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Estimating Incubation Period and Predicting Risk of Developing Hepatocellular Carcinoma in Patients with HCV Infection from the Clinic of a County-Level City in China*

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Abstract

***Background*: To estimate incubation period in patients infected with hepatitis C virus (HCV) and to predict patients' risk of developing hepatocellular carcinoma (HCC) for indicating health policy-making. *Methods*: An outbreak of HCV infection from a clinic was occurred due to the injection treatment of varicose veins called minimally invasive thrombolysis and meridian activating therapy (MITMT) in a county-level city of China. Conventional method and bootstrapping approach were used to estimate HCV incubation period. Risk assessment model generated from the previous study was applied to predict the risk of developing HCC in the coming 5-, 10-, and 15-year for HCV infected patients from the above outbreak. *Results*: A total of 120 subjects were underwent MITMT between October 22, 2012 and January 28, 2013; of those, 99 subjects were diagnosed with HCV infection. Mean age of the subjects was 54.42 years old, and females were accounted for 51.5% in contracted subjects. Mean incubation period of hepatitis C (HC) was 45.76 days using first MITMT as infected date and 30.68 days using bootstrapping approach, their standard deviations were 23.01 and 15.05 days, respectively. A total of 1.3, 4.7, and 10.6 persons will be developed into HCC in the coming 5, 10, and 15 years in infected subjects based on a risk prediction model. *Conclusion*: Some patients with HCV infection from a nosocomial clinic in a county-level city will be suffered**

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from more severe HCC in the coming decade; effective measures and controls were urgently needed to manage these patients with high risk of developing into end-stage liver disease.

Keywords

Hepatitis C Virus, Incubation Period, Hepatocellular Carcinoma, Prognosis

1. Introduction

Hepatitis C was caused by hepatitis C virus (HCV) that was successful cloning of its genome in 1989 and had been identified as one of leading causes of chronic hepatitis, cirrhosis, and liver cancer [1]. It was estimated that a global prevalence of HCV was 2.35%, affecting 160 million chronically infected individuals [2]. In China, the 1992-1995 national sero-epidemiology survey conducted in 145 Disease Surveillance Points (DSPs) demonstrated that the prevalence rate of anti-HCV was 3.2% [3], estimated 38.4 million persons were infected with HCV. Another sero-epidemiological study using the remaining blood samples of the nationwide survey of hepatitis B that was carried out in 2006 from 160 DSPs revealed that the prevalence rate of anti-HCV was 0.43% [4], it was speculated that about 5.6 million individuals were affected in China. Recently, the Global Burden of Disease Study 2010 (GBD 2010) showed that the median percent changes of all ages deaths, age-standardized death rate, all ages DALYs (disability-adjusted life years), and age-standardized DALY rate for acute hepatitis C had the fastest increase among all types of hepatitis [5]; therefore, HCV infection was a severe public health problem at present and in the near future in China.

An outbreak of HCV infection was occurred in a nosocomial clinic in a county-level city of China, a total of 99 cases with HCV-RNA positive were identified. These data were used to estimate the incubation period of HCV infection using two kinds of statistical approaches for better understanding the natural history and the epidemiology of HCV infection and to predict the risk of developing hepatocellular carcinoma (HCC) using risk prediction model for indicating health policymaking.

2. Materials and Methods

2.1. The Outbreak of HCV Infection

A clinic affiliated to the socio-medical insurance hospital in a county-level city of China began injection treatment of varicose veins called minimally invasive thrombolysis and meridian activating therapy (MITMT) on October 22, 2012. On January 28, 2013 municipal health bureau received report that some patients undergoing the MITMT were hospitalized due to elevated ALT (alanine amiotransferase). The local Center for Disease Control and Prevention (CDC) shut down the clinic in the same day and began investigating all 120 patients with varicose veins being treated in the clinic, including questionnaire survey, physical examination, and blood test. Epidemiological survey was completed on February 6, 2013, 117 subjects were surveyed and taken blood samples that were sent to the prefecture-level hospital for infectious diseases to test hepatitis C RNA, anti-HCV antibody, hepatitis B surface antigen, HIV, and syphilis; three subjects were not contacted owing to be hospitalized out of the town. Testing results showed 96 out of 117 subjects were tested positive for hepatitis C RNA; simultaneously 3 subjects out of the town were confirmed hepatitis C RNA positive as well. As such, a total of 99 subjects (82.5%) were infected with HCV out of 120 patients with varicose veins who had been treated using MITMT during the period of 98 days between October 22, 2012 and January 28, 2013.

The procedure of treating patients using MITMT illustrated in **Figure 1**. The doctor drew 5 - 10 ml blood from each patient's veins of lower extremity using a 5 ml syringe, disposed of the sampled blood and needle, drew physiological saline from a bottle with a needle hub (**Figure 1**), and washed the syringe. Then, using the same syringe drew 2.5 ml fluid drug from a medicine bottle with a needle hub (**Figure 1**), injected into the patient's veins of low extremity using a new needle. Each patient made use of their own syringe and needle, but the physiological saline bottle and medicine bottle with needle hubs had not been replaced for each patient and had been in place since the beginning of MITMT. This procedure of contracting patients was confirmed by epidemiological survey conducted by local CDC.

Figure 1. Simulated picture according to patients being treated in the clinic.

2.2. The Estimation of HCV Incubation Period

Ninety-six subjects out of 117 subjects were tested positive for hepatitis C RNA; three subjects out of the town for the treatment were confirmed positive for hepatitis C RNA as well, but no their epidemiological data; finally, a total of 99 subjects with HCV infection were left in the descriptive analysis, and 96 for the analysis of incubation period and risk prediction of developing HCC.

Incident date was considered as the onset date for 60 subjects who had a definite date of onset from the epidemiological survey; the rest of 36 subjects that had no date of onset, the date of blood sample taken (between February 1, 2013 and February 3, 2013) was defined as the incident date. Epidemiological survey had illustrated that none of subjects had been previously-infected with HCV. Two assumptions were proposed to determine the date of HCV infection, resulting in two statistical methods to estimate HCV incubation period. 1) The first date of MITMT was defined as the date of HCV infection; 2) The date of HCV infection was generated from a bootstrapping approach. Theoretically, patient's each MITMT had a chance to be infected, each date of MITMT could be considered as the date of HCV infection, so a number of incubation periods were calculated for a person using the incident date and a variety of HCV probable infection dates. For each patient, 1000 bootstrapping samples, each sample with identical number of incubation periods of the subject were selected, then mean of 1000 incubation periods was calculated to present final incubation period for the subject.

2.3. The Prognosis of HCV Infection

A risk prediction model was applied to predict the risk of developing HCC for individuals who had been identified to be seropositive of HCV infection [6].

Briefly, in the present analysis for 96 subjects with HCV infection seven statistically significant risk predictors of age, sex, serum ALT level, ratio of AST to ALT, liver cirrhosis status, HCV RNA, and HCV genotype were put in the model to predict the risk of developing HCC in the coming 5-, 10-, and 15-year. Each of seven coefficients was converted into an integer risk score by rounding the quotient of dividing the coefficient by a constant of the model; the constant was the regression coefficient for 5-year increase in age, making the integer risk score for a 5-year increase in age to be one [7]; the equation of predicting risks of HCC by the sum of risk scores was as followings [7]:

$$1-P_0^{\exp\left(\sum \beta_{age}\times score-\sum \beta_i \times M_i\right)}.$$

where P_0 was the baseline probability for the disease free, β_i was the regression coefficient for the *ith* variable X_i, and the M_i denoted the mean level of X_i. This risk prediction model was employed to predict the risk of developing HCC in the coming 5-, 10-, and 15-year after HCV infection using seven risk predictors of age, sex, serum ALT level, ratio of AST to ALT, liver cirrhosis status, HCV RNA, and HCV genotype.

All of the statistical analyses were conducted by SAS version 9.2 for Windows (SAS Institute Inc., Cary, NC, USA).

3. Results

A total of 120 subjects were underwent MITMT between October 22, 2012 and January 28, 2013; of those, 99

subjects were diagnosed with HCV infection, three subjects were out of the town for the treatment of hepatitis C, and 96 subjects had been interviewed and taken blood samples for the test. In 99 subjects, mean age was 54.42 years, with a minimum of 26 years and a maximum of 80 years; the characteristics of 99 patients with HCV infection were presented in **Table 1**.

Incubation period calculated from two types of methods was illustrated in **Table 2**. Mean, standard deviation, median, minimum, and maximum of HCV incubation period were 45.76, 23.01, 44.00, 0.00 and 102.00 days from the first MITMT method, and 30.68, 15.05, 29.00, 1.00 and 65.00 days from the bootstrapping approach. Mean incubation period from the first MITMT method was longer than that from the bootstrapping approach due to assumption made in the calculated.

The risk of developing HCC for patients with HCV infection was shown in **Table 3**. The risk of developing HCC in the coming 5-, 10-, and 15-year was 1.36%, 4.70%, 10.75%; based on the above values a total of 1.3, 4.7, and 10.6 persons will be developed into the HCC at 5, 10, and 15 years among 96 subjects infected with HCV.

4. Discussion

Most cases of hepatitis C were associated with transfusion at the time, came in 1975; so often the incubation period of HCV infection was derived from blood donors infected with HCV. Therefore, the estimation of incubation period using other data sources was necessary to better understand the nature history and epidemiology of hepatitis C. Furthermore, hepatitis C was linked to the risk of developing HCC in many areas of the world [8] [9]. These data were used to predict prognosis of patients with HCV infection using risk prediction model to indicate health policymaking.

Usually many HCV infections were insidious and 80% of persons developing acute hepatitis C had no any symptom. In a follow-up survey [10], a total of 30,140 subjects who participated in the National Health and Nutrition Examination Survey (NHANES) from 2001 through 2008 were tested for HCV infection, 393 persons who tested positive for past or current HCV infection were interviewed after 6-month examination, and only

Table 1. Characteristics of 99 patients with HCV infection.

Variables	No. of Subjects	Percent (%)	Variables	No. of Subjects	Percent (%)
Age (years)			**Education**		
20-	1	1.0	Illiteracy	2	2.0
30-	8	8.1	Primary school	35	35.4
40-	20	20.2	Middle or high school	57	57.6
50-	40	40.4	College or higher	5	5.1
60-	24	24.2	**Gender**		
70-	5	5.1	Male	48	48.5
80-	1	1.0	Female	51	51.5
Occupation			**Marital status**		
Cadres	1	1.0	Married	97	98.0
Workers	15	15.2	Unmarried	1	1.0
Peasants	35	35.4	Death of a spouse	1	1.0
Teachers	4	4.0			
Self-employed	13	13.1			
Others	31	31.3			

Table 2. Incubation period calculated from two types of methods.

Incubation Periods (Days)	First MITMT (Method 1)		Bootstrapping (Method 2)	
	No. of Patients	Percent (%)	No. of Patients	Percent (%)
1 - 7	5	5.3	8	8.5
8 - 14	3	3.2	3	3.2
15 - 21	6	6.3	17	18.1
22 - 28	9	9.5	16	17.0
29 - 35	9	9.5	17	18.1
36 - 42	13	13.7	13	13.8
43 - 49	11	11.6	9	9.6
50 - 56	6	6.3	5	5.3
57 - 63	11	11.6	5	5.3
64 - 70	10	10.5	1	1.1
71 - 77	3	3.2	0	0.0
78 - 84	2	2.1	0	0.0
85 - 91	6	6.3	0	0.0
92 - 98	1	1.1	0	0.0
Mean (days)	45.76		30.68	
Total	95	100.0	94	100.0

One subject was missed data for method 1; two subjects were missed data for method 2.

Table 3. Risk of developing HCC in a certain of years for patients with HCV infection using risk prediction model.

	Risk Score	No. of Subjects	5-Year Risk (%)	10-Year Risk (%)	15-Year Risk (%)
	3	1	0.24	0.86	2.04
	4	5	0.33	1.17	2.78
	5	9	0.45	1.59	3.76
	6	8	0.61	2.17	5.09
	7	9	0.84	2.94	6.88
	8	20	1.14	3.99	9.26
	9	15	1.55	5.39	12.40
	10	21	2.11	7.28	16.51
	11	2	2.86	9.78	21.80
	12	3	3.88	13.09	28.47
Mean	7.98		1.36*	4.70*	10.75*

* Mean was a weighted-mean by a proportion of the number at each category of risk score to the total number. Ninety-three subjects were successfully made risk prediction for HCC out of the 96 subjects.

49.7% of the positive were aware of their HCV infection before being notified by NHANES. This also implied exactly-exposed date was unknown for most cases of hepatitis C; therefore, the estimation of HCV incubation period was variable. From the previous studies, average incubation period was around 7 weeks ranged from 3 to 20 weeks. In our analysis, two methods were applied to estimate incubation period of HCV infection, mean, standard deviation, median, minimum, and maximum of HCV incubation period were 45.76, 23.01, 44.00, 0.00, and 102.00 days from the first MITMT method, and 30.68, 15.05, 29.00, 1.00, and 65.00 days from the bootstrapping approach. The first estimate of HCV incubation period (45.76 days) was close to 7 weeks, probably the first MITMT method was similar to that used in the previous studies; for example, the date of HCV infection was assumed to be the first exposed date. Frequently, HCV incubation period was derived from blood donors infected with HCV, this assumption might be plausible, but in most cases this might not be true. Bootstrapping approach obtained 30.68 days for HCV incubation period, 15.08 days fewer than conventional method. Bootstrapping was a novel approach used in the estimation of HCV incubation period, it considered each MITMT as a probable HCV infection and estimated mean using bootstrapping for each subject as their incubation period. There were few chances to exercise this approach using data with consecutive exposure to HCV, and it needs more evidence to illustrate the rationality and accuracy in the future.

Approximately 60% - 80% of HCV infection was progressed to chronic hepatitis C; of those, 20% - 30% were developed into cirrhosis after 20 - 30 years, and 1% - 4% of cirrhotic patients were advanced into HCC per year [8] [9]. A study conducted in China indicated that 9.2% and 15.3% of patients with 10- and 20-year HCV infection were developed into cirrhosis [11], respectively. At present there is neither a selective antiviral therapy nor a preventive vaccine, it seems far more important to predict the prognosis of HCV infection, hence appropriate actions will be taken to prevent patients with HCV infection from being progressed into the end-stage liver diseases.

A simple-to-use risk prediction model was developed to predict the risk of developing HCC for patients with HCV infection [6]. From the model estimation, subjects infected with HCV have risk of 1.36%, 4.70%, and 10.75% of developing HCC in the upcoming 5-, 10-, and 15-year. On one hand, risk prediction results could make patients come into notice and pay more attention to their HCV treatment; on the other hand, it also could inform health administrators of making health policy towards the prevention and treatment of end-stage liver diseases. Fortunately, all of 99 patients infected by HCV via MITMT were hospitalized to receive antiviral treatment for free of charge. After patients were discharged from the hospital the local CDC took responsibility for monitoring and following up patients, and they were required to get back to the hospital for the check up once every three months.

Recently, the GBD 2010 results showed that all ages deaths of acute hepatitis C had increased from 700 to 1200 cases from 1990 to 2010, with an increase of 88.2% of median percent change; age-standardized death rates were 0.1 and 0.1 per 100 000, with an increase of 16.0% of median percent change; median percent change of all ages DALYs (disability-adjusted life year) from 1990 to 2010 had increased by 66.0%, and 8.1% for age-standardized DALY rate [5]. All ages deaths, age-standardized death rate, all ages DALYs, and age-standardized DALY rate took up the fastest percent increase among all types of hepatitis virus infections. Therefore, HCV infection is a severe public health problem at present and in the near future in China. In the last two decades, most of the infectious diseases were declined, but the burden of hepatitis C was going up. Moreover, the cost of patients' treatment for hepatitis C and resulting end-stage liver diseases will add extra burden to health care system in China. Thus, the capacity of improved diagnosis and treatment for health services providers will be critical.

In general, some patients with HCV infection from a nosocomial clinic in a county-level city of China will be suffered from more severe HCC in the coming decade or so; effective measures and controls were urgently put in place to prevent those patients from being developed into end-stage liver diseases.

Disclaimers

All authors of this research paper have directly participated in the planning, execution, or analysis of this study; all authors of this paper have read and approved the final version submitted; the contents of this manuscript have not been copyrighted or published previously; the contents of this manuscript are not now under consideration for publication elsewhere; the contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration; there are no directly related manuscripts, pub-

lished or unpublished, by any authors of this paper.

Author Contributions

Conceived and designed the study: SCY FJC GSF MHL HL; Analyzed the data: SCY MHL FJC JQL GSF YHH WWL; Wrote the paper: SCY FJC.

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