Format for ANSWERING REVIEWERS

Jan 28, 2018

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 37872-review.doc).

Title: Epidemiological features of chronic hepatitis C infection caused by remunerated blood donors: A nearly 27-year period survey

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The manuscript has been improved according to the suggestions of reviewers:

Reviewer1#
As authors described, the natural history of HCV is still unclear as the natural history of HBV. One of the main reasons why natural history is not clear is that the time of establishment of the infection is unclear. In this report, authors followed many patients with HCV who can be estimated the time of infection. This paper is very interesting epidemiological study, I have several questions.
Major

Q1.Describe the inclusion criteria of blood donor and plasma donor.

A: The inclusion criteria were the following: (1) a history of remunerated blood donation between the late 1980s and the early 1990s, (2) age above 40 years, (3) voluntary provision of contact information, and (4) no HCV treatment performed.

Q2.Positivity of HCV is quite different between the two groups. Describe the difference of background of the two groups?

A: The results showed that the blood donation method is the main cause of transmission of hepatitis C, and plasma donation in particular is the main cause of hepatitis C infection.

This is due to the fact that plasma donations need to be separated from whole blood using separators,

and subsequent return of blood cells to the donor is the main cause of hepatitis C infection.

Q3.ALT level of plasma donor is higher than blood donor. How do you explain such difference?

A: Alanine aminotransferase (ALT) levels in plasma donors are higher than in blood donors, and blood donations from the plasma group are more frequently rejected because of elevated ALT levels. In other words, more plasma donors are likely to have been infected with hepatitis C virus.

Q4.Describe procedure of plasmapheresis. Which step is unhygienic? Did they share the needle or separation membrane?

A: Plasma donations need to be separated from whole blood using separators, and subsequent return of blood cells to the donor is the main cause of hepatitis C infection In other words, plasma donors share contaminated separation membranes. Blood donors simply have 200 ml-400 ml whole blood withdrawn through sterile needles.

Q5.Add the incidence of HCC in each group.

A: The incidence of HCC was added in Table 1

Reviewer 2#

Q6. The research design is better to add a normal or no donation group for comparison. Also, some writings and grammar need to be revised. For example, "rejection of blood donation" has no clear meanings and can be misunderstood.

The research design is better to add a normal or no donation group for comparison.

A: The research design does not include a normal or no donation group because the rate of HCV infection in blood donors is 3.3%, similar to the average anti-HCV-positive rate of 3.2% in the general

Chinese population, according to the national epidemiological survey of HCV conducted from 1992 to 1995.

Q7."rejection of blood donation" has no clear meanings and can be misunderstood.

A: "Rejection of blood donation" refers to rejection because of elevated ALT.

Reviewer3#

Dr. Tan and colleagues presented a cross sectional study in which they aimed to explore prevalence and epidemiological features of CHC caused by paid blood donors over a time period of 27 years. To show this the authors recruited nearly 1700 participants and analysed demographic, patient, and HCV data sets. As a result of the study the colleagues could demonstrate that there was a high prevalence of viremic HCV (68.5%) in the study population, most of them infected by HCV genotype 1b. Also a high number (28%) of the HCV infected individuals suffered from cirrhosis. As risk factors for CHC progression the colleagues identified age over 60 years and alcohol abuse. Overall, the study is well performed, concise in its content showing convincing data. The methodical design and English is adequate. Minor comment

Q 1. Because of the high number of viremic HCV infected individuals, how was the HCV RNA tests performed and how did the colleagues avoid contamination during HCV RT-PCR tests?

A: HCV RNA from subjects' sera was quantified in fresh or well-preserved stored samples by commercial quantitative assays, such as real-time PCR (COBAS AmpliPrep/COBAS TaqMan HCV Test, Roche, DaAn Gene Co, Nanjing, China).

There is no assurance that these reagents are entirely noninfectious. Therefore, test kit components should be treated as though they are capable of transmitting HCV. Consider all serum specimens for

analysis potentially positive for infectious agents including HIV, hepatitis B virus and HCV. Controls and samples should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Observe universal precautions when performing the assay, thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water, handle samples with extreme care to prevent sample contamination, use new, sterile aerosol barrier or positive displacement RNase-free pipette tips and sterile pipettes, wear personal protective apparel, disposable gloves and eyewear during all steps of this method to minimize both infectious and chemical contamination hazards. Use all pipetting devices and instruments with care and follow the manufacturer's instructions for calibration and quality control.

Q2. Do the colleagues have the HCV sequences from the participants on hand or did the authors perform HCV genotyping using commercial line tests? If there are sequences available it would be of interest to analyse the chain of infection since the participants were recruited in a defined region of three cities.

A: The HCV genotype was assessed in all patients with detectable HCV RNA. We used a PCR assay based on reverse transcription of the HCV core region with genotype-specific primers, in accordance with the international classification (i.e., Ⅰa, Ⅰb, Ⅱa, Ⅱb, Ⅲ, Ⅳ, Ⅴ, and Ⅵ) (DaAn Gene Co, Nanjing, China).

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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