays.

Dodig and Tavil[25] estimated that 30–50% of patients with HIV are co-infected with HCV. The current finding of an HIV/HCV co-infection rate of 0.6% may be due to the low prevalence of HIV and HCV, or may be due to prevailing risk factors associated with the transmission of the agents in the country.

The fact that HIV-1 co-infection with HCV serotypes 1 and 3 is consistent with that documented in developed countries.[2] Co-infection with HIV/HCV represents a major problem in developed countries and is a growing problem for sub-Saharan countries with a high prevalence of HIV and HCV.

Co-infection with HIV and HCV has been associated with higher HCV viral load, accelerated progression to HCV-related liver disease, and an increased risk for liver cirrhosis.[4,26,27] In sub-Saharan countries, study of HIV/HCV co-infection is particularly important, considering that the evolutionary route of some subtypes of HIV[26] and HCV[21]

have been traced to the continent. In this study, the comparatively high level of HIV-1/HCV serotype 1 co-infection has serious health implications, especially in terms of treatment, and highlights the need for provision of facilities to detect HCV infection and the identification of HCV serotypes. □

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References

1 Pawlotsky JM, Roudot-Thoraval F, Bastie A *et al*. Factors affecting treatment responses to interferon-α in chronic hepatitis C. *J Infect Dis* 1996; **174**: 1–7.
2 Haushofer AC, Kopty C, Hauer R, Brunner H, Halbmayer WM. HCV genotypes and age distribution in patients of Vienna and surrounding areas. *J Clin Virol* 2001; **20** (1–2): 41–7.
3 Hnatyszyn HJ. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. *Antivir Ther* 2005; **10** (1): 1–11.
4 Soriano V, Nunez M, Sanchez-Conde M *et al*. Response to interferon-based therapies in HIV-infected patients with chronic hepatitis C due to genotype 4. *Antivir Ther* 2005; **10** (1): 167–70.
5 De Cock L, Vranckx R. Serotyping and genotyping of hepatitis C virus in Belgium. *Infection* 2003; **31** (2): 92–7.
6 UNAIDS/WHO. United Nations report on the Global HIV/AID epidemics. UN AIDS Epidemic Update, 2002.
7 Galel SA, Strong DM, Tegtmeier GE *et al*. Comparative yield of HCV RNA testing in blood donors screened by 2.0 versus 3.0 antibody assays. *Transfusion* 2002; **42** (11): 1507–13.
8 Kirk GD, Lesi OA, Mendy M *et al.* The Gambia Liver Cancer Study: infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. *Hepatology* 2004; **39** (1): 211–9.
9 Ola SO, Otegbayo JA, Odaibo GN, Olaleye OD, Olubuyide OL. Serum hepatitis C virus and hepatitis B surface antigenaemia in Nigerian patients with acute icteric hepatitis. *West Afr J Med* 2002; **21** (3): 215–7.
10 Coursaget P, Bourdil C, Kastally R *et al*. Prevalence of hepatitis C virus infection in Africa: anti-HCV antibodies in the general population and in patients suffering from cirrhosis or primary liver cancer. *Res Virol* 1990; **141** (4): 449–54.
11 Ka MM, Herve P, Leguenno B *et al.* Low prevalence of anti-hepatitis C virus antibodies in hepatocellular carcinoma in Senegal. *Ann Gastroenterol Hepatol (Paris)* 1996; **31** (6): 329–32.
12 Aach RD, Stevens CE, Hollinger FB *et al.* Hepatitis C virus infection in post-transfusion hepatitis: an analysis with first- and second-generation assays. *N Engl J Med* 1991; **325** (19): 1325–9.
13 Terrault NA. Sexual activity as a risk factor for hepatitis C. *Hepatology* 2002; **36** (5 Suppl 1): S99–105.
14 Wasley A, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* 2000; **20** (1): 1–16.
15 Guadagnino V, Stroffolini T, Rapicetta M *et al.* Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy. *Hepatology* 1997; **26** (4): 1006–11.
16 Passos EP, Silveira TR, Salazar CC *et al*. Hepatitis C virus infection and assisted reproduction. *Hum Reprod* 2002; **17** (8): 2085–8.
17 Hyder Q, Burhan-ul-Haq M, Rashid R, Qazi S, Mehmood S, Hadi SF. Non-conventional transmission of hepatitis C: a true possibility ignored. *J Pak Med Assoc* 2009; **59** (7): 430–3.
18 Fletcher S. Sexual transmission of hepatitis C and early intervention. *J Assoc Nurses AIDS Care* 2003; **14** (5 Suppl): 87S–94S.
19 Bortolotti F, Resti M, Giacchino R *et al*. Hepatitis C virus infection and related liver disease in children of mothers with antibodies to the virus. *J Pediatr* 1997; **130**: 990–3.
20 Chen M, Xia S. A prevalence study on hepatitis C infection in 4055 healthy children of Beijing (in Chinese). *Zhonghua Yu Fang Yi Xue Za Zhi* 1999; **33** (3): 158–9.
21 Candotti D, Temple J, Sarkodie F, Allain JP. Frequent recovery and broad genotype 2 diversity characterize hepatitis C virus infection in Ghana, West Africa. *J Virol* 2003; **77** (14): 7914–23.
22 Mellors, JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996; **272** (5265): 1167–70.
23 Jeannel D, Fretz C, Traore Y *et al*. Evidence for high genetic diversity and long-term endemicity of hepatitis C virus genotypes 1 and 2 in West Africa. *J Med Virol* 1998; **55** (2): 92–7.
24 Songsivilai S, Kanistanon D, Dharakul T. A serotyping assay for hepatitis C virus in Southeast Asia. *Clin Diagn Lab Immunol* 1998; **5** (5): 737–9.
25 Dodig M, Tavill AS. Hepatitis C and human immunodeficiency virus coinfections. *J Clin Gastroenterol* 2001; **33** (5): 367–74.
26 Yoo TW, Donfield S, Lail A, Lynn HS, Daar ES; Hemophilia Growth and Development Study. Effect of hepatitis C virus (HCV) genotype on HCV and HIV-1 disease. *J Infect Dis* 2005; **191** (1): 4–10.