Distribution of hepatitis C virus genotypes in Canada: Results from the LCDC Sentinel Health Unit Surveillance System

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In a sentinel hepatitis surveillance study conducted by sentinel health units, 1469 patients were enrolled, and 959 (65.3%) were positive for antibody to hepatitis C virus (HCV). Samples from 387 patients (40.4%) were tested for HCV RNA, and 289 (74.7%) were positive for RNA. The major risk factor for HCV infection was injection drug use, reported in 71% of cases. The genotyping of HCV isolates showed that subtype 1a (48%) was predominant in Canada. The other subtypes detected were 1b (19%), 2a (6%), 2b (3%), 3a (22%) and 4a (1%). In Winnipeg, Manitoba, subtype 3a (47%) was more prevalent than subtype 1a (37%), and, in Guelph, Ontario, both subtypes 1a and 3a had equal (40%) distribution. The prevalence of subtype 3a was significantly higher in injection drug users (27%) than in nonusers (10%) ($P < 0.005$). In Canada, injection drug use is the major risk factor for HCV infections, and subtype 1a is more prevalent.

Key Words: *Genotyping, Hepatitis C virus, Sentinel Health Unit Surveillance System*

Distribution des génotypes du virus de l'hépatite C au Canada : Résultats du système de surveillance des unités de santé sentinelles du LLCM

RÉSUMÉ: Dans une étude sentinelle de surveillance de l'hépatite menée par des unités de santé sentinelles, 1469 patients ont été inclus, et 959 (65,3 %) étaient porteurs d'anticorps du virus de l'hépatite C (VHC). Des échantillons prélevés chez 387 patients (40,4 %) ont été analysés pour une recherche d'ARN du VHC, et 289 patients (74,7 %) testaient positifs pour l'ARN. Le principal facteur de risque pour une infection au VHC était l'utilisation de drogues intraveineuses, signalée dans 71 % des cas. Le génotypage des isolats de VHC a révélé que le sous-type 1a (48 %) prédominait au Canada. Les autres sous-types décelés étaient le 1b (19 %), 2a (6 %), 2b (3 %), 3a (22 %) et 4a (1 %). À Winnipeg, au Manitoba, la prévalence du sous-type 3a (47 %) était supérieure au sous-type 1a (37 %) et, à Guelph, en Ontario, la distribution des sous-types 1a et 3a était égale (40 %). La prévalence du sous-type 3a était nettement plus importante chez les usagers de drogues intraveineuses (27 %) que chez les non-usagers (10 %) ($p < 0,005$). Au Canada, l'utilisation de drogues intraveineuses représente le principal facteur de risque pour les infections au VHC et le sous-type 1a prédomine.

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Hepatitis C virus (HCV) was discovered in 1988, and scientific publication of the finding occurred in 1989 (1); subsequently a number of HCV isolates were cloned and sequenced. On the basis of sequence analysis, six major types were identified (2), and each type has one, two or three subtypes. Subtypes are designated as 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 5a and 6a. HCV can be genotyped by the polymerase chain reaction (PCR) with type-specific primers (3), specific endonuclease digestion (4), specific probes using hybridization (5) or by sequencing (2). Genotyping is an important tool for epidemiological studies and has a predictive value for the results of interferon therapy (6).

In the United States and Canada, type 1a predominates (7,8), whereas in Japan types 1b and 2a are more prevalent (3). This raises the question of whether genotypic differences are responsible for a more severe chronic form of HCV infection in Japan than in North America (9). Evidence indicates that infection with HCV subtype 1b produces more severe chronic liver disease in Japan (9). This indicates that there might be a relationship between genotype and the severity of the disease. Genotype was also reported to influence the results of treatment with interferon; patients infected with subtype 2a responded better than those infected with subtype 1a (6). However, recent data also suggest that response to interferon is dependent on the viral load (10).

When some Canadian isolates of HCV were genotyped (11-13), the dominant subtype was 1a. These studies were performed in a few small centres and do not represent the general Canadian population. The Laboratory Centre for Disease Control (LCDC) Sentinel Health Unit Surveillance System (SHUSS) consists of eight health units serving a combined population of 2,373,000 in 1993. SHUSS was used to collect data on newly identified cases of HCV (as well as incident cases of hepatitis B virus and hepatitis A virus). Reported cases were investigated by the health nurses of the health units, and blood samples were collected. Information on demographics and risk factors was also collected during an interview with the patient. Samples were forwarded to the LCDC for further testing.

In this study, we present data on HCV infection, virus genotypes and risk factors of patients referred to SHUSS health units in Canada.

MATERIALS AND METHODS

The Sentinel Hepatitis Surveillance Study was conducted at the eight SHUSS health unit sites from October 15, 1993, to March 31, 1995. The health units were in Kelowna, British Columbia; Edmonton, Alberta; Saskatoon, Saskatchewan; Winnipeg, Manitoba; Kingston, Ontario; Guelph, Ontario; Sherbrooke, Quebec, and Prince Edward Island. The cases were referred to the centres by physicians. Patients were approached to participate in the study by the health unit nurses and were asked questions by telephone.

This report deals with HCV cases only. Each participant was asked questions regarding risk behaviour. HCV samples were tested for antibody by different laboratories participating in the SHUSS.

The HCV RNA was detected by Amplicor HCV test (Roche Diagnostics System). The manufacturer's protocol was followed. HCV RNA was extracted from 100 L of serum and reverse transcribed with DNA polymerase from *Thermus thermophilus* at 60 C for 30 mins. The cDNA was amplified for 38 cycles using biotinylated primers from 5' end; each cycle comprised 15 s at 90 C, 20 s at 60 C and hold at 72 C for 15 mins. The amplified product was detected by an enzyme immunoassay system following denaturation with 1.6% sodium hydroxide. The HCV RNA-positive samples were genotyped using Inno-LiPA HCV II (Innogenetics, Zwijnaarde, Belgium). The test was performed according to the recommended protocol. In this assay, oligonucleotides derived from the 5' noncoding region act as specific probe for each genotype. The specific probes are immobilized as parallel lines on nitrocellulose strips and are hybridized with biotinylated amplified PCR product. Following hybridization and washing steps, the strips were treated with streptavidin labelled with alkaline phosphatase. Substrate nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl-phosphate toluidinium was used for colour development. The pattern of reactivity to different probes was noted, and subtypes were interpreted by comparison with a template. Z distribution was used to estimate and compare proportions (14).

RESULTS

In this study, 1469 patients were enrolled for investigation of hepatitis A, B and C. The majority of patients, 959 (65.3%), were positive for the antibody to HCV. Three hundred and eighty-seven samples (40.4%) were tested for HCV RNA, and 289 (74.7%) were positive. The HCV RNA-positive samples were tested for genotypes, and 263 (91%) were typed (Table 1). The major risk factor for HCV infection was injection drug use (Table 2) in 186 patients (72%). The remaining 71 patients (28%) infected with HCV had other risk factors such as blood transfusion, sexual contact, occupational exposure and contact with HCV-positive cases. No samples were received from Prince Edward Island.

The HCV genotyping results for different health units are given in Table 1. The results showed that subtype 1a was predominant in Canada (48%). The other subtypes found were 1b (19%), 2a (6%), 2b (3%), 3a (22%) and 4a (1%).

On a health unit basis, subtype 1a was predominant in Kelowna (61%), Edmonton (52%) and Kingston (49%). The prevalence rate for 1b varied (11% to 30%). Subtype 2a was not detected in Winnipeg and Sherbrooke, whereas the subtype 2b was not detected in Winnipeg, Guelph, Kingston and Sherbrooke. Subtype 3a was the second most prevalent subtype after 1a; it was detected in all provinces, and the rate varied from 15% to 50%. Subtype 4a was rare in Canada and was only detected in one person from Alberta and one from Manitoba.

The distribution of different subtypes in injection drug users (IDUs) is given in Table 2. Forty-seven per cent of IDUs were infected with subtype 1a. Other subtypes detected among IDUs were 1b (18%), 2a (5%), 2b (3%) and 3a (27%). Among non-IDUs, the prevalence rate for 1a, 1b, 2a, 2b and 3a was 55%, 21%, 10%, 4% and 10%, respectively.

Both IDUs and non-IDUs had similar prevalence rates for

TABLE 1

Results of hepatitis C virus subtyping from different sentinel centres participating in the Laboratory Centre for Disease Control Sentinel Health Unit Surveillance System

Site	Number of specimens tested	Number positive by PCR (%)	Number typed (%)	Number of different subtypes (%)					
				1a	1b	2a	2b	3a	4a
Kelowna, British Columbia	55	44 (88)	41 (93)	25 (61)	6 (15)	2 (5)	2 (5)	6 (15)	0
Edmonton, Alberta	130	102 (79)	92 (90)	48 (52)	16 (17)	6 (7)	2 (2)	19 (21)	1 (1)
Saskatoon, Saskatchewan	57	42 (74)	40 (95)	14 (35)	8 (20)	4 (10)	5 (13)	9 (23)	0
Winnipeg, Manitoba	28	21 (75)	19 (90)	7 (37)	2 (11)	0	0	9 (47)	1 (5)
Guelph, Ontario	15	11 (73)	10 (91)	4 (40)	0	2 (20)	0	4 (40)	0
Kingston, Ontario	94	65 (69)	57 (88)	28 (49)	17 (30)	2 (4)	0	10 (18)	0
Sherbrooke, Quebec	8	4 (50)	4 (100)	1 (25)	1 (25)	0	0	2 (50)	0
Total	387	289 (74.7)	263 (91)	127 (48)	50 (19)	16 (6)	9 (3)	59 (22)	2 (1)

PCR Polymerase chain reaction

different subtypes except for 3a, which was significantly higher ($P < 0.005$) in IDUs (27%) than in non-IDUs (10%). The high prevalence of subtype 3a in Guelph and Kingston was not significant.

DISCUSSION

This is the most extensive and representative Canadian report on HCV genotypes. The results show that the majority of viral hepatitis at SHUSS health unit sites was due to HCV. Prevalence of HCV was also reported in the United States, where the four counties sentinel study showed HCV to be the major etiological agent for viral hepatitis (15).

The major risk factor for HCV infection was injection drug use in 72% of patients tested. Other risk factors, such as blood transfusion, sexual contact, occupational exposure and family exposure, played a minor role. Injection drug use has been reported as a major risk for HCV in the United States (15), and a study at the National Institutes of Health, Maryland (9), showed that 80.0% of HCV carriers had apparent or covert parenteral exposure. In Canada, intravenous drug use has been noted as the major risk factor for HCV infection (16).

Genotyping results showed the prevalence of three major types of HCV in Canada (1a, 1b and 3a), and two of them (1a and 1b) were predominant in the United States (17). Subtype 4a was only detected in two cases. Currently, there are six major genotypes of HCV prevalent in the world (2), with 11 subtypes (1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 5a and 6a). The detection of subtypes 1a, 1b, 2a, 2b and 3a has been reported in Canada (11). In addition to these subtypes, the detection of 1c and 2c was reported (12) in Vancouver, British Columbia, and that of 5, 6a, 7c in Montreal, Quebec (13). The subtype 7c variant has been isolated in Vietnam (18); subtype 7c was isolated in Montreal from a Vietnamese patient. However, in this study subtypes 1c, 2c, 5, 6a and 7c were not detected. One of the reasons for this difference may be that these studies were local (few patients in small locations of the city were studied); subtypes 5, 6a and 7c were detected in immigrants at a very

TABLE 2

Hepatitis C virus subtypes distribution in injection drug users and nonusers

Injection drug use	Number of different subtypes (%)					
	1a	1b	2a	2b	3a	Total*
Yes	87 (47)	33 (18)	9 (5)	6 (3)	51 (27) [†]	186
No	39 (55)	15 (21)	7 (10)	3 (4)	7 (10)	71
Total	126 (49)	48 (19)	16 (6)	9 (4)	58 (23)	257

*Risk factor information was unknown for six cases; [†] $P < 0.005$, $\chi^2 = 0.05$

low level (0.3%). Overall, subtype 1a had a higher prevalence rate than other subtypes in Canada. However, in Winnipeg and Sherbrooke, subtype 3a was more prevalent, and in Guelph, the prevalence rates for both subtypes 1a and 3a were the same (40%). Subtype 1b was more prevalent (30%) in the SHUSS health centre in Kingston than in any other health centre. These results indicate that the distribution of different subtypes is not uniform in different locations. The genotypic differences may explain differences in clinical severity of cases (9); for example, the patients infected with subtype 1a and 1b in the United States had more severe disease and a lower rate of response to interferon than those infected with subtypes 2a and 2b (16). However, there are indications that the subtypes are not necessarily associated with different outcomes (9).

The distribution of HCV subtypes in IDUs and non-IDUs was similar for 1a, 1b, 2a and 2b. However, the prevalence of subtype 3a was significantly higher in IDUs than in non-IDUs. A similar observation was made in Vancouver where the prevalence of subtype 3a was significantly higher in IDUs than in non-IDUs (12).

CONCLUSIONS

Regarding the epidemiological pattern of HCV infection in Canada, injection drug use is the major risk factor for the transmission of HCV, and subtype 1a is the predominant subtype.

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