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Prevalence and incidence of hepatitis C in injecting drug users attending genitourinary medicine clinics

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SUMMARY

Surveillance reports and prevalence studies have indicated that injecting drug users (IDUs) contribute more to the hepatitis C epidemic in the United Kingdom than any other risk group. Information on both the prevalence and incidence of hepatitis C in IDUs is therefore essential to understanding the epidemiology of this infection. The prevalence of hepatitis C in specimens from the Unlinked Anonymous Prevalence Monitoring Programme collected in 1995, 1996, 1998, 1999, 2000, and 2001 was determined using residual syphilis serology specimens from IDUs attending 15 genitourinary medicine (GUM) clinics in and outside London. These specimens were tested for antibodies to hepatitis C virus (anti-HCV). Using this cross-sectional design, anti-HCV-negative specimens were tested for HCV RNA to identify incident infections during the 'window' period of infection, and thus to estimate HCV incidence. Results of the multivariable analysis showed that there was marked variation in prevalence by clinic ($P<0{\cdot}0001$) and age ($P<0{\cdot}0001$). Overall the majority of infections were in males and the overall prevalence in injectors declined over the study period from 36·9% to 28·7%. The annual incidence in these injectors was estimated as being 3·01% (95% CI 1·25–6·73). Over the study period HCV incidence decreased by 1·2% per year. Genotyping of the incident infections identified the most common genotype as type 1 with type 3 being more frequently seen after 1998. Of the prevalent infections, genotype 1 was the most common. The study has confirmed a higher prevalence of anti-HCV in IDUs in the London area compared to those outside London. How representative of the current injecting drug user population are IDUs attending GUM clinics is unclear. Even so, such studies allow prevalence and incidence to be estimated in individuals who have ever injected drugs and inform ongoing public health surveillance.

Key words: Genotype, hepatitis C virus, incidence, injecting drug users, prevalence, window period infections.

INTRODUCTION

National surveillance indicates that most diagnosed hepatitis C virus (HCV) infections in England and Wales are in current or ex-injecting drug users (IDUs) [1, 2]. It is therefore important to obtain accurate estimates of the incidence and prevalence of HCV infection in IDUs. A number of studies in IDUs in the United Kingdom, have found evidence of HCV infection in 30–60% of injectors [3–5]. There are, however, no proven laboratory markers of recently

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acquired (incident) infection and the incidence of HCV infection is therefore difficult to determine.

Two UK-based studies, one in Glasgow using a retrospective cohort method and the other in London using a more traditional prospective approach, estimated HCV incidence rates of 28·4 and 41·8/100 person-years in IDUs respectively [6, 7]. The first study used unlinked anonymous anti-HCV testing of serum residues collected from IDUs having two or more voluntary named HIV tests between 1993 and 1998. The second used a traditional prospective approach, testing oral fluids collected from IDUs in London in 2001–2002. Both studies were concentrated in areas of high HCV prevalence and included individuals who were injecting at the time of both their initial and subsequent tests. Prospective follow-up studies are both difficult and expensive and IDUs who are available for long-term follow-up may not be typical of the drug-using population as a whole.

Acute HCV infection is typically asymptomatic but when an illness occurs the average interval from exposure to symptom onset is 6–7 weeks (42–49 days) [8]. Anti-HCV antibodies can be detected in the blood of 80%, 90% and 97% of patients within 15 weeks, 5 months and 6 months, respectively, after exposure [8]. Studies of post-transfusion HCV infection have estimated that the average period before antibody is detected by third-generation HCV assays is 70 days [9, 10], whereas in another setting the average interval from exposure to seroconversion was 56–63 days [8]. The interval from exposure to the detection of anti-HCV is called the seroconversion window period. Before the appearance of anti-HCV antibody, however, individuals are typically viraemic, signified by the presence of HCV RNA in the blood. This is following an 'eclipse' phase immediately after exposure when no infectious virus is recoverable [11], HCV RNA can often be detected in serum or plasma within 7–14 days, but occasionally may not appear until 30–40 days [8–12]. The antibody-negative, HCV RNA-positive window period has been estimated in infected donors and blood product recipients to be around 60 days [13].

Detection of HCV RNA-positive individuals during this anti-HCV-negative window period can therefore be used to estimate the incidence of HCV infection using individual serum specimens from cross-sectional population surveys [14]. In the cross-sectional study described here the prevalence of anti-HCV in IDUs who attended genitourinary medicine (GUM) clinics was estimated over time. We also employed an effective approach to identify incident infections and to estimate HCV incidence in this high-risk group.

METHODS

Anti-HCV testing

The Unlinked Anonymous Prevalence Monitoring Programme (UAPMP) began in 1990 to measure the distribution of anti-HIV-1 in accessible groups of the adult population [15]. The survey includes attenders of GUM clinics in which residues of serum specimens collected for syphilis serology are unlinked and anonymized using established methods and tested for anti-HIV-1. Specimens were stored at $-20\,^{\circ}C$ and as part of the GUM survey, whether participants have ever injected drugs is recorded.

To determine the prevalence of hepatitis C in specimens collected in 1998, 1999, 2000 and 2001, remaining specimens from IDUs attending 15 GUM clinics (from Wales, Northern Ireland, and the following health regions in England: East of England, London, North East, North West, West Midlands, and Yorkshire & Humberside) were tested for anti-HCV. These specimens were tested individually with the Ortho® HCV 3.0 enzyme-linked immunosorbent assay (ELISA) test system (with enhanced SAVe; Raritan, NJ, USA). Each specimen that was reactive by the Ortho assay was also tested by Monolisa® anti-HCV Plus (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). Specimens with discordant results, and those that were weakly reactive in one or both assays were further tested with a recombinant immunoblot assay (Ortho® HCV RIBA-3), and results interpreted according to the manufacturer's instructions. We previously reported a prevalence study on 1329 specimens from IDUs collected during 1995 and 1996 as part of the UAPMP GUM survey, finding an unadjusted anti-HCV prevalence of 36·9% for both years [16]. Ethical clearance for the study was obtained from the ethics committee in each locality where the UAPMP operated.

Pooling and HCV RNA detection

To detect viraemic infections in the seroconversion window period (incident infections), the anti-HCV-negative specimens were tested in small pools for the presence of HCV RNA by a nested reverse transcriptase–polymerase chain reaction (RT–PCR)

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assay. A pool size of 10 was used as it provided significant time savings while having minimal impact on sensitivity (estimated detection limit: 120 copies/ml which, accounting for pooling, is equivalent to 1200 copies/ml in individual specimens). RNA was extracted from 100 μl of each serum pool using the Amplicor™ HCV Specimen Preparation kit (Roche Diagnostic Systems, Welwyn Garden City, Herts, UK). The RT–PCR assay utilized random priming of cDNA production and nested primer pairs which target the 5′-non-coding region (5′-NCR) of the HCV genome [17, 18]. If a pool was reactive, each specimen comprising the pool was tested individually for HCV RNA using the same PCR assay. Specimens that were indeterminate after Ortho® HCV RIBA-3 testing were tested individually for HCV RNA.

HCV genotyping using restriction fragment length polymorphism (RFLP) was performed on all individual RT–PCR-positive sera [17, 19]. In brief, products of the PCR process were digested with each of four restriction enzymes, the digests electrophoresed and the fragment pattern analysed to derive the genotype. As a sample of anti-HCV-positive samples from 1995 and 1996 had already been genotyped [16] a sample of anti-HCV-positive specimens from 2001 were tested individually for HCV RNA, and specimens found to be RT–PCR positive were genotyped to permit comparison.

Statistical analysis

Data from IDUs attending GUMs during 1995–2001 were analysed as a single group. Single variable logistic models were first fitted followed by multivariable models. Multivariable logistic regression was used to compare the prevalence of HCV by clinic, region, age, sex and sexual orientation, country of birth, and HIV serostatus. Statistical significance was taken at the 5% level. Two-way interactions between variables of interest ($P<0{\cdot}05$) in the multivariable model were also investigated.

Confidence intervals (CI) for the prevalence of HCV RNA in the anti-HCV-negative or indeterminate specimens were calculated using the Poisson distribution. Incidence in this study encompasses window-period detection as well as HCV RNA detection in indeterminate specimens. Window-period intervals were estimated to adjust for the uncertainty around the true seroconversion time and HCV RNA-positive window period. As HCV RNA appears between 7 and 14 days following exposure and the average time to seroconversion is between 56 and 70 days [8–10], it was assumed that the average duration of the HCV RNA-positive antibody- negative window period was between 42–63 days [13]. Using this assumption, incidence is proportional to the prevalence of specimens falling within the window period and can therefore be estimated from cross-sectional studies [20, 21] The uncertainty range for this incidence estimate is based upon the stated HCV RNA detection times. Incidence, in this instance, denotes the number of new infections for each 100 person-years lived in the susceptible population. The Cochran–Armitage test [22] was then used to test for linear trend in incidence by year.

RESULTS

Of a total of 1329 specimens from IDUs who had attended the collaborating GUM clinics in 1995 and 1996, 490 were anti-HCV seropositive, giving an unadjusted prevalence of 36·9% for both years [16]. For samples from the years 1998–2001, the overall prevalence estimate was 29·3% (747/2553). Prevalence fell from 36·9% in 1995 to 28·7% in 2001 (Table 1). Anti-HCV prevalence fell during 1998–1999 and then again during 1999–2000, but rose slightly in 2001. Multivariable logistic regression analysis of these data (1995–2001) showed that anti-HCV prevalence was higher in the London area compared to the geographical area outside London (39·3% *vs.* 21·0%, $P<0{\cdot}0001$) (Table 1). Results of the multivariable analysis showed that there was a marked variation in prevalence by clinic ($P<0{\cdot}0001$), this being largely explained by the higher prevalence seen in London. Overall the majority of infections were in males (834/1237, 67·4%). There was a highly significant variation in prevalence by age ($P<0{\cdot}0001$) with prevalence increasing with age (Table 1). In the multivariable analysis, year was significant ($P=0{\cdot}003$) and this can be summarized as a declining trend in the overall annual prevalence ($P=0{\cdot}0002$) where the odds ratio was 0·93 per year (95% CI 0·89–0·96).

Prevalence in those born abroad was 40·9% compared to 29·0% in UK-born attendees ($P=0{\cdot}002$). Male homosexual IDUs had a significantly lower anti-HCV prevalence (22·1%, $P=0{\cdot}0001$) than female and male heterosexual IDUs (35·1% and 33·6%, respectively). The overall prevalence of co-infection with HIV was low, with only 6·2% of anti-HCV positives also being anti-HIV-1 positive (77/1237). There was a significantly higher prevalence of

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Table 1. *Multivariable analysis of IDUs who attended GUM clinics (1995–2001)*

Factor	HCV positive	Total	% Pos.	Multivariable analysis		
				OR	95% CI	*P* value
Sex and orientation						<0·0001
Female heterosexual	403	1149	35·1	1·00		
Male heterosexual	691	2059	33·6	0·79	0·63–0·99	
Male homosexual	135	612	22·1	0·30	0·21–0·42	
Year						0·0032
1995	229	621	36·9	1·00		
1996	261	708	36·9	0·93	0·70–1·22	
1998	254	758	33·5	0·82	0·63–1·07	
1999	175	631	27·7	0·63	0·48–0·84	
2000	165	631	26·1	0·65	0·49–0·87	
2001	153	533	28·7	0·71	0·53–0·96	
HIV status						0·0046
Negative	1160	3678	31·5	1·00		
Positive	77	201	38·3	1·80	1·20–2·70	
Age group (yr)						<0·0001
<20	32	268	11·9	1·00		
20–24	110	790	13·9	1·04	0·67–1·61	
25–34	584	1800	32·4	2·88	1·93–4·30	
35–44	397	791	50·2	6·66	4·37–10·13	
⩾45	101	197	51·3	7·93	4·79–13·12	
Country of birth						0·0018
UK	798	2750	29·0	1·00		
Abroad	315	771	40·9	1·37	1·12–1·66	
Region						<0·0001
Outside London	334	1587	21·0	1·00		
London	903	2295	39·3	1·97	1·65–2·36	

IDU, Injecting drug user; GUM, genitourinary medicine; OR, odds ratio; CI, confidence interval.

anti-HCV in the anti-HIV-1-positive group compared to the anti-HIV-1-negative group (38·3% *vs.* 31·5%, $P=0·005$). There was a significant interaction between HIV status and sexual orientation ($P=0·002$). Comparison of HCV prevalence within the anti-HIV-1-negative and -positive groups gave the following results: in male homosexuals, anti-HCV prevalence was similar in anti-HIV-1-positive and -negative groups (25·0% *vs.* 21·5% respectively, $P=0·73$), whereas HCV prevalence was significantly higher in anti-HIV-1-positive than anti-HIV-1-negative male (71·4% *vs.* 32·8%, $P=0·041$), and female heterosexuals (90·0 *vs.* 34·2%, $P=0·009$), respectively.

In 1995–1996, of 19 indeterminate specimens tested for HCV RNA, four were positive [16]; all of the seven indeterminate specimens from later years were HCV RNA negative. A further 2506 anti-HCV-negative specimens (including 707 from 1995 and 1996) had sufficient volumes retrievable for PCR testing in pools (Table 2). Seven pools of anti-HCV-negative sera were found to contain HCV RNA and all constituent specimens were individually tested. Each of these seven reactive pools gave rise to a single RT–PCR-positive specimen. Inclusion of the four anti-HCV indeterminate/HCV RNA-positive specimens from 1995 and 1996, on the basis that these were

Table 2. *Specimens tested and found HCV RNA-positive by year*

Year	Anti-HCV negative (HCV RNA positive)	RIBA indeterminate (HCV RNA positive)	Total tested (HCV RNA positive)
1995	348 (1)	13 (2)	361 (3)
1996	359 (2)	6 (2)	365 (4)
1998	504 (3)	0 (0)	504 (3)
1999	452 (0)	4 (0)	456 (0)
2000	466 (0)	0 (0)	466 (0)
2001	377 (1)	3 (0)	380 (1)
Total	2506 (7)	26 (4)	2532 (11)

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Table 3. *Characteristics of IDUs on whom HCV RNA-positive/anti-HCV-negative or HCV RNA-positive/anti-HCV-indeterminate results were obtained*

Specimen	Year	Anti-HCV status	Genotype	Sex	Age group (yr)	Sexual orientation	Country of birth	Clinic
A	1995	Negative	1a	Female	35–44	Heterosexual	UK	Outside London
B	1995	Indeterminate	1b	Female	25–34	Heterosexual	Unknown	London
C	1995	Indeterminate	1b	Female	25–34	Heterosexual	UK	London
D	1996	Negative	1b	Female	25–34	Heterosexual	UK	London
E	1996	Negative	1a	Female	25–34	Heterosexual	UK	London
F	1996	Indeterminate	2b	Male	35–44	Heterosexual	UK	Outside London
G	1996	Indeterminate	3b	Female	35–44	Heterosexual	UK	London
H	1998	Negative	3a	Male	35–44	Heterosexual	UK	Outside London
I	1998	Negative	3a	Female	20–24	Heterosexual	UK	Outside London
J	1998	Negative	1a	Male	20–24	Homosexual	UK	Outside London
K	2001	Negative	3a	Male	20–24	Heterosexual	UK	Outside London

IDU, Injecting drug user.

Table 4. *Annual incidence estimates* (*interval estimates*) *of hepatitis C in IDUs attending GUM clinics*

Prevalence of HCV RNA (%)	Seroconversion time	Window period (HCV RNA positive/ anti-HCV negative)	Window period intervals	Incidence (%)	Incidence estimate intervals
0·43	70 days [9, 10]	56–63 days (mid-point: 60 days)	46–74 days	2·66	1·07–6·21
0·43	63 days [8]	49–56 days (mid-point: 52 days)	42–63 days	3·01	1·25–6·73
0·43	56–63 days [8]	42–56 days (mid-point: 49 days)	35–63 days	3·23	1·25–8·07

IDU, Injecting drug user; GUM, genitourinary medicine.

likely to be early seroconverters, gave a total of 11 HCV RNA-positive specimens classified as incident infections (Table 3). None of these specimens were anti-HIV-1 positive.

Genotyping of the incident infections identified the most common genotype as type 1 (Table 3), with type 3 being more frequently identified after 1998. Sixty anti-HCV-positive specimens from 2001 were tested for HCV RNA for comparative purposes. Of the 60 tested, 15 (25%) contained HCV genotype 1a, three carried 1b, eight carried 3a, one carried 3b, and two carried 4d. One specimen was identified as of indeterminate genotype, six had insufficient volume for genotyping and 24 were RNA negative.

The estimated prevalence of HCV RNA in the anti-HCV antibody-negative and indeterminate specimens was 0·43% (11/2532, 95% CI 0·22–0·78). Assuming that the mean window period is between 42 and 63 days, the observed prevalence can be converted to an incidence estimate. This gives an overall point estimate of incidence of between 2·66% and 3·23% (Table 4) taking account of the uncertainty about the true window period. The annual incidence in these injectors was estimated as being 3·01% (95% CI 1·25–6·73).

A significant downward trend in incidence was detected between 1996 and 2001 ($P=0·009$). The average annual decrease in the proportion of HCV RNA-positive specimens over the period 1995–2001 was estimated as 0·17%. This converts to an annual decrease in HCV incidence of 1·2% on the basis of a midpoint duration of pre-seroconversion viraemia of 52 days.

DISCUSSION

The prevalence and incidence of HCV in IDUs attending GUM clinics fell between 1995 and 2001. It has previously been shown that in GUM clinic attendees in England, the prevalence of HIV infection was highest in homosexual men [23]. The present study confirms the small extent of overlap between the HIV and hepatitis C epidemics in IDUs. The prevalence of HIV infection in IDUs in London was previously found to be 4·2% while that for HCV in the same study population was 43·7% [7].

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In our study, the estimated prevalence of HCV RNA positivity in anti-HCV-negative specimens was low, at 0·43%. HCV genotyping identified that types 1a, 1b and 3a were equally dominant HCV genotypes in new infections, suggesting that different transmission chains are occurring and similar to the seroconversions identified in a Netherlands study [24]. The distribution of genotypes in new infections was broadly similar to that in prevalent infections.

From these incident infections, we were able to estimate an annual incidence of 3·01% for the period 1995–2001, lower than previous UK-based studies. As outlined below, the differences may be due to several factors. The only other national estimates of HCV incidence in IDUs in England and Wales were based on HCV prevalence data in those attending specialist services. This used a mathematical modelling approach and estimated that the incidence in susceptible IDUs (force of infection) over the period 1999–2003 was 16% in the first year of injecting, declining to 6% thereafter [25]. Other UK studies have been conducted in areas of high hepatitis C prevalence, where HCV incidence is also expected to be high. The annual incidence was estimated to be 28·4% in Glasgow IDUs during the 1990s [6] and 41·8% during 2001 and 2002 in London [7]. In the London study, participants reported high levels of injecting risk behaviour in the previous 4 weeks. Another London study that tested stored serum from anti-HCV-negative IDUs for HCV RNA between 1999 and 2001 estimated the incidence to be 14·3% [26]. Based on a low rate of RNA positivity in stored samples from anti-HCV-positive IDUs, however, the authors of the latter study suggested that the specimens may not have been stored optimally, the implication being that the true HCV incidence may have been greater [26]. In our study, 29/54 (54%) specimens from anti-HCV-positive IDUs in 2001 were RNA positive, somewhat lower than the 74% expected [27]. This suggests that our specimens may not have been stored optimally for RNA detection, and the true number of acute infections may be greater. In addition, the specimens tested were from GUM clinic attendees who had admitted ever injecting drugs and may include ex-users who had not been at recent risk of HCV infection. As the GUM clinic survey principally targets sexual risk, detailed risk information on current and past injecting history is not available. Data from the National Survey of Sexual Attitudes and Lifestyles (NATSAL) survey in 2000–2001 suggest that in those with a history of injecting who also attended GUM clinics only 21% are current injectors [28]. Using this figure, this would suggest that annual incidence in current IDUs may be around five times higher than our estimates, i.e. about 15%, more consistent with previous studies.

Another possible explanation for the low HCV incidence observed in IDUs who attended GUM clinics, would be an incorrect assumption about the duration of the anti-HCV-negative/HCV RNA-positive window period. Estimates for the window period are generally derived from studies of infected donors and blood-product recipients using third-generation ELISAs [13]. We assume that the natural history of HCV in injectors does not differ from those studied but if the infectious window period in our population was shorter, then the true incidence could be higher. A cohort study of 358 IDUs in The Netherlands, however, observed a prolonged period of seronegative HCV viraemia in five (all HIV negative) of 19 HCV seroconvertors [29]. In addition, to maximize our identification of incident infections, we included HCV RNA-positive/anti-HCV antibody-indeterminate specimens on the basis that these probably represent recently acquired HCV infections.

The wide range of HCV incidence estimates obtained in IDUs in other countries is also consistent with differing levels of risk between IDUs in different settings, at different times and recruitment methods. In Europe, annual HCV incidence was found to be 4·2% and 11·7% in IDUs on methadone maintenance programmes in Switzerland [30, 31], 26·3% in those attending syringe exchanges in Sweden [32] and 9% in a prospective study in France [33]. In the United States, annual HCV incidence rates of 6·4%, 16% and 11% were found in retrospectively identified IDUs in Baltimore [34], prospective studies of IDUs in Baltimore [35] and young (aged <30 years) street-recruited IDUs in San Francisco, respectively [36]. In Canada, a prospective study in street-recruited injectors found an incidence of 29% [37] and the incidence was 27% in a retrospective study in Quebec [38]. Two recent cohort studies in Australia have found HCV incidence rates of around 30% [39, 40].

We demonstrated that incidence in our study population declined over the period 1995–2001. Although the proportion of anti-HCV-positive samples that were HCV RNA positive was slightly higher (64%) in 1995–1996 [16], storage conditions for the specimens were similar, and we do not believe that this decline could be explained by declining sensitivity of the technique. Evidence from Scotland suggests that the

incidence of HCV infection declined in the early to mid 1990s [5], but no major change in the force of infection was detected in England and Wales over the period 1999–2003 [25]. It is possible, that the decline in incidence observed in our study is due to a change in the population under study, for example, by including more ex-users in later years. The accompanying decline in prevalence is also consistent with lower risk in our study population in later years. Incidence may have increased after 2001, consistent with the higher prevalence rates in the UAPMP [41].

Further large scale cross-sectional studies are necessary to examine the validity of estimating HCV incidence through PCR approaches such as that described here in comparison with the conventional follow-up studies. The approach adopted in the present study presents an efficient means of estimating HCV incidence in unlinked anonymous surveys. Such strategies can be used effectively to inform ongoing public health surveillance. Assuming that the sensitivity of assays does not vary with time, the results obtained are valid for inferring trends in incidence in IDUs over time and between localities.

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DECLARATION OF INTEREST

None.

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