

Detection of hepatitis C virus (HCV) among health care providers in an Egyptian university hospital: different diagnostic modalities

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Background: Hepatitis C virus (HCV) infection has received much attention and is placed at the core of the infection control agenda. It is considered as a major public health problem in Egypt, where the highest prevalence of HCV exists. The great risk of exposure to infection of health care providers (HCPs) has highlighted the urgent need for implementing an infection control program.

Objective: The purpose of this study was to detect the prevalence of HCV infection among HCPs in Zagazig University Hospitals and to assess the performance of different diagnostic modalities.

Methodology: Blood, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and saliva tests were performed in enrolled HCPs.

Results: This study compared HCV diagnosis Hapanostika HCV Ultra ELISA as a screening test and PCR as gold standard test, which resulted in 40.6% positive results by ELISA compared to 34.8% by PCR ($p < 0.0001$), while OraQuick HCV rapid antibody compared to PCR shows that 37.7% of the participants were positive by OraQuick HCV rapid antibody test. Application of standard precautions while dealing with blood has negative significant correlation with HCV infection ($r_s = -0.265$, $p = 0.03$).

Conclusion: HCPs at Zagazig University Hospitals are at high risk for HCV infection. Lack of compliance and awareness of prevention and control of the infection are associated cofactors. Serum HCV-Ab detection by Hapanostika HCV Ultra ELISA and OraQuick HCV rapid antibody test are sensitive and specific serologic assays for diagnosis with correspondent results to that obtained by quantitative real-time PCR.

Keywords: HCV, ROC curve, OraQuick HCV, infection control

Introduction

Hepatitis C virus (HCV) is a global health threat, with Egypt having the highest prevalence worldwide.¹ Most HCV transmission opportunities in Egypt are related to medical injections and procedures.² Health care workers (HCWs) represent a high-risk population for sharps injuries, needles, and scalpels during the execution of their health care duties. In addition, HCW's mucosa may be exposed to droplets or splashes of blood, saliva, and urine. These occupational exposure accidents carry an estimated risk of 2% HCV transmission.^{3,4} World Health Organization (WHO) estimates that each year more than 3 million health workers hurt themselves with an object/edge definitely contaminated with at least one HIV (~170,000 exposures), hepatitis B (~2,000,000 exposures), and hepatitis C (~900,000 exposures).⁵ As there is no available vaccination for HCV up till now, the use of standard precautions coupled with strict adherence to post-exposure prophylactic measures to HCV are the available measures for prevention.

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Yet, lack of compliance hampered HCW response.⁶ Personnel with HCV antibody-positive and HCV RNA-positive status by polymerase chain reaction (PCR) or those in whom HCV RNA PCR status is yet to be determined should be excluded from exposure prone procedures.⁷

Different methods assist in HCV diagnosis including third-generation enzyme-linked immunosorbent assay (ELISA) for the detection of anti-HCV antibodies.⁸ It includes multiple recombinant HCV antigens with subsequent reduction of the window period and improvement in the detection of patients exposed to HCV. Several points of care testing have been developed to detect HCV-specific antibodies with a relatively high sensitivity and specificity. The only test currently approved by the Food and Drug Administration is the OraQuick HCV rapid assay (OraSure Technologies Inc., Bethlehem, PA, USA). This test detects HCV antibodies in fingerstick and venipuncture whole blood, serum, plasma, or oral fluid specimens by an indirect lateral flow immunoassay. Core, NS3, and NS4 antigens are immobilized on a nitrocellulose membrane, and the results are directly visualized using colloidal gold labeled with protein A. Molecular assays to detect the HCV genome are used for the diagnosis of active HCV infection in patients with a positive antibody test, an early diagnosis of acute HCV infection, as the HCV-RNA can be detected before specific antibodies become detectable (within 1–3 weeks after exposure). Moreover, the diagnosis of a chronic HCV infection is confirmed by the presence of both HCV antibodies (with the exception of severely immune-suppressed patients) and HCV RNA over 6 months.⁹

As accurate diagnosis of HCV is a corner stone in control of disease spread, the current work was conducted to detect the prevalence of HCV occupational infection among health care providers in Zagazig University Hospitals and to assess the performance of the different diagnostic modalities.

Subjects and methods

Study design and settings

A cross-sectional study was conducted in Zagazig University hospitals, in the eastern province of Egypt with more than 2000 beds. It took place over a period of 18 months. Infection control unit-Zagazig University hospitals has a reporting system for needle stick and sharps injuries (NSSIs).

Study participants

Sixty-nine cases were enrolled for the study. They were selected as shown in Figure 1. Over 18 months, a total of 104 NSSIs reports were delivered to the unit. After excluding 28 cases, 12 cases were positive for HCV at the time of reporting, 10 cases had a history of other risk factors of HCV infection (eg, previous blood transfusion, liver disease, etc), and 6 cases had no index patients. Seventy-six HCWs were interviewed after explaining the study protocol. Three refused to participate in the study, four agreed to participate in saliva test but refused to give blood sample for the other two tests. Sixty-nine individuals accepted to participate in the study by the 3 diagnostic modalities.

The selection and consent of participants were done at the time of reporting of sharp injury. Each filled a structured

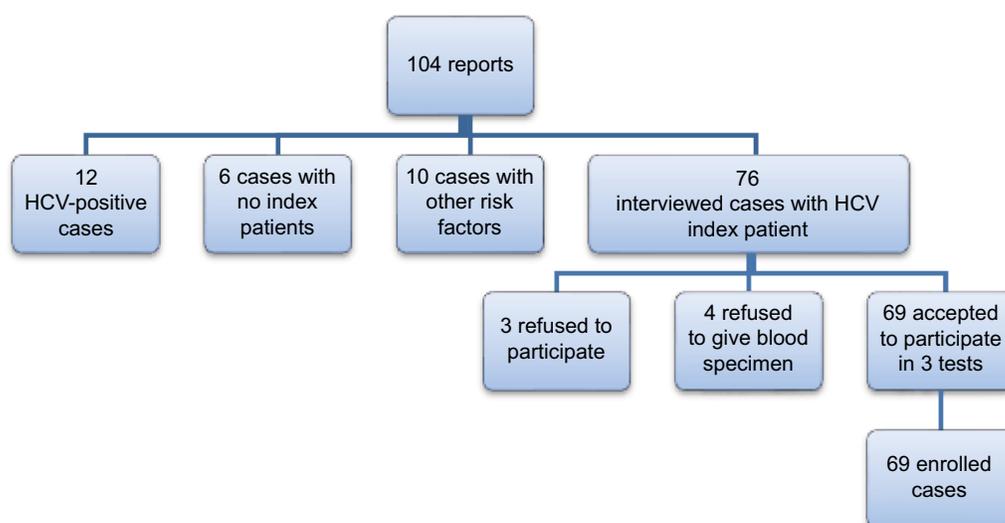


Figure 1 Frame work of the participants in the study during study period.

Abbreviation: HCV, hepatitis C virus.

questionnaire; eight weeks later, blood was sampled from participants for PCR and ELISA anti HCV Ab detection, and saliva was collected for screening of HCV Ab.

Tools used for data collection

- a- A structured questionnaire was designed consisting of several parts. The first part included questions about general characters of the participants such as gender, age, residence, profession, smoking, allergy to medications, presence of chronic diseases, and history of HBV vaccine. The second part of the questionnaire asked participants about their history, current incident, and the circumstances of the most sharp injury that predispose to HCV infection (eg, direct contact with blood, application of standard precautions on dealing with blood, using protective personal equipments, frequency of attending dental clinics, and presence of case of HCV within family).
- b- Microbiological investigations: The following three tests for HCV were performed for each of the 69 participants: HCV-antibody detection by saliva test, HCV-antibody detection by ELISA, and HCV RNA detection by reverse transcription (RT) real-time PCR. Oral cavity was swabbed for saliva, and the test was performed immediately. For the other two tests, blood sample was aseptically collected; a volume of 2 mL serum and 2 mL plasma were separated by centrifugation. They were frozen to -80°C within 2 hours of collection until use.
 - a. Detection of HCV-Ab was carried out in serum samples using a commercially available 3rd generation enzyme immunoassay (Hepanostika HCV Ultra; UBI Diagnostics, Beijing, China). As stated in manufacturer's guide, initially, samples were tested in duplicate. Samples which did not react in any of the tests were considered non-reactive for HCV antibodies. Reactive samples in one or two tests were considered as positive for HCV antibodies.
 - b. Saliva test for anti-HCV antibody testing: An indirect lateral flow qualitative immunoassay was used (Ora-Quick HCV rapid antibody test; OraSure). According to the manufacturer's instructions, swiping gums with the collection pad of the device was performed. A reddish-purple line develops within 20 minutes in the presence of HCV-specific antibodies.⁹
 - c. RT real-time PCR for quantitative HCV RNA detection:
 - i. RNA extraction: RNA was extracted from 140 μL of plasma using QIAamp UltraSense virus kit (Qiagen, NV, Venlo, the Netherlands) according to the

manufacturer's protocol. The extracted RNA was eluted in elution buffer and used as the template for the quantitative RNA PCR.

- ii Taqman real-time PCR was implemented using HCV quantification kit with a lower limit of sensitivity of 50 IU/mL (Amplicor 2.0; Roche Diagnostics, Meylan, France). Procedures were done according to the manufacturer's instructions using *Rotor* gene 2000/3000 real-time PCR machine (Applied Biosystems Sequence Detector 7500 machine; Thermo Fisher Scientific, Waltham, MA, USA). The threshold cycle values from the clinical samples were plotted on the standard curve, and the numbers of copies were automatically calculated. For each run, positive and negative controls were included.

Data management and analysis

The data were analyzed by SPSS 19 (IBM, Armonk, NY, USA) using the suitable test based on the type of the collected data. Descriptive statistics such as frequency, percent, mean, and SD were determined. The performance of ELISA and saliva as screening tests compared to PCR as a gold standard test was assessed using the area under receiver operating characteristic (ROC) curve to define a cut-off value of model score among study participants; the sensitivity, specificity, accuracy as well as agreement using Cohen kappa test (k) with cutoff points <0.4 was considered as poor agreement, >0.75 was considered as excellent agreement, and in-between as good one. Factors that correlate to the infection by HCV virus diagnosed by PCR were determined by calculating Spearman correlation coefficient (r_s). P -value <0.05 was considered as statistically significant in all analyses.

Ethical considerations

Before collecting data, all the required official permissions were obtained, and the study protocol was approved by Institutional Review Board of Faculty of Medicine, Zagazig University. Participants were informed about the objectives and the importance of the study. Those who agreed to participate signed an informed consent. Also, the participants were informed that their participation is voluntary assuring the confidentiality of the collected information through anonymity of the participant, and that, study results will be used only for the purpose of research.

Results

Regarding the general characters of the study participants, their mean age was 31.8 ± 8.06 , most of them were males

(52.2%), from rural areas (71.0%), the majority of them were nurses (62.3%), and least were laboratory technicians (1.4%). Most of the participants were non-smokers (91.3%); they did not have any chronic diseases (92.8%), and 71 % have history of hepatitis B virus vaccination as revealed in Table 1.

Regarding the distribution of factors that might be associated with increasing risk of HCV infection among participants, it was found that (60.9%) of them had their work exposing them to direct blood contact, 87.0% of them apply standard precautions while dealing with blood, only 27.5% had direct blood contact without protection. All of them reported using personal protective equipment in work; 98.6% rarely attend to dental clinics, and the presence of HCV infection within the family was confirmed by 31.9% of them (Table 2).

HCV detection methods

Comparing HCV diagnosis by serum HCV-Ab detection using Hepanostika HCV Ultra ELISA as a screening test and PCR as a gold standard test, resulting in that 40.6% of participants had positive results for HCV by ELISA compared with

34.8% by PCR with high significant difference ($p < 0.001$), and level of agreement is good ($k = 0.69$) as shown in Table 3. Using ROC curve, the area under the curve was 86.0% giving a sensitivity of 87.5%, specificity 84.4, 1-specificity (false positive = 15.6%), 1-sensitivity (false negative = 12.5%) with diagnostic accuracy (85.5%) as shown in Figure 2. Similarly, on assessing the performance of OraQuick HCV rapid antibody test as a screening test compared to PCR in diagnosis of HCV infection shows that 37.7% of the participants were diagnosed positive by OraQuick HCV rapid antibody test compared to 34.8% by PCR with high significant difference ($p > 0.001$) with good level of agreement ($k = 0.69$) as shown in Table 4, and from ROC curve, the area under the curve was (85.0%) ($p = 0.000$) with a sensitivity of 83.33%, specificity 86.67%, 1-specificity (false positive = 13.33%), 1-sensitivity (false negative = 16.67%), resulting in 85.5% diagnostic accuracy as shown in Figure 3.

Regarding the relation between PCR results and predisposing factors of HCV infection among participants, Table 5 represents that, among the enlisted factors, the direct blood contact without protection was significantly positive, correlated to HCV infection as diagnosed by PCR ($p = 0.02$), whereas application of standard precautions while dealing with blood has negative significant correlation with HCV infection ($r_s = -0.3$, $p = 0.03$)

Table 1 General characters of the health care providers

Variables	N (69)	% (100.0)
Age		
Mean age \pm SD, years	31.8 \pm 8.06	
Gender		
Female	33	47.8
Male	36	52.2
Residency		
Urban	49	71.0
Rural	20	29.0
Profession		
Physicians	6	8.7
Nurses	43	62.3
Laboratory technicians	1	1.4
Dentists	6	8.7
Interns	9	13.0
Students	4	5.8
Smoking		
Yes	6	8.7
No	63	91.3
Having chronic diseases		
No	64	92.8
Auto immune disease	1	1.4
Hypertension	1	1.4
Diabetes	3	4.3
HBV vaccination		
History vaccination		
Negative	20	29.0
Positive	49	71.0

Abbreviation: HBV, hepatitis B virus.

Table 2 Distribution of factors associated with increasing risk of HCV infection among participants

Variables	N (69)	% (100.0)
Direct blood contact in work		
Yes	42	60.9
No	27	39.1
Application of standard precautions while dealing with blood		
Yes	9	13.0
No	60	87.0
Direct blood contact without protection		
Yes	19	27.5
No	50	72.5
Always using personal protective equipment		
Yes	69	100.0
No	0	0.0
Frequency of attending dental clinic		
Rare	68	98.6
Sometimes	1	1.4
Presence of HBV or HCV infection within the family		
No	46	66.7
HCV	22	31.9
HBV	1	1.4

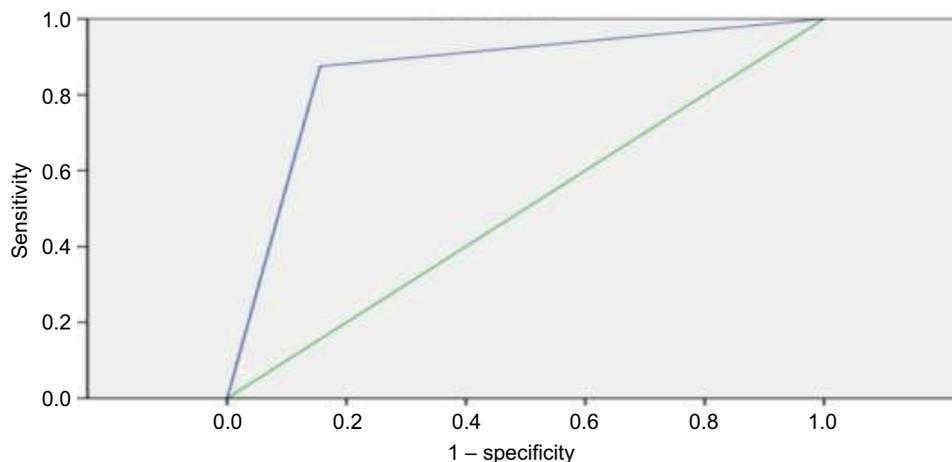
Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 3 ELISA as a screening test compared to PCR for diagnosis of HCV infection

Test	PCR		Total	Cohen's kappa	P-value
	Positive	Negative			
ELISA					
Positive	21 (30.4%)	7 (10.1%)	28 (40.6%)	0.6925	0.0000
Negative	3 (4.3%)	38 (55.1%)	41 (59.4%)		
Total	24 (34.8%)	45 (65.2%)	69 (100.0%)		

Note: Significance difference $p \leq 0.05$.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

**Figure 2** Receiver operating characteristic curve for ELISA to diagnose hepatitis C virus infection.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; ROC, receiver operating characteristic.

Table 4 OraQuick HCV rapid antibody test as a screening test compared to PCR in diagnosis of HCV infection

Test	PCR		Total	Cohen's kappa	P-value
	Positive	Negative			
OraQuick HCV rapid antibody test					
Positive	20 (29.0%)	6 (8.7%)	26 (37.7%)	0.6866	0.0000
Negative	4 (5.8%)	39 (56.5%)	43 (62.3%)		
Total	24 (34.8%)	45 (65.2%)	69 (100.0%)		

Note: Significance difference $p \leq 0.05$.

Abbreviations: HCV, hepatitis C virus; PCR, polymerase chain reaction.

Quantitative HCV-RNA showed different values of HCV-RNA number, 65.2% of them (45 out of 69) having undetectable level of HCV-RNA, while positive subjects had their viral loads ranged from 39.000 to 131.000 IU/mL.

Discussion

The prevalence of anti-HCV in Egypt was 14%, about 11,826,360 persons infected with HCV in 2010.¹⁰ HCWs are at increased risk of contracting HCV. At the individual level, this risk has two dimensions. In the first dimension, HCWs are performing hazardous and unsafe injection delivery practices, and in the second, they seek care as a patient in a system with lax infection control regulations. At a broader level of health system operations, this is due to quality issues in health care delivery.¹¹

Current study clarified that the prevalence of HCV among investigated HCWs by real-time PCR was 34.8%. A study carried out in 2012 among Egyptian HCWs at a national liver disease referral center detected a prevalence of hepatitis C virus antibody (HCV-Ab) to be 16.6%.¹² Another study in Cairo found that, among 597 HCWs who reported a blood exposure, the anti-HCV prevalence was 7.2%.¹³ Okasha et al⁶ found a prevalence of 8% in Ain Shams University Hospitals. El-Melligy et al¹⁴ detected HCV-Ab in 6.14% of Egyptian HCWs at Teaching Hospital being significantly higher in individuals aged ≥ 40 years when compared with those < 40 years. Half of those with positive HCV-Ab had HCV-RNA below the detection limit.¹⁴ Lower figure of 5.2% of HCWs were anti HCV positive as reported by Zayet et al in 2015 in Aasuit General Surgery Department, Egypt.¹⁵

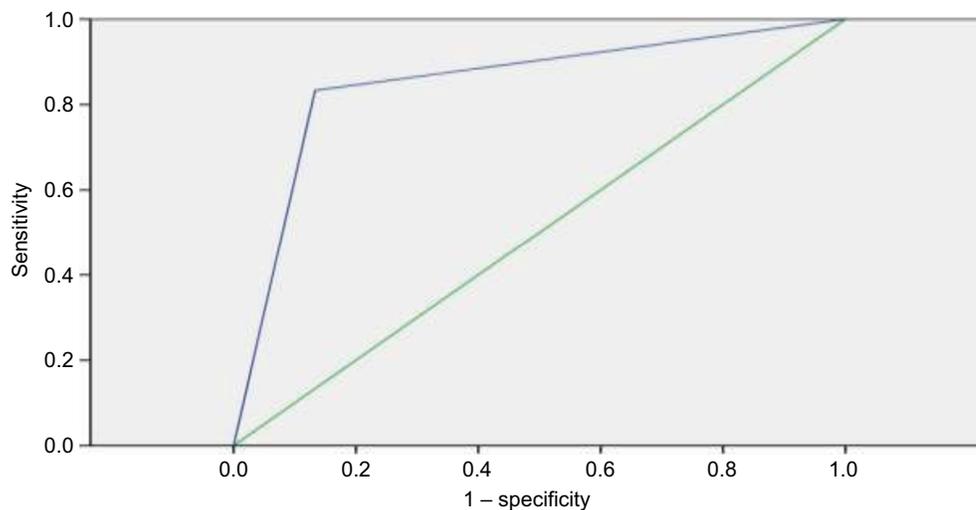


Figure 3 Receiver operating characteristic curve for OraQuick HCV rapid antibody test to diagnose HCV infection.
Abbreviation: ROC, receiver operating characteristic.

Table 5 The correlation between PCR readings and factors increasing infection among participants

Variables	* r_s	**P-value
Direct blood contact in work	0.100	0.421
Application of standard precautions while dealing with blood	-0.265	0.028
Direct blood contact without protection	0.027	0.019
Frequency of attending dental clinic	0.089	0.469
Presence of HBV or HCV infection within the family	0.007	0.952

Note: *Spearman correlation, ** $p < 0.05$ is significant.

Abbreviations: PCR, polymerase chain reaction; HBV, hepatitis B virus; HCV, hepatitis C virus.

Eskandarani et al¹⁶ in 2014 argued that even with frequent exposure to blood and body fluids (BBF) among HCWs, the risk of HCV infection among Danish hospital staff was low represented 3.8%. However, they could not rule out the possibility of transmission due to significant underreporting and disobedience to follow-up blood testing, and they proposed improving surveillance of BBF exposure.¹⁶

Although the detected prevalence is much higher than those from different studies, yet this can be explained by the high prevalence of HCV among the general population. In a finding reported earlier by Munier et al in 2012, the HCV prevalence among HCWs was similar to that of the general population.¹³ Also, this difference may be due to the number of HCWs in the facility, different work environment, training, culture, availability of resources,¹⁷ and to the implementation of infection control guidelines.¹⁴ To further understand the gap that exists between written policies and procedures and actual practice, we distributed self-administered

questionnaires about factors associated with increased risk of infection. The results showed that 50% of study participants have direct contact to blood or other body fluids during their routine work, 30% of them confirmed adherence to personal protective equipment with non-compliance of the remaining majority. In like manner, different studies^{2,3,17} have reported suboptimal and non-uniform adherence to standard precautions by HCWs in developing countries as in developed. A large-scaled study done in West India reported that the majority (81.5%) of the respondents experienced splashes from body fluid.¹⁸

In this study, over three-quarters of medical doctors (78%) and two-thirds of nurses (64%) reported having experienced NSSIs, while the incidence among medical technologists was remarkably lower (26%). In a previous Indian study, 84 respondents reported NSSIs. The majority of the respondents (59%) experienced low accident incidence while just over one-tenth (14%) reported high incidence.¹⁸ Talaat et al¹⁹ in 2003 found 35.6% of HCWs from different types of health care facilities from two governorates in Egypt (Nile Delta and Upper Egypt) were exposed to at least one needle stick injury during the past 3 months with an estimated annual number of 4.9 needle sticks per worker.¹⁹ Another Egyptian study found that, out of 215 HCWs 86.5% was exposed to NSSI, and 68% was exposed to patients body fluid.¹⁴

Occupational exposure to percutaneous injuries and NSSIs are a substantial source of infections with blood-borne pathogens among HCWs causing substantial health consequences and psychological stress for them and accounts for almost 40% of the HCV infections.²⁰ WHO estimated that

of 35 million HCWs worldwide, ~3 million experienced percutaneous injuries each year, and of those injured 15,000 were likely to be infected with HCV.²¹ As stated before, the current work was done at a university hospital where an infection control program was recently implemented with the compliance of the HCW to practice infection control standard precautions which was still unsatisfactory. Infection prevention and control campaign is mandatory toward raising awareness of HCW about risks, post-exposure management, and prophylaxis during their everyday activity, being the mainstay to ensure their strict adherence with preventive behaviors, including safe procedures for using and disposing of sharps, and banning recapping via constant surveillance, monitoring response, and follow-up.⁶

Serological and molecular assay methods for diagnosing HCV infection should be helpful to help reduce the burden of this infection.²² Serum Ab ELISA has undergone some modifications over decades, seeking to improve their diagnostic capability and increasing sensitivity and specificity of the assay. Current study revealed specificity of 84.44% (CI 71.22–92.25) and sensitivity 87.5% (CI 69–95.66) by Heganostika HCV Ultra ELISA. Other serum Ab ELISA kits were assessed elsewhere and showed absolute sensitivity (100%) and a high specificity ranged from 88.1% to 100%.²³

The ELISA results were debated by other researchers since the attained results cannot distinguish between active infection and those who have cleared the virus. This is coupled with absence of an efficient culture system for HCV, denoting the necessity of confirmatory nucleic acid amplification technology techniques.²⁴ Others claimed for a lower sensitivity and specificity of serum Ab ELISA,²⁵ whereas, studies done in subSaharian Africa displayed high false positive ELISA Ab results in African populations.²⁶ However, ELISA is still a recommended test for screening as it is a cheap and easy test.

One step further on evaluation of OraQuick HCV rapid antibody test, it represented sensitivity of 83.33% (CI 64.15–93.32), specificity of 86.67% (CI 73.82–93.74). Practically, this test is easy to apply using saliva, an acceptable screening tool even for the public sector with a valuable cost-benefit. Our results were supported by other studies, recommending rapid oral HCV antibody test in particular in the developing countries, highlighting it as being a modified approach in HCV diagnosis facilitating the possibility of testing millions of people worldwide.^{27,28}

Accurate quantitation and detection of HCV infection with a lower limit of 5–50 IU/mL can be obtained by RT real-time HCV PCR in the favor of using a single assay and

to eliminate the need for other qualitative and quantitative tests.^{29,30} Evidently, confirmation of both ELISA serum Ab and saliva Ab kit results was got by RT real-time HCV PCR in 24 out of 54 participants, and their viral loads ranged from 39,000 to 131,000 IU/mL. In like manner, other studies mentioned real-time HCV RNA, high sensitivity, simplicity, reproducibility, and wide dynamic range especially, of available cost, make this method to be a suitable approach for monitoring viral load during therapy and tailoring of treatment schedules accordingly.³¹

To summarize, HCW at Zagazig University Hospitals are at risk for HCV infection with estimated prevalence of 34.8%. Lack of compliance to infection prevention and control guidelines are associated cofactors. Serum HCV-Ab detection by Heganostika HCV Ultra ELISA, is a sensitive and specific serologic assay 84.44% and 87.5%, respectively, each for diagnosis with correspondent results to that obtained by quantitative real-time PCR. OraQuick HCV rapid antibody test represented a sensitive (83.33%) and specific (86.67%) assay. With best concordance with the reference assay, real-time PCR is easy to apply using saliva and an acceptable screening tool even for the public sector with a valuable cost-benefit. Strict implementation of infection control and post exposure preventive measures should be a part of higher system management plan in all hospital departments.

Recommendations

Based on the findings from the current study, the authors recommended:

1. Reconstruction of curricula and modification of educational programs to include standard precautions and preventive behaviors to be a habit on daily basis.
2. Knowledge, attitude, and practice to NSSIs of health care beginners should be taken into consideration as a policy statement, being a challenging priority in career objectives.
3. Strict implementation of infection control and post exposure preventive measures should be a part of higher system management plan in all hospital departments.
4. Use of rapid oral test to screen for HCV infection
5. Different health care groups should be included with an appropriate sample size help to attain risk assessment.

Limitations of this study

The participants obtained by using a convenient sampling technique may not be representative of all hospital departments and limit the generalization of the study results

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Disclosure

The authors report no conflicts of interest in this work.

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