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Analysis of HTLV Infection Status among Voluntary Blood Donors in Yulin City

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Abstract

Objective: To comprehensively understand the infection status of human T-lymphotropic virus (HTLV) among the voluntary blood donors in Yulin City and accurately assess the impact of HTLV on the current situation of blood safety in Yulin City. **Methods**: A total of 113,588 blood samples from voluntary blood donors collected in Yulin City from January 2023 to June 2024 were selected. The HTLV-I/II antibody screening was carried out on these samples using the HTLV enzyme-linked immunosorbent assay (ELISA) kit. For the samples with reactive screening results, further confirmation was performed by means of immunoblotting assay and real-time fluorescent quantitative polymerase chain reaction (qPCR). **Results**: Among the 113,588 blood samples from voluntary blood donors, 20 samples showed positive reactions for HTLV-I/II antibodies, with an initial screening positive reaction rate of 1.76‰. After confirmation, 8 of them were confirmed to be HTLV-I positive, with a positive rate of 0.7‰, and no HTLV-II positive blood donors were detected. Moreover, all the HTLV-I positive blood donors were Han people from Yulin. In addition, by following up the 4 children of a female positive blood donor, it was found that her eldest daughter was confirmed to be HTLV-I positive, while the test results of the other children were negative. **Conclusion**: At present, there is no HTLV-II infection among the voluntary blood donors in Yulin City. There is a relatively low level of HTLV-I infection mainly among the local people. Continuous monitoring is still needed in the future. Emphasis should be placed on strengthening the publicity, education and testing work for the close relatives of the confirmed positive blood donors and women during pregnancy and childbirth periods, so as to prevent and reduce the HTLV infection caused by family transmission.

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Keywords

Human T-Lymphotropic Virus, Enzyme-Linked Immunosorbent Assay, Immunoblotting Assay, Real-Time Fluorescent Quantitative Polymerase Chain Reaction

1. Introduction

Human T-cell lymphotropic virus (HTLV) holds a special position in the field of pathogenic human retroviruses. It is the first virus of this kind to be discovered and can be subdivided into two types: type I and type II. Globally, its known main endemic areas cover many regions, such as Japan, sub-Saharan Africa, Central America, South America, the Caribbean Islands, key areas in the Middle East, and Australia-Melanesia. In China, a large-scale survey study of the blood-donor population indicates that, overall, it is a low-prevalence area for HTLV. However, there are certain characteristics in the geographical distribution, mainly concentrated in coastal areas such as Fujian, Zhejiang, and Guangdong provinces. Among them, it is particularly noteworthy that the prevalence rate in Fujian Province is much higher than that in other provinces [1].

The infection mechanism of HTLV is rather special. It mainly causes a lifelong infection in human CD4+ T cells. For most individuals infected with this virus, there may not be obvious symptoms for a long time after infection. However, after an incubation period of several years or even decades, it may lead to a series of serious health problems, such as neurodegenerative diseases, like HTLV-1-associated myelopathy (HAM) or tropical spastic paraparesis (TSP). More seriously, it may also induce fatal malignant tumors such as adult T-cell leukemia (ATL) [2].

In terms of transmission routes, HTLV has similarities with viruses such as HIV and can be transmitted through multiple routes such as mother-to-child, blood, and sexual contact. Among the many transmission routes, blood transfusion is the main transmission route of HTLV-1, apart from intra-family transmission. The reason for transmission through blood transfusion is that if there are lymphocytes carrying HTLV-I in blood products, the virus can easily be transmitted to the recipient during the blood transfusion process, thereby causing infection [3].

In view of the above characteristics of HTLV and its potential threat to blood safety, in order to further improve the blood safety level in Yulin City, our laboratory has specifically conducted an in-depth study on the infection status of human T-cell lymphotropic virus (HTLV) among the voluntary blood donors in Yulin City. The specific detection method is to use the enzyme-linked immunosorbent assay (ELISA) double-antigen sandwich method to detect HTLV-I/II antibodies in the voluntary blood donors in Yulin City. For those specimens that show positive reactions during the initial screening process, they are uniformly sent to the confirmation laboratory of the Guangxi Zhuang Autonomous Region Blood Center for further confirmation, so as to comprehensively and accurately assess the impact of HTLV on the current situation of blood safety in our city.

2. Materials and Methods

2.1. General Information

A total of 113,588 blood donor specimens of those who voluntarily donated blood at this station from January 1, 2023 to June 30, 2024 were collected. All these blood donors underwent a comprehensive and strict health consultation and examination process in accordance with the "Requirements for the Health Examination of Blood Donors". Among them, rapid tests were carried out for hepatitis B virus surface antigen (HBsAg), treponema pallidum antibody (TP), and alanine aminotransferase (ALT) of the blood donors to preliminarily evaluate the blood health status of the blood donors and ensure the safety and quality of the collected blood.

2.2. Reagents and Instruments

The HTLV-I/II antibody ELISA kits produced by Beijing Wantai were used in this study, and their lot numbers are T20160101, T20160502, T20160503, T20160804, and T20171105 respectively. The instruments used in the detection process include the fully-automatic sampler (EVO) produced by TECAN Company in Switzerland and the fully-automatic enzyme-linked immunosorbent analyzer (FAME) produced by Hamilton Company in Switzerland. These high-quality reagents and advanced instruments provide a reliable material basis and technical guarantee for the subsequent detection work.

2.3. Methods

2.3.1. HTLV-I/II Antibody Detection

ELISA reagents were used to detect HTLV-I/II antibodies. During the entire detection process, every step was strictly carried out in accordance with the operation process specified in the reagent instructions, from sample processing reagent addition to the control of reaction conditions. At the same time, the judgment of the results was also completely in accordance with the standard of the instructions. When the test result was reactive (that is, S/CO value ≥ 1.0), the specimen was re-examined in double wells. During the re-examination process, if any one of the wells was reactive, the specimen was judged to be initially screened as a positive reaction. This rigorous detection and judgment method helps to improve the accuracy of the detection results.

2.3.2. Processing of Blood and Specimens with Positive Initial Screening Reaction

Special processing was carried out for the blood specimens with positive initial screening reactions of HTLV-I/II antibodies. First, the specimens were centrifuged at a speed of 3000 r/min for 10 minutes, and then the centrifuged plasma and buffy coat layer were carefully transferred to screw-capped cryopreservation tubes. To ensure the stability of the specimens, the cryopreservation tubes containing the specimens were placed in a refrigerator below −20˚C. After that, under the condition of cold-chain transportation, the cryopreservation tubes and the

corresponding plasma of blood bags were sent to the Guangxi Zhuang Autonomous Region Blood Center for immunoblotting test and real-time fluorescent quantitative polymerase chain reaction (PCR) confirmation test to further confirm the reliability of the detection results.

2.4. Statistical Processing

In terms of data processing, we made full use of the powerful tool of Microsoft Excel 2010. First, the original data of the 113,588 collected specimens were comprehensively and meticulously organized, including the basic information of each blood donor and the values of various detection indicators. During the organization process, the integrity and accuracy of the data were strictly checked, and any data that might be wrong or missing were excluded. Then, the data analysis function in Excel 2010 was used to classify and statistically analyze different types of data. For example, the blood donors were grouped according to demographic characteristics such as age and gender, and the incidence of positive initial screening reaction of HTLV-I/II antibodies in each group was statistically analyzed. At the same time, the data from different detection stages (initial screening and re-examination) were statistically analyzed, and relevant indicators such as positive rate and false-positive rate were calculated to evaluate the effectiveness and reliability of the detection methods. Through these detailed and in-depth statistical processing, we can more comprehensively and accurately understand the data characteristics and laws in this study and provide strong data support for the subsequent research conclusions.

3. Results

3.1. HTLV Detection Results

In this study, in-depth detection was carried out on 113,588 blood specimens of voluntary blood donors. After these specimens underwent a strict double-well re-examination process, 20 specimens showed positive reactions of HTLV-I/II antibodies. Through calculation, the initial screening positive rate was 1.76‰, and this data provides an important basis for our preliminary understanding of the carrying situation of HTLV-I/II antibodies among blood donors. Subsequently, the specimens with positive initial screening were further sent to the Guangxi Zhuang Autonomous Region Blood Center for immunoblotting test and real-time fluorescent quantitative PCR confirmation test. After this series of more accurate detections, 8 of them were finally determined to be HTLV-I positive, and thus the HTLV positive rate was 0.7‰. It is worth noting that all the blood donors confirmed to be HTLV-I positive are Han people from Yulin local area. In addition, there was a special situation during the detection process. One specimen had an uncertain result during the initial screening, but after resampling and testing the blood donor two months later, it was finally confirmed to be negative. This situation also reflects some possible complex factors in the detection process. For a clearer presentation of information, the detailed detection results of the 8 HTLV-

I confirmed-positive specimens and the basic information of their blood donors have been sorted in Table 1 and Table 2 for easy reference and comparison in subsequent research and analysis.

Table 1. Detection results of 8 HTLV-I confirmed positive samples.

Specimen number	S/CO Value	Western blotting confirmation	Real-time fluorescence quantitative PCR	Final result judgment
1	11.8	+	+	HTLV-I positive
2	11.8	+	−	HTLV-I positive
3	11.8	+	+	HTLV-I positive
4	12.1	+	−	HTLV-I positive
5	10.9	+	+	HTLV-I positive
6	11.4	+	−	HTLV-I positive
7	11.4	+	−	HTLV-I positive
8	12.2	+	−	HTLV-I positive

Table 2. Basic information of blood donors of 8 HTLV-I confirmed positive samples.

Specimen number	Gender	Age (years)	Ethnic group	Native place	Occupation	Number of blood donations (n)
1	Male	35	Han	Luchuan County, Yulin City, Guangxi	Worker	3
2	Female	39	Han	Xingye County, Yulin City, Guangxi	Civil servant	25
3	Male	58	Han	Luchuan County, Yulin City, Guangxi	Others	18
4	Male	26	Han	Bobai County, Yulin City, Guangxi	Others	1
5	Male	44	Han	Bobai County, Yulin City, Guangxi	Worker	2
6	Female	40	Han	Bobai County, Yulin City, Guangxi	Others	1
7	Female	50	Han	Bobai County, Yulin City, Guangxi	Farmer	1
8	Female	34	Han	Luchuan County, Yulin City, Guangxi	Others	1

3.2. Tracking Situation of Family Members of Confirmed Positive Blood Donors

Among the 8 blood donors confirmed to be HTLV-I positive, only the family situation of blood donor No. 7 was successfully tracked. This blood donor No. 7 is a female and in a widowed state. Her family members include one son and three daughters, totaling 4 children. During the testing of these family members, it was found that the test result of the eldest daughter was positive, the initial test result of the second daughter was uncertain. While the test results of the other two children, namely the son and the youngest daughter, were both negative. It is worth mentioning that regarding the uncertain initial test result of the second daughter, her blood specimen was recollected for testing 4 months later, and finally, it was confirmed to be negative, which provided more comprehensive information for the analysis of the entire test results.

3.3. Detection Situation of Other Pathogens

For the 20 blood specimens of voluntary blood donors with positive initial screening reactions of HTLV-I/II antibodies, we simultaneously carried out the detection of multiple other pathogens. Using the ELISA method, the hepatitis B virus surface antigen (HBsAg), hepatitis C virus (HCV) antibody, human immunodeficiency virus (HIV) antibody, specific antibody of syphilis, and alanine aminotransferase (ALT) were detected. After a strict detection process, the test results of all these items showed to be qualified. This indicates that among these blood donor specimens with positive initial screening of HTLV-I/II antibodies, no abnormalities in the infection situation of other common pathogens were found within the scope of this test, providing an important basis for the subsequent further analysis of the safety and disease association of blood samples.

4. Discussion

In this study, we screened the HTLV-I/II antibodies in as many as 113,588 blood specimens of voluntary blood donors. Through screening, it was found that a total of 20 cases showed positive antibody reactions, and the calculated initial screening positive reaction rate was 1.76‰. This data is at a lower level compared with 9‰ of the Guangxi Blood Center [4] and 3.88‰ of Nanning [5]. Among these 20 cases with positive initial screening, 8 cases were further confirmed to be HTLV-I positive, with a positive rate of 0.7‰, and no HTLV-II positive cases were found. This result is basically consistent with the survey situations in low-prevalence areas such as Nanning [5] and Laibin [4] within Guangxi region.

We know that various factors such as the purity of antigens and antibodies in ELISA reagents, the concentration of factors to be tested, and the operation techniques of ELISA will all affect the sensitivity, specificity, and repeatability of the test results [6]. It is worth noting that our station, the Guangxi Blood Center, and the Nanning Central Blood Station all selected ELISA reagents from the same manufacturer. However, the initial screening positive rate of our station is much lower than that of the other two units within the region. This is probably because the sensitivity and specificity of the reagents have been continuously improved over time. The Guangxi Blood Center used this reagent from 2016 to 2017, and the Nanning Central Blood Station used it from 2016 to 2018, while our station used it more than 5 years later than theirs. It can be seen that when laboratories in various regions conduct tests, they should carefully select blood screening reagents with high sensitivity and strong specificity according to their actual conditions. Doing so can not only effectively reduce the false positive rate of HTLV, avoid the waste of precious blood resources, but also reduce the unnecessary psychological burden on blood donors caused by false positive results.

The results of this study show that all 8 blood donors confirmed to be HTLV-I antibody positive are from Yulin local area. Among them, 4 cases are repeated blood donors, and the one who has donated blood the most has already carried out 25 voluntary blood donations. The repeated blood donation behavior of

HTLV-positive individuals will undoubtedly pose a serious threat to blood safety. The HTLV-I positive rate of voluntary blood donors in Yulin City in this study is 0.7‰, which is at a lower level compared with the HTLV positive rates in regions such as Ningde 18.44‰, Putian 4.15‰, and Sanming 4.11‰ in Fujian reported [7]. And from the current data, the infected population is mainly local. However, with the increasingly frequent exchanges between different regions and the continuous expansion of the scale of personnel movement, there is a high possibility that HTLV infection will spread from high-prevalence areas to low-prevalence areas or even non-prevalence areas. Based on this, it is necessary to continuously carry out HTLV screening and confirmation work for voluntary blood donors.

In addition to regional differences, the level of HTLV positive rate in the population is also closely related to the selection of screening methods and confirmation methods. This study shows that the confirmation positive rate of HTLV for blood donors is 40% (8/20), which is much higher than other related reports, such as the highest 26.52% (247/939) in Fujian [1], 16.67% (4/24) in Nanning [5], and 2.13% (1/47) in the Guangxi Blood Center [4]. Even in some regions, there is currently no reported confirmed positive situation [1]. In this study, among the 8 positive results of immunoblotting test, only 3 cases were positive in real-time fluorescent quantitative PCR; there was also 1 blood donor whose immunoblotting test result was uncertain and real-time fluorescent quantitative PCR was negative. After tracking for 2 months and recollecting samples for immunoblotting test and real-time fluorescent quantitative PCR experiments again, the results showed negative for both. Given that the real-time fluorescent quantitative PCR detection technology has relatively complex conditions and requirements, at present, the confirmation work of HTLV should mainly rely on immunoblotting tests. When the result of immunoblowing test is uncertain, it is advisable to carry out tracking verification after an interval of time, and the combined detection method of immunoblotting test and real-time fluorescent quantitative PCR can be adopted to ensure the reliability of the confirmation result of HTLV. At the same time, if conditions permit, it is best to detect the gene sequence of the HTLV-confirmed positive samples so as to track and determine the source of the HTLV virus strain of the blood donor.

Among the 8 blood donors confirmed to be HTLV-I positive, only the family situation of blood donor No. 7 was tracked, including one son and three daughters. Among them, the eldest daughter was confirmed to be positive, and the other children were negative. This situation is most likely caused by mother-to-child transmission. Therefore, we should strengthen the tracking and confirmation work of the close relatives of HTLV-confirmed positive blood donors, and carry out targeted publicity and education activities for this part of the population, so as to effectively reduce the transmission of HTLV. Since no relevant vaccines and safe and effective treatment drugs have been developed for HTLV at present, it is the most effective preventive and control measure to detect HTLV-infected individuals as early as possible, isolate them, and block the transmission routes [8].

5. Conclusion

The results of this study clearly indicate that only HTLV-I infected individuals have been found among the voluntary blood donors in Yulin City at present, which means that Yulin City belongs to a low-prevalence area of HTLV. However, although the number of infected individuals is relatively small, there are indeed a certain number of infected individuals. In order to effectively ensure blood safety and avoid the transmission of HTLV through the blood transfusion route, it is imperative to continue carrying out screening work for voluntary blood donors. At the same time, in order to better prevent and reduce the harm that HTLV infection may bring, we should focus on strengthening the publicity, education, and testing work for the close relatives of the confirmed positive blood donors and women during pregnancy and childbirth.

6. Research Limitations

1) Only part of the blood donors' family members were tracked, with limited sample tracking, which affects the understanding of the transmission mode.

2) The detection methods may have the possibility of missed detection and incorrect detection, and there is a lack of method comparison.

3) The study is limited to the voluntary blood donors in Yulin, with narrow geographical and population coverage, and the representativeness of the results is limited.

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Conflicts of Interest

The authors declare that there is no conflict of interest in this study. The research process is independent and objective, and all data collection, analysis, and conclusions have not been affected by any commercial institutions, organizations, or individuals, ensuring the scientificity and fairness of the research.

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