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Performance of a rapid diagnostic test for screening of hepatitis C in a real-life prison setting

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ABSTRACT

Background: Hepatitis C virus (HCV) point-of-care testing using rapid diagnostic test (RDT) is the solution for large-scale, feasible, fast and reliable screening of HCV infection.
Objectives: The aim of this study was to evaluate the diagnostic performance of HCV RDT for screening of HCV infection in a real-life prison setting.
Study design: This study was conducted on individuals admitted and incarcerated in the Central Prison of Karaj, 2017–2018. For all inmates, anti-HCV testing using a RDT on finger-stick blood in the prison and ELISA at the laboratory were performed. For evaluation of reproducibility, more than 1000 cases were recruited for re-evaluation of the HCV RDT using anticoagulated blood in the laboratory.
Results: Among 1788 participants, 76 (4.25%) and 106 (5.93%) were positive for anti-HCV using RDT and ELISA, respectively. Among 34 cases with discordant results using the RDT and ELISA, 17 were the result of testing error in prison, 7 false positive of ELISA and 10 false negative of RDT in individuals with HCV spontaneous clearance. The sensitivity of the RDT with inclusion of testing error in prison for detection of anti-HCV was 75%. However, with exclusion of testing error in prison and considering HCV RNA as the reference method for diagnosis of current HCV infection the sensitivity reached 100%. The RDT was 100% reproducible using both evaluations in prison and the laboratory.
Conclusions: The RDT is a reliable and feasible method for screening of anti-HCV in settings such as a prison. However, the testing should be performed in a standard procedure to have the optimal diagnostic performance.

1. Background

Hepatitis C virus (HCV) infection has become a global health challenge with more than 71 million chronic infection and annual mortality of 400,000 cases from chronic sequelae [1]. In 2011, with the introduction of a new line of HCV antiviral therapy with direct-acting antiviral agents (DAAs), the management of hepatitis C has been greatly transformed [2–5]. This pipeline of new HCV therapeutics resulted in Interferon-free regimens with more than 95% treatment success in most of the patients with chronic HCV infection [3,6–8]. With wider availability of these new HCV treatments the goal of HCV elimination by 2030 has been set by healthcare systems in a number of countries around the world [9].

The issue of screening and case finding has been identified as one of the barriers for elimination of hepatitis C [10]. Hepatitis C is mostly observed in low socio-economic populations especially in groups with high-risk behaviors such as people who inject drug (PWID) [11]. Definitely, the screening for hepatitis C should be targeted to specific groups based on the risk factors for HCV transmission and target groups (i.e. PWID, thalassemia, hemophilia and etc.) in each country based on local epidemiologic studies. Given access to the groups with high-risk behaviors is hard, the screening of HCV infection with standard strategies for case finding in these special groups is a great challenge. In the setting of clinics, screening is performed by detection of anti-HCV

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antibodies (HCV Abs) using ELISA and other immunoassay-based techniques. However in community-based settings, blood sampling, specimen transfer and the turn-over time for the testing results make the process of testing and screening laborious and time-consuming. With the development of rapid diagnostic tests (RDT) for detection of antigens and antibodies of infectious agents the procedure of screening can be performed in a point-of-care (POC) setting in a short turn-over time followed by POC treatment of cases with confirmed reactive results. The RDT kits for screening of anti-HCV has been commercially available since the early 2000s. However, since the treatment with interferon-based therapies was a troublesome process, there was no interest in wide screening of hepatitis C in community-based settings. The recent revolution in treatment and diagnosis of HCV infection, make the opportunity to conduct wide screening in prisons and community-based settings a necessity.

2. Objectives

The aim of this study was to evaluate the diagnostic performance of an HCV RDT using finger-stick blood on a large cohort of inmates in a prison setting.

3. Study design

3.1. Study population

This prospective study is a subsidiary of a national pilot on "Screening, diagnosis and treatment of hepatitis C in Iranian prisons". The study was conducted in the Central Prison of Karaj (Karaj, Alborz, Iran) from September 2017 to January 2018. In this time span, all the individuals admitted and incarcerated in the prison were included in the study consecutively. All study participants provided informed consent explaining the aims of our study. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study protocol was approved by the institutional review board of the Digestive Disease Research Institute of Tehran University of Medical Sciences.

3.2. Testing and laboratory procedures

All included individuals were tested for anti-HCV on a finger-stick blood specimen using a rapid anti-HCV test (InTec Products Inc., Advanced Quality Rapid anti-HCV test). Briefly, 10 μL blood was provided by pricking the tip of the alcohol-wiped index or middle finger. The finger-stick blood sample was transferred to the test cassette using a single-use disposable micropipette. After applying 2 drops (100 μL) of diluent, the test was read at 15 min. All tests were quality controlled based on the presence of a control band and in cases without the control band the test result was rejected and the inmate was retested using a new kit. The tests were performed by the healthcare worker of the prison whom routinely tests the inmates for anti-HIV using a RDT and was trained for the HCV testing using the HCV RDT as well.

After HCV rapid testing, a blood sample was drawn from all included inmates and transferred to the Middle East Liver Disease (MELD) Laboratory (Tehran, Iran). In the laboratory, the plasma samples were separated and stored at −20 °C for subsequent evaluations. All plasma samples were tested for anti-HCV using a fourth generation enzyme-linked immunosorbent assay (ELISA) kit (DIA. PRO, Italy) according to the manufacturer's instructions. The samples with a borderline result (signal-to-cutoff ratio = 0.9–1.1) for anti-HCV using ELISA were re-evaluated in duplicate using the same method to obtain a definitive result.

In cases with discordant results for anti-HCV using the RDT and ELISA, the patients' plasma samples were re-evaluated for anti-HCV using the RDT of the same lot number in the laboratory to identify testing errors in the setting of the prison. The inmates (those were not released from prison at follow-up) with discordant results were retested for anti-HCV using RDT on finger-stick blood as well. Finally, the samples with confirmed discordant results for anti-HCV using the RDT and ELISA following RDT retesting in the laboratory were evaluated for anti-HCV using a recombinant immunoblot assay (RIBA) (HCV BLOT 3.0, MP Biomedicals) and HCV RNA using reverse transcription-polymerase chain reaction (RT-PCR) (Artus HCV RG RT-PCR Kit, QIAGEN) according to the manufacturers' instructions.

For evaluation of the reproducibility of finger-stick blood testing, 1096 EDTA-anticoagulated blood samples were selected for detection of anti-HCV using the same lot number of RDT kit at the laboratory according to the manufacturer's instructions.

3.3. Statistical analysis

Categorical variables were expressed as frequencies and percentages and continuous variables as mean ± standard deviation (SD). For evaluation of diagnostic performance of the HCV RDT, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated taking the number of tests with false- and true-positive results and false- and true-negative results. All statistical analyses were performed using SPSS version 20.0 (IBM SPSS).

4. Results

4.1. Characteristics of study population

In this study, 1788 incarcerated males, aged 36.5 ± 11.1 (mean ± SD) years old were registered and finally included. Anti-HCV testing using a RDT at inmate admission to the prison resulted in detection of 76 (4.25%, 95% CI = 3.41%–5.29%) inmates with reactive anti-HCV, while the testing of plasma samples for anti-HCV using ELISA in the laboratory resulted in 106 (5.93%, 95% CI = 4.93%–7.12%) positive cases. Moreover, 34 (1.9%, 95% CI = 1.36%–2.64%) inmates had discordant results for anti-HCV with 2 RDT positive/ELISA negative and 32 RDT negative/ELISA positive samples.

4.2. Assessment of cases with discordant anti-HCV results with the RDT and ELISA

Re-evaluation of plasma samples from 34 cases with discordant results with the RDT and ELISA tests for anti-HCV using the RDT in the laboratory showed that 17 were caused from a testing error in the prison using the RDT including 2 RDT positive/ELISA negative and 15 RDT negative/ELISA positive samples. Among these 17 cases with presumed error in initial testing in prison, 12 (2 RDT positive and 10 RDT negative) were retested using RDT on finger-stick blood in prison and all had the same results as those obtained by anti-HCV retesting using RDT on plasma in the laboratory. The remaining 17 cases (RDT negative/ELISA positive) with confirmed discordant results of anti-HCV using the RDT and ELISA underwent further evaluations for anti-HCV using RIBA and for HCV RNA using RT-PCR. Among these 17 cases, 7 cases were negative for anti-HCV using RIBA and for HCV RNA using RT-PCR and as a result, they were considered as false positive results of ELISA. The other 10 cases were confirmed to be anti-HCV positive while they were negative for HCV RNA using RT-PCR and as a result, they were considered as cases with spontaneous clearance of HCV. The study design and results are presented in Fig. 1.

4.3. Diagnostic performance of the HCV RDT in the prison setting

Based on testing errors, the false positive results of ELISA and RDT-negative cases with spontaneous clearance of HCV, the diagnostic performance of the HCV RDT in the prison setting was calculated in

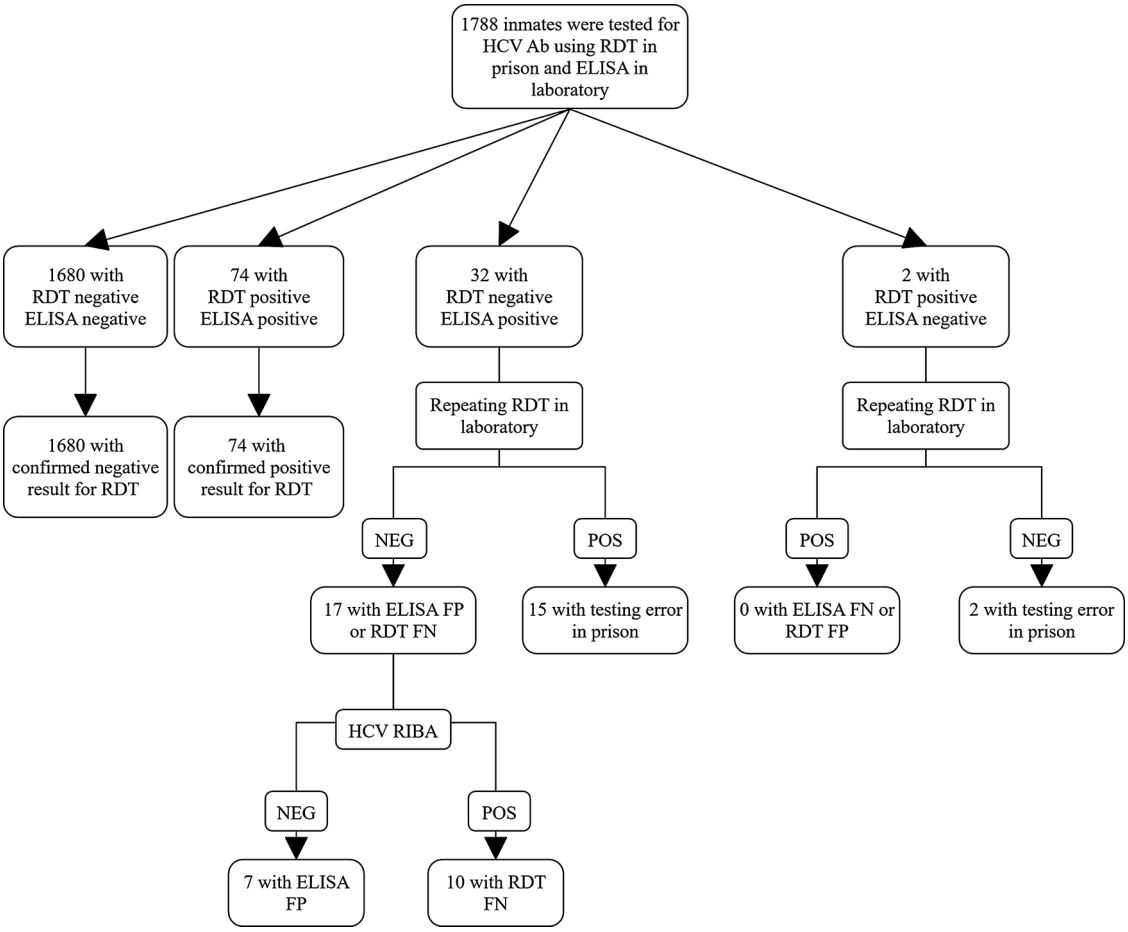

Fig. 1. Study Plan and Anti − HCV Results Using the Rapid Diagnostic Test, Enzyme-linked Immunosorbent Assay and Recombinant Immunoblot Assay.
Abbreviations: RDT rapid diagnostic test; ELISA enzyme-linked immunosorbent assay; RIBA recombinant immunoblot assay; NEG negative; POS positive; FP false positive; FN false negative.

four different models (Table 1). In all models, the specificity of the HCV RDT for detection of anti-HCV or infection was higher than 98%. The sensitivity of anti-HCV RDT for detecting anti-HCV antibody using ELISA as the reference method was 75% with inclusion of testing errors in prison, and 90% when excluding these errors. The sensitivity of anti-HCV RDT for detecting current HCV infection as defined by positive ELISA anti-HCV and positive HCV RNA was 100% (Table 1).

4.4. Reproducibility of HCV RDT using finger-stick blood and anti-coagulated blood samples

For evaluation of reproducibility, 1096 individuals with confirmed results of anti-HCV RDT in prison were retested using anti-coagulated blood in the laboratory using the same lot number of the HCV RDT kit. The results were concordant in all 1096 (100%) individuals. Among these 1096 samples included for evaluation of reproducibility, 28 samples had discordant results of anti-HCV using ELISA and initial RDT in prison. The results of these 28 samples in evaluation of reproducibility were the same as results obtained by anti-HCV retesting using RDT on plasma samples in the laboratory.

5. Discussion

This study included 1788 participants in prison- and laboratory-based evaluations and identified RDT as an alternative for laboratory testing for large-scale screening of HCV infection. We used the available infrastructure of the prison in which HIV was tested routinely. The healthcare workers in the prison whom routinely test for HIV were

Table 1
Diagnostic Performance of the HCV Rapid Diagnostic Test in Prison.

	Sensitivity (95%CI), %	Specificity (95%CI), %	PPV (95%CI), %	NPV(95%CI), %
Diagnostic performance-model 1[a]	69.81 (60.13-78.35)	99.88 (99.57-99.99)	97.37 (90.02-99.33)	98.13 (97.52-98.59)
Diagnostic performance-model 2[b]	74.75 (65.02-82.94)	99.88 (99.57-99.99)	97.37 (90.02-99.33)	98.54 (97.96-98.96)
Diagnostic performance-model 3[c]	89.90 (82.21-95.05)	100 (99.78-100)	100 (95.86-100)	99.41 (98.95-99.67)
Diagnostic performance-model 4[d]	100 (94.87-100)	98.89 (98.28-99.33)	78.65 (70.20-85.21)	100 (99.77-100)

[a] Model 1: diagnostic performance with inclusion of HCV testing errors in prison using the RDT and false positive results of ELISA.
[b] Model 2: diagnostic performance with inclusion of HCV testing errors in prison using the RDT and correction of false positive results of ELISA.
[c] Model 3: diagnostic performance with correction of both HCV testing errors in prison using the RDT and false positive results of ELISA.
[d] Model 4: diagnostic performance with correction of HCV testing errors in prison using the RDT and inclusion of HCV RNA data as reference for cases with anti − HCV.

educated and trained in a single-session course for using the HCV RDT. As a result, the data reported in this study reflected real-life experience with rapid testing for HCV in prison, which can be taken into account for decision making regarding implementation of screening for HCV in prisons.

The current study found a prevalence of 5% for anti-HCV in the Central Prison of Karaj which is approximately half the prevalence reported in a national bio-behavioral surveillance survey of prison inmates in 2015 [12]. This finding may be the result of the study been performed in a single prison. The prevalence of anti-HCV in the general population of Iran is around 0.5% [13]. Our results indicate a 10 fold higher prevalence in a prison in Iran, which creates the opportunity to screen and treat inmates as one of the targets for HCV elimination [14].

In the real-life setting of a prison, among 89 cases with a positive result for anti-HCV using a RDT, 15 (16.8%) were read as anti-HCV negative by mistake (false negative). However, among 1699 cases with a negative result for anti-HCV using the RDT, only 2 (0.1%) were read as anti-HCV positive by mistake (false positive). Based on these results, it seems that the reactive bands of cases with true anti-HCV positivity were missed in the reading stage by the users. The problem with testing errors using a malaria RDT was previously observed and documented as well [15].

All samples with anti-HCV missed by RDT were negative for HCV RNA and considered as cases of spontaneous HCV clearance. Such cases presumably have low anti-HCV titer. Missing these cases by the HCV RDT caused a 10% reduction in sensitivity of anti-HCV detection. However, given all these cases were HCV RNA negative, the sensitivity of RDT for diagnosis of current HCV infection would be 100%. In a study in chronic kidney disease patients, the sensitivity of the RDT for detection of anti-HCV was 85–89%, which was the cause of missing 5–7 patients with anti-HCV [16]. In the latter study, all the missed cases were HCV RNA negative except one with low HCV RNA level which is similar to the results of our study. In another study, the sensitivity of the RDT using oral mucosal transudate for detection of anti-HCV in anti-HCV positive/HCV RNA negative individuals following HCV antiviral therapy was 82%, which suggests that after clearance of HCV viremia, the anti-HCV titer decreases, causing false negative results as in those with HCV spontaneous clearance [17].

The specificity of RDT for detection of anti-HCV was found to be perfect (100%) after exclusion of test errors in prison and no cases with false positive results for anti-HCV was found. Similarly, previous studies reported high (> 98%) specificity for detection of anti-HCV using RDT kits [16–18]. Moreover, reproducibility assessment showed that the performance of the RDT on finger-stick blood specimens was the same as using anticoagulated blood for testing.

This study has few strengths over previous studies such as using a real-life prison setting and healthcare workers of the prison for POC testing. Moreover, the complete testing profile of individuals with discordant results was used to identify testing errors. The main weakness of the current study was the lack of data on the type of the errors that occurred in testing for HCV using the RDT to have appropriate intervention to reduce such testing errors.

In conclusion, the screening for HCV using RDT make the opportunity to implement POC testing for hepatitis C in the setting of prison possible. The results of the current study support the implementation of HCV RDT testing in prisons. However, using the HCV RDT should be implemented in a standard procedure to have optimal diagnostic performance. The future of HCV community-based screening and diagnosis will be a single step POC testing for HCV RNA and/or HCV core antigen [19,20].

Credit author statement

HSH, HP, FA, MS, RA, SMA, and SM had contributed to conception and design; HSH, HP, FA, BT, RR, FH, and MT had contributed in acquisition of data, study management and administrative support; HSH, HP, MMG, SMA, and SM had contributed in data management, statistical analysis and interpretation of results; HSH, HP, and SM had contributed in drafting the manuscript. All authors contributed to critical revision of the manuscript for important intellectual content, and approved the final version of the manuscript.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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