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The Prevalence of Hepatitis C in England and Wales

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Objectives: To estimate the background population prevalence of hepatitis C in England and Wales, observe the prevalence over time and assess the extent of infection outside of known risk groups.
Methods: Sera from residual specimens from adult patients submitted to laboratories in England and Wales were tested for anti-HCV. Testing was carried out using a cost-effective pooling strategy.
Results: Although the prevalence of anti-HCV was highest in 1986 (1.07%), in the multivariable analysis, prevalence did not vary significantly between the 3 periods 1986, 1991 and 1996 ($P = 0.14$). The prevalence of infection was higher in males than in females ($P = 0.0013$). An age-period-cohort analysis revealed a cohort effect due to a lower HCV prevalence in the most recent birth cohorts, that is, those born between the calendar years 1971–1975 and 1976–1980.
Conclusions: The majority of HCV infections in England and Wales were probably acquired before 1986. Infections in younger males identified in 1996 may signify more recent acquisition by injecting drug use.

Introduction

The hepatitis C virus (HCV) was first identified in 1989 [1]. The World Health Organisation estimates that there are 170 million carriers worldwide who are at risk of developing severe liver disease such as cirrhosis or hepatocellular carcinoma [2]. Population-based studies of hepatitis C infection indicate that the prevalence of infection varies between countries [3–6]. Countries known to have a high general population prevalence (>10%) include Egypt [7,8] other parts of Africa [9] and some parts of South America [10], whereas Japan and parts of Southeast Asia [11,12] are intermediate prevalence areas (2–10%). Compared to these countries, the prevalence of HCV in Western Europe [13] and the USA [14] is low (<2%).

Transmission of HCV occurs most effectively by injecting drug use and by the transfusion of infected blood and blood products [15]. Transmission via other routes such as from mother to baby and from sexual exposure is less efficient [16]. In England and Wales the majority of cases with HCV have been reported in people who have injected drugs [17].

The majority of the seroprevalence studies have been conducted in selected high risk groups, such as injecting drug users [18,19]. Studies in lower risk groups are mainly confined to blood donors [20,21] or antenatal women [22,23] who are likely to be at lower risk of bloodborne infection than the general population. Because of uncertainty about the numbers of people in high risk groups and the prevalence outside of these groups, the true prevalence of HCV in England and Wales is unclear. Estimates have ranged from 0.1% to 1% [24,25] but as up to 20% of chronic carriers go on to develop chronic liver disease [26] a more precise estimate will be useful for health care planning. Using sera from adult patients that had been submitted to

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laboratories in England and Wales for routine diagnostic examination, the prevalence of anti-HCV over time was investigated.

Materials and Methods

Stored specimens

In 1986, 1991 and 1996 the PHLS Seroepidemiology unit based at Preston Public Health Laboratory and the Communicable Disease Surveillance Centre collected between 4000 and 10,000 age–sex defined sera from residual specimens submitted to Public Health and National Health Service Laboratories in England and Wales for routine diagnostic examination. In 1986 and 1991 specimens from immunocompromised patients and specimens sent for testing for antibody to hepatitis B and the human immunodeficiency virus (HIV) were excluded. In 1996 all specimens were included irrespective of whether they were from immunocompromised patients or submitted for HIV or hepatitis B testing. Sera are stored at −20 °C with relevant details such as source laboratory, age, sex, identifying number and year of specimen collection retained. Infections investigated using this sera include measles, mumps, rubella, varicella-zoster, pertussis, and hepatitis B [27]. Anonymised serum residual specimens remaining from adults ($\geq$16 years of age) were therefore available for testing for anti-HCV. Specimens with sufficient volumes available for testing were taken from the years 1986, 1991 and 1996. Five laboratories contributed to the study in 1986 compared to six in 1991 and 17 in 1996. In 1986, public health laboratories (PHLs) from the Northern and Yorkshire, South East, South West and North West regions were included. In 1991, a PHL from West Midlands was added. In 1996 PHLs from Eastern and Wales were included and, in an attempt to get better representation of the population in greater London, 4 laboratories (2 NHS, 2 PHL) submitted specimens.

Laboratory methods

A pooling strategy similar to that described previously for testing specimens for anti-HIV was investigated and validated by the PHLS Hepatitis and Retrovirus Laboratory for anti-HCV prevalence testing [28]. This HCV pooling strategy was shown to have a sensitivity of approximately 99% (95% CI: 96.5–99.9) using pools of 12 specimens when compared with testing individual specimens for anti-HCV [22]. The most cost-effective pool size is dependent on the prevalence in the survey population [29] and for anti-HCV testing, pools of 12 were found to be cost-effective. Using these sample sizes retained adequate sensitivity.

Serological testing

Pooled serum specimens of 12 were tested using the Ortho® HCV 3.0 ELISA Test System (enhanced SAVe) in the Omni autoanalyser (Biotek Instruments, Vermont, USA). The optical density (OD) of the end product of the antigen–antibody–enzyme complex was used to compare each of the pool reactivities. Each specimen incorporated in a reactive pool was subsequently tested individually by the standard (long) 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 be discordant or weakly reactive by either or both assays were further tested with a recombinant immunoblot assay (Ortho® HCV RIBA 3). Specimens that were positive by the two separate ELISAs or one ELISA and a RIBA were identified as being serologically HCV positive. Specimens that were weakly reactive by one or more ELISAs but RIBA indeterminate were classified as indeterminate and excluded from the analysis of prevalence. One hundred and thirty-eight specimens from 1996 which were found to be positive for antibody to hepatitis B core antigen (anti-HBc) in a previous study [30] had been tested individually for anti-HCV by a single Ortho ELISA. Seventy-three of these specimens had insufficient volumes remaining for inclusion in the pools in this study but these results have been included in the overall analysis to obtain a better prevalence estimate. A total of 500 specimens from 1986, 332 from 1991 and 164 from 1996 were excluded from the study because of insufficient volumes remaining for testing.

Genotyping and serotyping

Serologically positive or indeterminate specimens with sufficient volumes remaining were genotyped using restriction fragment length polymorphism (RFLP) [31] and serotyped using the Murex HCV Serotyping 1–6 assay. HCV RNA was extracted using the Amplicor HCV Specimen Preparation Kit (Roche Diagnostic Systems, Welwyn Garden City, Herts, UK). The HCV 5′ noncoding region (5′ NCR) was amplified by nested PCR. The serotyping assay detects type specific antibodies, HCV types 1–6, against NS4 derived epitopes. Serotype identification was carried out according to the manufacturer's interpretative criteria.

Statistical analysis

Birth cohorts by calendar year were constructed according to the age and time period in which the specimens were collected. Initially the proportions of positive specimens by sex, year, birth cohort and laboratory were compared using a chi-square test, and Student's t test to compare prevalence by age. Multivariable logistic regression using an age-period-cohort model [32] was used to compare the prevalence of HCV by age, sex, laboratory, year and birth cohort and to determine the separate contributions of each of these factors. Interactions between these factors were also examined. Statistical significance was taken at the 5% level. Confidence intervals for the unadjusted prevalence by age were calculated using the exact binomial distribution.

Results

Anti-HCV prevalence

Testing of serum samples in each of the years has given an overall anti-HCV estimated prevalence of 1.07% (39/3647) in 1986, 0.55% (31/5634) in 1991 and 0.70% (45/6401) in 1996. In 1996, prevalence was 11/865 (1.27%) in the Greater London area, higher than the prevalence of 34/5536 (0.61%) in the rest of England and Wales.

The mean age of individuals with specimens used was 32.1 years (standard deviation (SD) 15.4; range: 16–99) in 1986, 42.1 years (SD 22.1; range: 16–107) in 1991 and 41.2 years (SD 19.4; range: 16–95) in 1996.

Two specimens from 1991 (persons aged 51 and 57 years, male and female respectively both from laboratory B) and two specimens from 1996 (24 and 25 years, both males and both from laboratory D) were identified as being of an indeterminate nature following RIBA testing. These 4 specimens were excluded from the serologically HCV positive group. Results for 73 anti-HBc positive specimens tested for anti-HCV by single Ortho ELISA in a previous study [30] which had insufficient volumes for inclusion in this study were included in the overall analysis (6 positive, 67 negative).

Serologically HCV positive serum specimens were identified in all age groups in the three years investigated. The mean age of the serologically HCV positives in 1986 was 27.1 years (SD 10.6; range: 16–75) compared to 41.6 years (SD 8.3; range: 17–82) in 1991 and 37.3 years (SD 14.6; range: 17–78) in 1996. In 1996 the mean age of positives was 38.9 (SD 15.90; range: 17–78) outside of London and 32.2 years (SD 8.00; range: 18–47) inside London. Overall, the crude anti-HCV positivity rate was higher in males (1.04%) than females (0.46%) ($P < 0.0001$). The greatest difference in prevalence was seen in 1986 where the prevalence in men was 29/1808 (1.6%) compared to 10/1839 (0.54%) in women ($P < 0.002$), the difference between males and females was also significant in 1996 ($P = 0.002$).

Amongst men in 1986, the highest prevalence was seen in younger males aged 20–24 years (2.79%; 11/394) whilst in 1991 and 1996 the prevalence was higher in all age groups up to 55 years. The highest prevalence in 1991 was in the 40–44 year age group (2.94%; 4/136) while in 1996 the highest prevalence was seen in the 30–34 year age group (2.67%; 8/300). In females in 1986, the majority of serologically HCV positive individuals were aged between 20 and 29 years but by 1996 the only infections identified were in women aged over 25 years. In males in 1996, prevalence in those aged under 25 was 7/731 (0.96%; 0.39–1.96), 6 of these positive specimens were from laboratory D.

For each of the three time periods 1986, 1991 and 1996, in those aged over 65 years, anti-HCV positivity was low and no positives above 85 years of age were identified. In those aged >65, the prevalence in males was 1/107, 1/499 and 1/546 in 1986, 1991 and 1996 respectively compared to 0/126, 3/588 and 3/545 in females.

The variation in prevalence between laboratories was significant in 1986 ($P = 0.007$), 1991 ($P = 0.04$) and 1996 ($P = 0.001$). In the seven laboratories that had data for more than one time period, only laboratory N showed a significant period effect ($P < 0.0001$). This period effect (year of specimen) in laboratory N was influenced by a high proportion of anti-HCV positives amongst males aged between 16 and 35 years in 1986. In 1986, a total of 17/400 males in the 16–35 year age group, 1951–1970 birth cohort (year of birth) from laboratory N were serologically positive compared to 0/224 in 1991 and 2/120 for 1996. For females, the prevalence was 3/410, 0/141 to 0/97 for 1986, 1991 and 1996, respectively.

Multivariable analysis: age-period-cohort

An age-period-cohort analysis was carried out to investigate the separate effects of age and period (year) and of birth cohort and period. Crude prevalence rates by five year birth cohorts after 1946 are shown in Table I. This

Table I. Unadjusted prevalence of anti-HCV by birth cohort and sex.

Birth cohort	Positive/number tested (%)							
	Male				Female			
	1986	1991	1996	Total	1986	1991	1996	Total
1976–1980	–	–	5/424 (1.18)	5/424 (1.18%)	–	–	0/457 (0.00)	0/457 (0.00%)
1971–1975	–	2/388 (0.52)	2/360 (0.56)	4/748 (0.53%)	–	1/454 (0.22)	0/399 (0.00)	1/853 (0.12%)
1966–1970	6/364 (1.65)	0/364 (0.00)	5/317 (1.58)	11/1045 (1.05%)	1/391 (0.26)	3/364 (0.82)	3/397 (0.76)	7/1152 (0.61%)
1961–1965	11/414 (2.66)	1/327 (0.31)	9/302 (2.98)	21/1043 (2.01%)	4/389 (1.03)	3/412 (0.73)	0/389 (0.00)	7/1190 (0.59%)
1956–1960	6/355 (1.69)	1/220 (0.45)	3/279 (1.08)	10/854 (1.17%)	2/343 (0.58)	2/360 (0.56)	4/360 (1.11)	8/1063 (0.75%)
1951–1955	4/185 (2.16)	4/185 (2.16)	3/247 (1.21)	11/617 (1.78%)	1/221 (0.45)	0/286 (0.00)	1/282 (0.35)	2/789 (0.25%)
1946–1950	0/130 (0.00)	3/132 (2.27)	4/164 (2.44)	7/426 (1.64%)	2/116 (1.72)	0/190 (0.00)	1/168 (0.60)	3/474 (0.63%)
1936–1945	0/138 (0.00)	3/256 (1.17)	0/252 (0.00)	3/646 (0.46%)	0/159 (0.00)	1/247 (0.40)	0/299 (0.00)	1/705 (0.14%)
1926–1935	1/86 (1.16)	1/237 (0.42)	1/286 (0.35)	3/609 (0.49%)	0/77 (0.00)	2/170 (1.18)	2/271 (0.74)	4/518 (0.77%)
1880–1925	1/136 (0.74)	1/472 (0.21)	0/365 (0.00)	2/973 (0.21%)	0/143 (0.00)	3/570 (0.53)	2/383 (0.52)	5/1096 (0.46%)
Total	29/1808 (1.60%)	16/2581 (0.62%)	32/2996 (1.07%)	77/7385 (1.04%)	10/1839 (0.54%)	15/3053 (0.49%)	13/3405 (0.38%)	38/8297 (0.46%)

shows a low prevalence rate amongst the most recent five year birth cohorts (1971–1975 and 1976–1980). It was not possible to assess the separate effects of age and cohort due to the extent of confounding. A significant period and laboratory interaction ($P = 0.0013$) due to the effect of laboratory N was detected. This skews the interpretation of the analysis and so laboratory N was investigated separately and excluded from the overall analysis.

The first analysis looked at age, period, laboratory and sex with birth cohort held constant. This "age model" showed no significant interactions. There was a clear laboratory ($P = 0.00005$) and sex ($P = 0.0013$) effect while the age effect was not significant ($P = 0.15$). The period effect when considered with age was significant ($P = 0.043$).

The second model looked at birth cohort, period, laboratory and sex with age held constant. This 'cohort model' (Table II) showed no significant interactions. Similar to the 'age model', a highly significant laboratory ($P = 0.00003$) and sex ($P = 0.0013$) effect was seen. The period effect was not significant ($P = 0.14$). The cohort effect was close to significance ($P = 0.065$). This can be seen to be largely due to the lower prevalence in the most recent birth cohorts, that is, in those born between the calendar years 1971–1975 and 1976–1980.

Analysis of laboratory N alone showed a highly significant period effect using both models, $P = 0.0026$ and $P = 0.006$, respectively. The difference between sexes was significant in both models ($P = 0.002$) as was the difference between age groups in the "age model" ($P = 0.049$) and birth cohort in the "cohort model" ($P = 0.048$).

Genotypes and serotypes

Of the serologically positive specimens tested, HCV RNA was extracted from only five. RFLP analysis identified genotype 1a (4 specimens in 1986) and one type 2b in

Table II. Cohort Model (multivariable analysis): Anti-HCV by laboratory, period, birth cohort and sex (adjusted).

Factor	Level	Odds ratio (95% CI)	*P*-value
Laboratory	A	1.00 (baseline)	0.00003
	B	0.95 (0.42–2.16)	
	C	1.05 (0.40–2.75)	
	D	3.94 (1.37–11.3)	
	E	0.90 (0.10–8.1)	
	F	0†	
	G*	0.69 (0.08–6.27)	
	H*	2.77 (0.62–12.4)	
	I	0.44 (0.14–1.43)	
	J	0†	
	K	0.24 (0.08–0.70)	
	L	0.28 (0.10–0.80)	
	M	0†	
	O	1.51 (0.42–5.42)	
	P	0.89 (0.10–8.0)	
	Q*	2.56 (0.57–11.5)	
	R*	4.10 (1.03–16.3)	
Period	1986	1.00 (baseline)	0.14
	1991	0.95 (0.41–2.19)	
	1996	0.44 (0.16–1.19)	
Cohort	1976–1980	0.63 (0.21–1.89)	0.065
	1971–1975	0.44 (0.15–1.28)	
	1966–1970	1.07 (0.49–2.34)	
	1961–1965	1.61 (0.78–3.30)	
	1956–1960	1.30 (0.60–2.83)	
	1951–1955	1.34 (0.57–3.16)	
	1946–1950	2.36 (1.00–5.58)	
	1880–1945	1.00 (baseline)	
Sex	M	2.03 (1.31–3.16)	0.0013
	F	1.00 (baseline)	

Note: laboratory N excluded from overall analysis, see Results.
*Laboratories in the Greater London area.
†95% CI could not be calculated for odds ratio of zero.

Table III. HCV serotype distribution in residual specimens.

	Serotype						Indeterminate	Total
	1	2	3	4	5	6		
Year								
1986	19*	–	9	1	–	–	1	30
1991	7	–	6	–	–	–	6	19
1996	11	3*	8	3	–	–	8	33
Total	37	3	23	4	–	–	15	82

Note: Table excludes two RIBA indeterminate specimens from 1996 that were indeterminate after serotyping.
*Includes one specimen that had been genotyped that was identified as indeterminate after serotyping.

1996. A total of 30/39 (77%), 19/31 (61%) and 33/45 (73%) specimens from 1986, 1991 and 1996 respectively where sufficient volumes remained were serotyped. Two indeterminate specimens from 1996 were also serotyped but were also indeterminate by serotyping. Three of the four specimens identified as genotype 1a corresponded to serotype 1. Serotyping of the remaining genotyped specimens (1a from 1986 and 2b from 1996) revealed that they were of an indeterminate nature. The results from the serotyping and genotyping are summarised in Table III. The most prevalent serotypes were types 1 and 3. HCV types 5 and 6 were not identified. Numbers were too small to determine any significant differences by age, birth cohort, gender or laboratory.

Discussion

The overall prevalence of HCV infection in residual specimens from adult patients suggests that transmission of HCV outside of high risk groups is low in England and Wales. Prevalence of anti-HCV was highest in the 1986 sample population. Prevalence varied by laboratory and was higher in laboratories from Greater London compared to the rest of England and Wales. The majority of infections were seen in men and the prevalence was twice as high in males compared to females. An ageing birth cohort effect probably contributed to the older profile of anti-HCV positive males and females seen in the 1990s. It is not known what the specimens in this study had been originally tested for. Hepatitis C was identified in 1989 and therefore, some specimens in 1991 and 1996 could have been investigated for this particular infection. If this had indeed been the case, such sampling bias would have potentially increased prevalence in the late periods, however unless this bias was age-dependent it would not effect prevalence by age or cohort after adjusting for period in the multivariable models. In fact a lower anti-HCV prevalence was observed in 1991 and 1996 compared to 1986.

In this study HCV RNA detection was poor and prevented genotype identification. These specimens had been in storage for many years and had not been handled specifically to optimise HCV RNA recovery. It is likely that in many of the specimens HCV RNA degraded to undetectable levels. Serotyping has been shown to correlate well with genotyping [33,34] and even though it is unable to differentiate between subtypes, it is able to detect type specific antibodies when HCV RNA is undetectable. Serotype analysis showed that types 1 and 3 were the most prevalent in each of the three years studied. The most prevalent genotypes in England and Wales are 3a and 1a [31], with type 3 most common among IDUs [35]. It has been suggested that type 3a and 1a have been introduced into Europe by needle sharing amongst injecting drug users [36], and our findings are therefore consistent with the majority of infections having been acquired by injecting drug use. A study of the serotype profiles from Australian IDUs showed that the HCV serotypes identified in specimens from the 1970s differed from those in specimens from IDUs in the 1990s [37], with a decrease in serotype 1 and an increase in serotype 3. In this study in 1986, only one type 4 was identified, although by 1996 serotypes 2 and 4 were detected more commonly. This suggests a changing profile of circulating types in England and Wales as strains from specific geographical regions of the world are introduced into the country. HCV types 1, 2 and 3 are commonly found in Europe whereas type 4 is common in Northern and Central Africa and the Middle East [38].

This overall prevalence observed in this survey is in line with other studies from Western Europe [13] and the USA [14]. In France, surveys of persons having a Social Security health check found an overall prevalence of 1.2% and prevalence was strongly associated with age and sex [39]. A serosurvey of 2,203 adults in South-east Spain found an overall anti-HCV prevalence of 1% and reported an increasing trend with age and no difference by sex [40]. A study from the USA found a prevalence of anti-HCV of 1.8% with the highest prevalence both in older age groups and in males [14]. A high prevalence was observed in southern Italy where 12.6% of subjects were positive for anti-HCV [41] and prevalence increased from 1.3% in subjects younger than 30 years to 33.1% in those aged over 60 years of age. Unlike our study, a higher positivity rate was seen in females compared to males. It has been suggested that the high prevalence in the elderly population in Italy was due to an epidemic of HCV during and after the Second World War [42]. The

use of reusable glass syringes and other non-disposable materials for tuberculosis treatment have been implicated as the cause. The authors suggested a cohort effect to explain the high anti-HCV prevalence rates in the elderly population with a decreased risk of infection amongst younger generations. In Japan, higher HCV positivity rates are also seen in the older generation and this has been attributed to the re-use of needles and syringes for vaccination, surgical operations for tuberculosis infected patients and blood transfusions from paid donors during the 1930s and 1940s [11]. In this study, however, the prevalence in those aged over 65 years was lower than amongst younger adults. A UK study of only 25 anti-HCV positive patients aged more than 65 years identified a history of blood transfusion as the most common risk factor [43], although another possible risk included overseas service during the Second World War. Transmission of HCV infection in England and Wales took place via transfusion and use of blood products prior to the introduction of heat treatment processes (in 1985) and donor screening (in 1991). The age distribution of infections identified in this survey, however, suggests that the numbers of infections acquired from contaminated blood and blood products is small in comparison to the number associated with injecting drug use. If a large epidemic of HCV infection had occurred in England and Wales as a result of blood transfusion, a higher prevalence of infection may be expected amongst the elderly. Exposure during overseas service in the war, would be expected to lead to a higher prevalence amongst older males, whereas the prevalence of anti-HCV in both 1991 and 1996 was slightly higher in older females than older males.

Most of the HCV infections in the population studied in this survey were probably acquired before 1986, mainly amongst people born between 1946 and 1970. The low prevalence in the more recent birth cohorts, implies that the incidence of HCV infection has declined. This epidemic is probably primarily associated with acquisition of HCV through injecting drug use. Seroprevalence studies both in the UK and Europe have found prevalence levels ranging from 50% to 90% in injecting drug users and the importance of drug use as a major risk factor for infection has been well documented [18,19,44,45]. The use of recreational drugs in the UK increased steadily during the 1960's and into the 1970s [46]. During this time, non-therapeutic heroin misuse emerged in London and spread to neighbouring counties. More widespread injecting of other illicit drugs, such as barbiturates, also increased during this period. The age profile of persons now presenting with HCV liver complications who have acquired HCV through injecting drug use reflects these historical patterns of injecting drug use [47]. It is also likely that injecting drug use explains the high prevalence seen in young males in 1986, in particular in laboratory N. In the mid-eighties there was an epidemic of hepatitis B infection amongst injecting drug users in England and Wales, facilitated by sharing of injecting equipment [48]. This hepatitis B epidemic may have occurred concurrently alongside an epidemic of HCV within the same group.

Although the prevalence in females is consistent with the ageing of individuals infected prior to 1986, in 1996, a number of infections were identified in males under 25 years of age (birth cohort 1971–1975, 1976–1980). This finding may signify more recent HCV acquisition, most likely due to injecting drug use. The excess in young males and in the higher number of cases in the London area in 1996 reflects the prevalence of injecting drug use [49]. This emphasises the continual need for the targeting of public health intervention strategies at young injectors and the ongoing need for seroprevalence studies to monitor trends.

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