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The prevalence and genetic diversity of hepatitis C infection in antenatal clinic attenders in two regions of England

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SUMMARY

The prevalence and genetic diversity of hepatitis C infection in women attending antenatal clinics in two regions of England was investigated to inform future surveillance and control measures. Women booking into antenatal care are routinely offered a test for immunity to rubella. Serum residues from these tests were unlinked, anonymized and archived as part of the Unlinked Anonymous Prevalence Monitoring Programme (UAPMP). The serum specimens were tested for anti-HCV using a cost-effective pooling strategy. After taking into account differential sampling from the UAPMP serum archive, the adjusted overall prevalence of anti-HCV was 0·43% (95% CI: 0·32–0·53) in London and 0·21% (95% CI: 0·14–0·28) in the Northern and Yorkshire region. Restriction fragment length polymorphism of amplified HCV RNA identified type 3a as the most common HCV genotype in these antenatal women. The prevalence of anti-HCV in antenatal women in the UK is low and consistent with that expected from injecting drug use.

INTRODUCTION

In the UK, national surveillance of hepatitis C virus (HCV) infection and studies in blood donors indicate that injecting drug use is the predominant risk behaviour associated with HCV transmission [1, 2]. A number of studies have established that transmission of HCV from mother to child can also occur [3–6], but with an efficiency estimated to be less than 10% [7–9]. Concurrent maternal HIV infection [10, 11], high titres of maternal plasma HCV RNA [12], and acute HCV infection in the last trimester of pregnancy [13] may each increase the risk of transmission by this route.

A UK study of an antenatal population in the West Midlands found an overall HCV prevalence of 0·14% [14], although smaller studies in London and Glasgow found higher prevalences [15, 16]. Mother-to-child transmission of hepatitis C is of particular concern because of the long-term sequelae of chronic infection, including chronic liver disease, cirrhosis and hepatocellular carcinoma [17, 18]. At present, it is not known whether maternally acquired infections have a higher likelihood of progression to chronic infection and liver disease than infections acquired by other routes

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or at other ages. In addition, the absence of an effective intervention to reduce transmission from mother to baby means that the potential public health benefits and economic implications of identifying HCV infected pregnant women are not clear.

Since early 1990, selected antenatal clinics in England have participated in prospective studies in which unlinked serum specimens have been tested for antibodies to the human immunodeficiency virus (HIV) as part of the Unlinked Anonymous Prevalence Monitoring Programme (UAPMP) [19]. Serum specimens from these antenatal clinic attenders were available for testing for the presence of antibody to HCV (anti-HCV). We report our findings from testing an antenatal population in two regions of England to determine the baseline prevalence and the genetic diversity of HCV infection, and to contribute to the future surveillance and control of HCV infection.

METHODS

Serum archive

The UAPMP collaborating laboratories received specimens from pregnant women attending antenatal clinics and general practices. Residues remaining from routine antenatal rubella serology were unlinked from patient identifiers using established methods [19]. Data retained were age group, centre (hospital) of antenatal booking and quarter year of collection. The antenatal survey was conducted in centres in Greater London and in the Northern and Yorkshire region. The study was co-ordinated by the Communicable Disease Surveillance Centre (CDSC) and the Hepatitis and Retrovirus Laboratory (HRL) of the Public Health Laboratory Service (PHLS), where the survey's serum specimens were stored. Selected archived specimens from women who during 1996 attended one of 14 antenatal clinics in Greater London and 11 antenatal clinics in the Northern and Yorkshire region were tested for anti-HCV (Table 1). Ethical clearance for the study had been obtained from the ethics committee in each locality where the UAPMP operated.

Pooling

A pooling strategy for anti-HCV testing was developed and validated similarly to that described previously for screening specimens for anti-HIV [20]. For pools of 12 blood donor serum specimens, the protocol was shown to have a sensitivity of approximately 99% (95% CI: 96·5–99·9) when compared with testing specimens individually for anti-HCV. The most cost-effective pool size is dependent on the prevalence in the survey population as reactive pools may have to be re-examined, specimen by specimen [21]. For anti-HCV testing, pools of 12 were expected to be cost-effective, and employing these pool sizes retained adequate sensitivity.

Serological testing

Pools of 12 serum specimens were tested using the Ortho® HCV 3·0 ELISA Test System (enhanced SAVe). To compare each of the pool reactivities the optical density (OD) of the end product of the antigen-antibody-enzyme complex was divided by the cut-off (CO) value to calculate an OD/CO ratio. Pools that gave an OD/CO of 1·0–1·5 were retested and investigated further only if repeatedly reactive. Each serum specimen incorporated in a reactive pool was subsequently tested individually by the standard (short) protocol for the Ortho® HCV 3·0 ELISA Test System (enhanced SAVe). Each individual serum specimen that was reactive by the Ortho assay was tested also by the Monolisa® anti-HCV Plus, (Sanofi Diagnostics Pasteur). Specimens that were found to give discordant results or were weakly reactive by either or both assays were further tested with a recombinant immunoblot assay (Ortho® HCV RIBA 3). Specimens from HIV-1 positive women were not tested in pools due to small volumes, but were tested individually. Any individual sample that was positive by the Ortho assay, but only a small volume remained, went straight to RIBA testing. Specimens that were positive by the two separate ELISAs or by one ELISA and RIBA (manufacturer's interpretative criteria) were identified as being serologically HCV positive. Specimens that were positive by one or both ELISAs, but RIBA indeterminate were classified as indeterminate and excluded from the analysis of prevalence.

PCR and genotyping

All serum specimens found to be anti-HCV positive by individual screening by the single Ortho assay were tested by RT-PCR for HCV-RNA. RNA was extracted from each specimen using the Amplicor™ HCV Specimen Preparation Kit (Roche Diagnostic

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Table 1. *Total number of samples selected for HCV testing*

Age group (years)	Total†	Number tested	% tested
London			
< 20	3230	3060	94·74
20–24	9884	9326	94·35
25–29	16096	7798	48·45
> = 30	25676	5754	22·41
Total	54886	25938*	47·26
Northern and Yorkshire			
< 20	3388	2340	69·07
20–24	7862	5413	68·85
25–29	11348	5239	46·17
> = 30	11361	3683	32·42
Total	33959	16675	49·10

* Excludes two samples age group not known.
† Total, the total number of specimens available for archiving.

Systems, Welwyn Garden City, Herts. UK). This RT-PCR assay amplifies the HCV-5′-non-coding region (5′NCR) of HCV. The products of this process were digested with restriction enzymes and the digests analysed using restriction fragment length polymorphism (RFLP) to determine HCV genotype [22].

Statistical analysis

Multivariable logistic regression was used to compare the prevalence of HCV by age, region, centre and HIV status. Interactions between these factors were also examined. Statistical significance was taken at the 5% level. Confidence intervals for the unadjusted prevalence by age and region were calculated using the binomial distribution. To adjust for the differential sampling from the archive in each of the regions, the observed prevalence by age was applied to the totals (Table 1) to give an adjusted estimate of the overall prevalence. Within each age group we assumed the samples selected for testing were representative.

RESULTS

A total of 25940 serum specimens from antenatal clinics in the Greater London area and 16675 in the Northern and Yorkshire region collected during 1996 were tested for anti-HCV. Among the Greater London specimens, 104 were anti-HCV reactive by the Ortho ELISA. Of these, 82 were also strongly positive in the Monolisa assay. Supplemental RIBA testing was performed on the 22 specimens with discordant results, which were weakly reactive by both ELISAs, or which had small residual volumes. This supplemental RIBA testing confirmed 4 to be anti-HCV positive, 12 to be anti-HCV negative and 6 to be indeterminate. In total, 86 specimens were confirmed to be serologically HCV positive and 6 specimens were classified as indeterminate. Among the Northern and Yorkshire region specimens, 45 were found to be reactive by the Ortho ELISA. Thirty-four of these specimens were strongly reactive in the Monolisa assay. Nine specimens required RIBA tests of which 3 were confirmed to be anti-HCV positive and 6 to be negative. Two specimens that were positive on individual testing by the Ortho assay had insufficient volumes for further testing and were not included in the confirmed positive group. The prevalence of confirmed anti-HCV was therefore 86/25940 (0·33%) in Greater London and 37/16675 (0·22%) in Northern and Yorkshire. Taking into account the proportion of the total specimens selected for testing (Table 1), adjusted prevalences were 0·43% (95% CI: 0·32–0·53) for the Greater London area and 0·21% (95% CI: 0·14–0·28) for the Northern and Yorkshire region. Had the 6 serologically indeterminate specimens been classified as positive, the prevalence in Greater London would have been 92/25940 (0·35%). Had the two specimens which were Ortho positive but insufficient for RIBA testing been classified as positive the prevalence in Northern and Yorkshire would have been 39/16675 (0·23%).

Multivariable logistic regression analysis demonstrated a significant ($P = 0·0047$) variation in preva-

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lence by age (Table 2). Prevalence also varied by centre in the Greater London area (range: 0·04–0·75%) with four centres having an overall prevalence of more than 0·5%. In the Northern and Yorkshire region (range: 0·00–0·87%) only one centre had a prevalence of greater than 0·5%. Overall variation between centres was significant, even after controlling for age, HIV status and region ($P = 0·0004$).

Narrow age groupings were known for 5738/5754 (99·7%) women over 30 years of age in London and suggested that the prevalence declined with increasing age. The prevalence was 24/3549 (0·68%), 8/1483 (0·54%) and 1/706 (0·14%) in 30–34 year olds, 35–39 year olds and over 40 year olds respectively (Chi square test for trend, $P = 0·10$). For Northern and Yorkshire, narrow age groupings were only known for 2027/3683 (55·0%) specimens from women over 30 years of age and numbers were therefore too small to observe a trend. The prevalence was 1/1601 (0·06%), 1/253 (0·40%) and 0/173 (0·00%) in 30–34 year olds, 35–39 year olds and over 40 year olds respectively. A significant interaction was observed between age and region ($P = 0·023$) indicating that the age-specific prevalence differed between Greater London and Northern and Yorkshire (Table 2). In Greater London, the highest prevalence was observed in women aged over 30 years, whereas in Northern and Yorkshire, the peak prevalence was in the 20–24 year old age group. Amongst women over 30 years of age, therefore, anti-HCV prevalence was significantly higher in Greater London than in Northern and Yorkshire ($P = 0·0013$).

The HIV findings on the antenatal serum archive have been described elsewhere [19]. Overall, prevalence of HIV-1 in London antenatal clinic attenders tested in this study was 0·26% (67/25940). Six anti-HIV-1 positive specimens from the London area were also anti-HCV positive (of which five were viraemic). One anti-HIV-1 positive specimen was of indeterminate HCV status and HCV-RNA negative. An additional specimen from London was HIV-1 negative, HIV-2 positive and anti-HCV negative. All of the specimens from Northern and Yorkshire were anti-HIV negative. The overall prevalence of anti-HCV in the 67 anti-HIV-1 positive specimens was 8·95%, significantly higher than amongst the anti-HIV-1 negatives after controlling for age and centre ($P < 0·0001$). Although significant variation in anti-HCV prevalence was found between centres, the centres with a high anti-HCV prevalence were not always those with a high anti-HIV prevalence.

Table 2. *Overall prevalence (95% confidence interval) of hepatitis C antibody in antenatal clinic attenders*

Region	Age group (years)	< 20	20–24	25–29	> = 30	Age not known	Total
London	No. positive/total tested	3/3060	23/9326	25/7798	35/5754	0/2	86/25940
	% anti-HCV positive	0·10%	0·25%	0·32%	0·61%	0·00%	0·33%
	95% CI	(0·02–0·29)	(0·16–0·37)	(0·21–0·47)	(0·42–0·84)	—	(0·26–0·41)
Northern and Yorkshire	No. positive/total tested	3/2340	18/5413	10/5239	6/3683	0/0	37/16675
	% anti-HCV positive	0·13%	0·33%	0·19%	0·16%	—	0·22%
	95% CI	(0·03–0·37)	(0·20–0·53)	(0·09–0·35)	(0·06–0·35)		(0·16–0·31)

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Table 3. *HCV genotype distribution in antenatal clinic attenders* (*London and Northern and Yorkshire*)

Age group…	< 20	20–24	25–29	> = 30	Total
Genotype					
1*			1	1	2
1a		8	4	9	21
1b	1		1	2	4
2a		1	1		2
3a	3	11	12	12	38
3b		1	1	2	4
4		1			1
6			1		1
Total	4	22	21	26	73

* Existing subtype unable to be assigned.

Amongst women under 35 years of age there was no significant difference in anti-HCV (0·32%; 75/23733) and anti-HIV (0·21%; 65/23733) prevalence. However, amongst women over 35 years of age, the prevalence of anti-HCV was 9/2189 (0·41%) compared to 0/2189 (0·00%) for anti-HIV ($P = 0·004$). Sixteen specimens, 2 of which were both anti-HCV and anti-HIV positive, were excluded from this analysis because their age in years was recorded as over 30 years only.

Fifty-five (64·0%) of the 86 anti-HCV positive specimens from the London area and 16 (43·2%) of the 37 anti-HCV positive specimens from the Northern and Yorkshire region contained detectable HCV RNA. In addition, 2 of the 6 indeterminate specimens (both from London) contained HCV RNA. RFLP identified type 3a as the most prevalent genotype (52·1%) followed by type 1a (28·8%) (Table 3). The number of specimens genotyped was too small to demonstrate significant differences in genotype by age or region. However, the proportion of type 3a infections was slightly lower in the London area than in the Northern and Yorkshire region and a correspondingly higher proportion of infections were type 1a.

DISCUSSION

Several studies of pregnant women in Europe reported relatively low anti-HCV prevalences (< 2%) when second and third generation ELISAs were used in conjunction with supplemental testing. The reported prevalence of anti-HCV was 1·55% in France [23], 1% in both Italy [12] and Spain [24] and 0·94% in Germany [25]. The prevalence of 0·43% in London is consistent with a more recent study in London in which prevalences of 0·38% and 0·20% were seen in inner and outer districts respectively [26]. Outside of London no large studies have been conducted, but our findings are similar to an earlier study in the West Midlands [14]. Outside of Europe a study from South Australia found a prevalence of anti-HCV of 1·1% [27], and a prevalence of 3·2% was reported in a US study [28]. A prevalence of 1·3% was observed in Taiwan [29], and prevalence estimates of between 0·7–2·6% have been reported in Japan [30–32]. The prevalence of anti-HCV in antenatal clinic attenders in the UK is therefore lower than observed in other antenatal populations in Western Europe, Australia, North America and Asia.

A higher adjusted anti-HCV prevalence was seen in the Greater London area (0·43%) compared to the Northern and Yorkshire region (0·21%). This was mainly due to the higher prevalence of anti-HCV in women over 30 years of age. In a national survey in 1990–1, around 0·8% of women in the London area reported ever having injected drugs [33]. This compared with 0·3% of women in the rest of England and Wales, and may explain the higher anti-HCV prevalence observed in London. In addition, the survey in 1990–1 indicated that, compared to younger women, a very low proportion of women aged over 35 years reported ever having injected drugs. This may explain the low prevalence of anti-HCV in women aged over 40 in this study. Alternatively low risk women may be over-represented amongst women who have babies at this age. As the prevalence of anti-HCV in injecting drug users in England and Wales ranges from 46–67% [34–36] the prevalence of HCV infection in this study may be completely attributable to injecting drug use.

The majority of anti-HCV positive sera were also positive for HCV RNA. Studies of the natural history of hepatitis C suggest that 80% of anti-HCV positive individuals are viraemic [37], a higher proportion than observed in this study. As these specimens had been in storage for some time and had not been handled specifically to optimize RNA recovery, it is likely that the HCV RNA in a proportion of the specimens had degraded to undetectable levels and more would originally have contained HCV RNA. Alternatively, anti-HCV positive patients whose serum does not contain detectable HCV RNA may represent past resolved infections. The possibility of intermittent viraemia in these patients cannot be excluded, but the risk of transmission from individuals who are persistently PCR negative is extremely low [38]. Even in viraemic anti-HCV positive women, studies suggest

that the rate of mother to infant transmission of HCV in HIV negative women is less than 10% [7, 8]. Co-infection with HIV, however, may result in increased maternal HCV viraemia and increased risk of transmission. Even though HCV infection was more common amongst HIV infected women, it was reassuring that few co-infected women were identified in London and that none were identified in Northern and Yorkshire.

The distribution of HCV genotypes showed a high frequency of type 3 infections, nearly all of these being subtype 3a. The most commonly occurring genotypes in Northern Europe are 1a, 1b and 3a [39] and genotypes 4, 5 and 6 are rarely seen in Europe. In the UK, type 1a has been found to occur frequently in haemophiliacs and others in whom HCV infection was a consequence of receipt of blood products [22, 40]. Recent UK studies of circulating HCV genotypes suggests that injecting drug use is the main source of type 3a infections in the UK [22]. This supports the hypothesis that genotype 3a was introduced into Northern Europe in association with increased injecting drug use over the last 20 years. The genetic diversity in this study is therefore consistent with the main exposure category for acquisition of HCV in antenatal women being injecting drug use. This emphasizes the need for public health intervention strategies to be aimed at individuals who continue to inject drugs.

Before universal anti-HCV testing of pregnant women could be adopted, screening tests and algorithms with a low cost and a high sensitivity and specificity are required [9]. The low prevalence of anti-HCV in UK antenatal women means that the positive predictive value of current screening assays would be low. In this study, many samples which were reactive on a single assay could not be confirmed and samples from six women were classified as anti-HCV indeterminate. Testing is therefore likely to raise unnecessary concern in women who have false positive screening tests and indeterminate confirmatory results. Two anti-HCV indeterminate samples were HCV–RNA positive. Although these findings may be due to a false positive PCR, they probably indicate recent seroconversion or, possibly, an inability to mount a detectable antibody response due to immunosuppression. The risk of HCV transmission from women with such markers is unclear, and for confirmed anti-HCV positive mothers, there is no intervention which has been shown to reduce or prevent the risk of transmission to their babies. Unlike hepatitis B, no vaccine is currently available and passive immunisation with immunoglobulin containing HCV antibody appears to be ineffective [41, 42]. Mother to child HIV transmission has been shown to be substantially reduced by antiretroviral therapy, delivery by caesarean section and avoidance of breastfeeding [43]. Although antivirals are used in the treatment of hepatitis C, there is as yet no evidence that such therapy can similarly reduce HCV transmission [44]. Breastfeeding does not increase the risk of HCV infection [45]; however there is contradictory evidence that elective caesarean delivery may reduce the risk [8]. One argument for antenatal testing would be to identify women who could themselves benefit from treatment but, compared to testing groups at higher risk (eg. injecting drug users), a larger number of tests would be required to identify a single positive. An alternative approach would be selective antenatal testing on the basis of known exposures. In South Australia, it was demonstrated that selective screening of women on the basis of risk factors would have detected 16 of the 17 positive women identified by universal testing [27]. At present, therefore, targeted testing would seem a more appropriate strategy for identifying infected women in the UK. The adoption of such a policy would require further analysis of costs and the effectiveness of testing and treatment and require the availability of local treatment services.

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