

# HEPATITIS C INFECTION AND DETECTION OF ANTIBODIES, RNA AND GENOTYPES AMONG FEMALE HEALTHCARE WORKERS IN BAGHDAD

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## Abstract

**Background:** Hepatitis C Virus (HCV) is a major public health problem worldwide. About 130- 200 million people are infected with HCV worldwide leading to 500,000 deaths annually (WHO 2014). Healthcare workers (HCWs) have played an important role in the transmission of HCV infection, either as victims or as sources of infection.

**Objectives:** To determine the prevalence of HCV, antibodies (Abs) RNA and genotypes among the female HCWs in Baghdad and to identify whether HCWs were infective or only infected.

**Subjects and Methods:** A cross-sectional study involving 1001 women attending 17 health care centres in Baghdad, Iraq, was carried out. Information on type and duration of their occupation was obtained. HCV Abs (anti-HCV) were tested using a third generation enzyme immunoassay (EIA-3) and immunoblot assay (Lia Tek-111). Molecular analysis using RT-PCR and DNA enzyme immunoassay (DEIA) for HCV-RNA and genotype detections were carried out for 63 serum samples.

**Results:** Only 160/1001 (15.98%) were HCWs. Anti-HCV and HCV- RNA seroprevalence were significantly higher (6.37%,  $p=0.0057$ , 88.83%,  $p= 0.011$  respectively) among HCWs than non HCWs. HCWs were at a significantly higher risk of exposure to HCV infection (OR=2.75, 95% C.I. =1.31-5.79). There was no significant association between HCV genotypes and the HCWs. HCV-4 showed higher expression (62.5%) among HCWs.

**Conclusion:** Female HCWs were infective and infected with HCV, thus there is a need for medical equipment to be sterilized and cleaned thoroughly.

**Keywords:** Healthcare Worker, Hepatitis C Virus, Hcv-Genotype, Nosocomial Infection, Ribonucleic Acid, Risk Factor.

## Introduction

A healthcare worker (HCW) is defined as an employee in the healthcare setting who comes into contact with patients or the patients' body (1). HCWs who are exposed to blood and body fluids in the workplace are at risk of being infected with blood-borne pathogens such as HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) (2). HCV continues to be a major public health concern worldwide. It is estimated that 130 -200 million people are infected worldwide (3) leading to 500,000 deaths annually (4, 5). Sharing of needles among intravenous drug users (IVDUs) is one of the major risk factors of HCV infection in developed countries; however,

in low-income countries, HCV transmission is frequently due to the re-using of needles for injections and other inadequately sterilized medical instruments (6). HCWs exposed to HCV-infected blood may acquire HCV infection (7). Bruno *et al* (2014) reported that HCWs may either be victims, or more rarely, a source of infection if they are already infected by HCV (7). About 10% or more of HCWs have been infected following exposure to blood from a HCV-RNA positive patient. This rate may vary according to the HCV-RNA load and/or HCV-genotype of the source (8).

The risk of HCV infection among HCWs is higher in percutaneous than in mucosal-cutaneous exposure

(75% vs. 25%) (9). Nicola *et al.* (2016) estimated that the incidence of sharp object injuries among healthcare workers ranges from 1.4 to 9.5 per 100 HCWs /year (9). Elseviers *et al.* (2014), in his literature review, stated that, "according to the data provided by the World Health Organization (WHO)", there are approximately 36 million HCWs worldwide, of whom around 3 million per year are exposed to injuries from sharp instruments, and therefore about one million subjects are contaminated with HCV (10).

Use of virologic assays have become essential in the management of HCV infection in order to improve the diagnosis, guide treatment decisions and assessment of the virologic response to antiviral therapy (11). Moreover, RNA detection used is as a measure of infectivity. Therefore, besides detecting HCV RNA, HCV genotype recognition is important for diagnosis, management and epidemiological purposes to allow tracing of the source of infection as well as the route of transmission (12). There are six major genotypes (1-6) of HCV that have been detected and are of various geographical distributions worldwide. Among those, HCV genotype 1 is the most prevalent (9).

In Iraq, variation in the prevalence of HCV was noticed in different population segments. The prevalence was 7.1% in the general population (13), 3.21% among pregnant women (14) and about 1% among blood donors (15). However, such prevalence was remarkably higher among haemophiliacs patients, which was estimated at 66.0% (16). Presently in Iraq, very few studies are available on the epidemiology of HCV in HCWs. Moreover, the molecular epidemiology of HCV and its associated occupational risk factors have never been investigated before. Thus, this study aims to determine the prevalence of HCV, antibodies, RNA, genotypes and risk of infection among the HCWs in several healthcare centres in Baghdad, and also to identify the predominant HCV genotypes among the Iraqi HCWs.

### **Materials and Methods**

The study protocol was approved by the Ethics Committee, Ministry of Health, Iraq and Dajilah University. Formal approval from the chosen healthcare clinics in Baghdad, Iraq, was also obtained. Sample size was calculated in order to achieve at least 0.8 power of study (sample size was calculated considering 80% power) with the given maximum probability of committing a Type 1 error of 0.05. The prevalence of HCV in the general population of Iraq was 7.1% (13), while the estimated prevalence in the study population was 13%. The estimated sample size was 814 subjects. Taking into account a 20% defaulter, therefore, the final estimated total sample size will be 977 women.

Data were collected from November 2009 - August 2011. Out of 122 healthcare centres in Baghdad, 17 were randomly chosen. A cross-sectional study was carried out with a random sample of 1001 women attending the 17 healthcare centres. Informed and voluntary consent were obtained from participants. HCWs with a working period of less than six months were excluded. Face to face interview

was conducted by the researchers to obtain information on age, occupation, and duration of working in the healthcare setting for HCWs.

Blood sample of 5–10 ml was obtained from each participant. The serum was separated immediately to prevent viral RNA degradation. Each serum sample was dispensed into two screw-capped vials and then stored at  $-20^{\circ}\text{C}$  and  $-70^{\circ}\text{C}$  for antibody testing and molecular analysis respectively. Initial screening of HCV antibody was carried out using a third generation enzyme immunoassay (EIA-3) (UBI HCV EIA, United Biomedical, USA). Confirmation of positive results was done using a third generation immunoblot assay (LiaTek-III kit, Organon, Amsterdam). The results were interpreted as Positive, Indeterminate and Negative. LiaTek-III only reactive serum samples were regarded as positive HCV antibodies serum. In addition, 63 serum samples (stored at  $-70^{\circ}\text{C}$ ) containing 33 positive, 20 indeterminate & 10 negative anti-HCV LiaTek-III sera were transported in an ice card to Sorin Diagnostica laboratories (Sallugia, Italy) for molecular analysis. All the 63 serum samples were tested for HCV-RNA positivity followed by HCV-genotyping using an advanced molecular method based on the combination of two well-established techniques, namely, reverse-transcription polymerase chain reaction (RT-PCR) and DNA enzyme immunoassay (DEIA). Each sample was subjected to extraction of RNA, followed by synthesis of complementary DNA (cDNA). According to the protocol by Sorin Biomedica laboratory amplification of the newly developed cDNA at the 5 untranslated region, using single-step PCR, was performed. Then hybridization to specific oligonucleotide probe was done using a fixed solid phase avidin-biotin bridge (Genentech, San Francisco, USA). The outcome of this procedure was detected by standard enzyme linked immunosorbent assay (ELISA) using monoclonal antibody specific for double-stranded DNA. The absorbance of the coloured reaction was obtained at 450 and 630 nm.

For HCV genotype detection, DEIA was carried out, as described previously, using different oligonucleotide probes, according to 6 HCV genotypes and subtypes following the manufacturer's instructions. Classification of HCV genotypes/subtypes were instituted according to Simmond's nomenclature (1994) (17).

Data analysis was performed using SPSS 21.0. Unadjusted association between outcome variables (HCV status) and occupational characteristics was tested using bivariate analysis via  $\chi^2$  test for comparison. Risk factors for HCV were estimated using odds ratio (OR) with associated 95% confidence intervals (CIs). Depending upon the probability of exposure to an overt or covert risk factor, the participants occupation was classified into health care workers (HCWs) and non-HCWs (i.e, housewives (HW) and other types of job).

### **Result**

Most (54%) of the respondents were between 25 and 40 years old, 38% were younger than 25 years, and 6%

were more than 40 years of age and the majority -889 respondents (89%)- were married. Out of 1001 participants, 160 (15.98%) were HCWs and 841(84.01%) were non-HCWS, the latter included 636 (63.5%) housewives (HW) and 205 (20.5%) others (students, office workers, house maid, farmers etc). Most of the HCWs were nurses (111 or 69.4%), followed by 23 laboratory workers (14.38 %) and 13 dentists (8.12%).In addition, 13 (8.12%) were medical doctors comprising seven house officers, three medical specialists with three obstetricians and gynecologists (O&G) qualification.

Positive anti-HCV antibodies was detected in the sera of 11 HCWs and 22 non-HCWs. The HCWs demonstrated a significantly higher (6.88%) anti-HCV seroprevalence rate compared to the non-HCWs (2.62%),  $\chi^2=7.649$ ,  $p=0.0057$  (Table 1). Anti-HCV seroprevalence was more than three times significantly higher among the HCWs (6.88%) compared to HW (2.36%) and the others (3.41 %,) with  $\chi^2= 8.19$ ,  $p=0.0042$  (Table 2). Positive anti-HCV serum was found in 15.4% (2/13) dentists, and in 8.7% (2/23) laboratory workers and 6.3% (7/111) nurses. None of the 13 medical doctors were infected with HCV.

**Table 1.** Comparison of anti-HCV antibody rate between HCWs and non- HCWs women in Baghdad, Iraq

Occupation	Total No. (%)	Anti-HCV sera status		$\chi^2$ value	P value	Crude OR (95% CI)
		Positive No. (%)	Negative No.			
HCWs	160 (15.98)	11 (6.88)	149	7.649	0.0057	2.75 (1.31-5.79)
Non-HCWs	841 (84.02)	22(2.62)	819			

**Table2.** Anti-HCV antibody rate among HCWs and subset of non- HCWs women in Baghdad, Iraq

Occupation	Total No. (%)	Anti-HCV sera status		$\chi^2$ value	p-value
		Positive No. (%)	Negative No.		
HCWs	160 (15.98)	11 (6.88)	149	8.19	0.0042
HW	636 (63.54)	15 (2.36)	621		
Others	205 (20.48)	7 (3.41)	198		
Total	1001	33 (3.3)	968		

In developing the hypothesis on modes of transmission of HCV, the association between potential risk factors and anti- HCV seropositivity was analysed. Our study revealed that the odds of exposure to HCV infection was almost three times significantly greater in HCWs than in non HCWs, OR=2.75, 95% C.I. =1.31-5.79 (Table 1).

Molecular analysis for the 63 samples showed that 34 (54%) were positive for HCV-RNA. Out of the 33 positive LiaTek-III examined sera, 27 (81.82%) showed positive HCV-RNA. On the other hand, none of the negative anti-HCV LiaTek-III sera exhibited positive HCV-RNA. Interestingly seven out of 20 (35.0 %) indeterminate LiaTek-III sera showed a positive HCV-RNA (Table 3).

**Table 3.** HCV- RNA Sera status among HCWs and Non HCWs women in Baghdad, according to their anti-HCV (positive, indeterminate and negative) Lia Tek-111 Sera status

Lia Tek-111 Sera status	HCV-RNA Sera status		HCV-RNA n=53			
	Negative N, (%)	Positive N, (%)	Positive N, (%)		Negative N, (%)	
			HCWs	Non HCWs	HCWs	Non HCWs
Positive N= (33)	6	27(81.82)	10	17	1	5
Indeterminate N= (20)	13	7 (35)	6	1	1	12
Negative N= (10)	10	0	-	-	-	-
TOTAL	29	34 (54)	16(88.9)	18 (51.43)	2	17

With respect to the individual’s occupation and its relation to HCV- RNA seropositivity, our study revealed that HCWs were significantly associated with a higher rate (88.9%) of positive HCV-RNA compared to the non-HCWs (51.43%),  $\chi^2= 7.25$ ,  $p= 0.007$  (Table 4). Additionally, HCWs showed a significantly higher positive HCV-RNA rate (88.9%) compared to HW and other jobs (58.3% and 36.4% respectively),  $\chi^2=6.904$ ,  $p= 0.0317$  (Table5). Moreover, there was significant evidence that HCWs were exposed to a higher risk for HCV infection, which was more than 6 times higher, compared to non-HCWs (OR = 6.74, 95% CI=1.15 to 2.31) (Table 4). The rate of HCV viremia among HCWs was 10% (16/160), which was much higher than 2.1% in non HCWs (18/841).

**Table 4.** Comparing the risk of HCV infectivity (HCV-RNA) between HCWs and Non HCWs women in Baghdad, Iraq

Occupation	Total 53	HCV- RNA		$\chi^2$ value	P-value	Crude OR (95% CI)
		Positive=34 No. (%)	Negative=19 No.			
HCWs	18	16(88.9)	2	7.253	0.0071	6.74 (1.15 -2.31)
Non- HCWs	35	18 (51.43)	17			

**Table 5.** Comparing the risk of HCV infectivity (HCV-RNA) between HCWs and subset of Non HCWs women in Baghdad, Iraq

Occupation	Total 53	HCV- RNA		X <sup>2</sup> value	P-value
		Positive=34 No. (%)	Negative=19 No.		
HCWs	18	16(88.9)	2	6.904	0.0317
HW	24	14(58.3)	10		
Others	11	4 (36.4)	7		

Five HCV genotype/subtypes (1,1a, 1b, 3a or 4) were detected among the Iraqi population, either as a single (1, 1a, 1b or 4) or mixed (1+4, 1b+4; 3a+4) infection. There was no significant association between HCV genotypes and occupation of women ( $\chi^2=1.421$ ;  $p=0.964$ ). However, the most predominant HCV-genotypes among Iraqi HCWs was HCV-4 (10/16-62.5%), followed by HCV-1b (5/16-31.25%), and HCV-1 (4/16-25%) genotypes/subtypes (Table 6).

**Table 6.** HCV genotypes/subtypes distribution among women's HCWs & non-HCWs in Baghdad, Iraq

	Lia Tek-111		HCV-RNA		HCV-genotypes/subtypes							Total
	+ve	Indeterminate	-ve	+ve	1	1a	1b	4	1& 4	1b&4	3a&4	
HCWs (n=18)	11	7	2	16	2	1	3	4	2	2	2	16
Non HCW (n=24)	22	13	17	18	2	3	3	4	3	1	2	18
Total	33	20	19	34	4	4	6	8	5	3	4	34

$\chi^2= 1.421$ ;  $p = 965$

## Discussion

Healthcare workers (HCWs) are usually exposed to human blood and other potential infectious biological products compared to the general population. Blood-borne transmission is caused by more than 60 pathogenic agents, with HCV and HBV are those most frequently transmitted to HCWs (18).

Anti-HCV seropositive prevalence worldwide, among HCWs, range from 0% to 9.7% (9). The prevalence of anti-HCV seropositivity in our study (6.88%) was found to be within this range. However, it is higher than those reported by others in Italy (1.2%) (19), Japan (3%) (20), Pakhtunkhwa (4.13%) (21), Germany (5.8%) (1), Brazil (4.8%) (22), and Georgia (5%) (2). On the other hand, our finding is much lower than that in Egypt (7.2%, 8.0 %) (18,23). A meta-analysis study done by Claudia *et al.* (2015) demonstrated that HCWs worldwide (except in Japan) have a significantly increased risk (OR =1.5) of HCV infection due to exposure (24). Our findings support that of Claudia *et al.* (2015) where we detected that the HCWs were significantly exposed to risk of HCV infection more than 2.5 (2.75) times greater than the non-HCWs. High prevalence and risk of HCV infection might be related to the absence of good preventive measures and the high workload. Availability of electrical power, well- trained health care workers, availability of spare parts, good management and control procedures are factors affecting the decontamination and sterilization process. Lack of one or more of these essential conditions (as is the case in Iraq) leads to contamination of medical equipment such as needles and syringes, which may contribute to the high prevalence of anti-HCV. In addition, non-adherence to guidelines on infection control,

inadequate provision of protective devices for HCWs may also be contributory factors for HCV transmission. High prevalence of HCV among the general population placed the HCWs at a higher risk of acquiring HCV infection. Various methods used, difference in the population socio-demography and HCV genotype variations may play a role in the variation of anti-HCV antibody prevalence.

HCV transmission that occurred among HCWs was either through mucosal-cutaneous or percutaneous exposure to potentially infectious material (9). Infection via mucosal-cutaneous method occurs when patients' blood, blood products or any other infected biological material accidentally enters or come in contact with mucous membranes of the mouth or eyes of HCWs. Percutaneous exposure is when the HCWs are injured by sharp contaminated objects, such as a needles, blades or pieces of glass (18).

Several factors affecting the likelihood of HCV transmission through contaminated biological material are mentioned in some of the studies done worldwide. Okasha *et al.* (2015) reported that the highest rate of transmission of HCV infection was through exposure to contaminated blood and/or its products (18). A study done by Jagger *et al.* (2002), found that 10% of the healthcare workers were infected due to exposure to blood of HCV-RNA-positive patients (8). Another factor that contributes to the likelihood of HCV transmission is the extent and/or depth of the cutaneous or mucosal wound (18). Non-adherence to guidelines on infection prevention control, measures such as unsafe disposal of used needles/syringes, non-sterilized syringes, and unscreened blood transfusion are among the factors affecting the likelihood of transmission (25).



In Iraq, a study done by Salih *et al.* (2014), concluded that awareness of protective measures among the HCWs was insufficient, considering 67.8% of HCWs experienced at least one occupational percutaneous injury and mucosal exposures (PME) within a year (26). A study done in Egypt showed that 64% of the HCWs did not follow the guidelines for safe disposal of used needles or syringes, and only 32% of the HCWs used gloves during medical procedures (2). Low educational levels and high HCV prevalence rate (3.21- 7.1%) in the general population (13, 14), and infected blood donors 1% (15), are among the contributory factors to HCV transmission among HCWs in Iraq.

According to the categories of HCWs, an estimation of HCV prevalence had been demonstrated in our study, and we found that the dentists showed the highest HCV prevalence, which supports the findings of Al-Kubaisy *et al.* (2015), in which dental surgery acts as a risk factor for HCV infection (27). In contrast, Butsashvili *et al.* (2012), who found that physicians were almost twice as infected with HCV compared to that in nurses (2). Interestingly, our study revealed that none of the medical doctors showed positive anti-HCV, while the nurses showed a 6.3% anti-HCV seropositive rate. Most probably this is related to the fact that recapping of needles and decontamination/cleaning of instruments after surgery were usually done by the nurses (2, 28). Salih *et al.* (2014) in their study reported a rate of 13.3/nurse/year PME (per mucous exposure) (26).

The most important finding in our study is the high rate of HCV viremia (HCV-RNA) (10%) among HCWs, which was almost five times higher than that in non-HCWs (2.1%). This was much higher (2.79%, 3.7%, and 3%) than those reported, respectively by several authors (21, 22, and 29). On the other hands, this rate is lower than the 12.3% detected by Aline *et al.* (2013) (23). This discrepancy could be due to the variation of HCV prevalence in different communities or different HCV marker detection method used and the variation of HCV genotypes.

The high (35%) rate of HCV-RNA among the LiaTek-III indeterminate sera is higher than that detected by another study (12%) (23). Aline *et al.* (2013) stated that subjects of high risk exposure to HCV such as HCWs, could induce cell-mediated immunity in the absence of detectable viremia or seroconversion (23). Our findings showed a significantly high rate of positive HCV- RNA (88%) in sera of the HCWs (positive and indeterminate LiaTek-III) with a significant risk of exposure (OR 6.7368) which is more than 6 times higher. These findings are interesting in terms of nosocomial infection indicating that Iraqi HCWs were not just infected but they were also infective and might be considered as an important source of HCV infection. Hasegawa *et al.* (2003) stated that anti-HCV and HCV-RNA in haemostatic gauzes from infected patients referred to dental clinics were found to be positive even after being kept at room temperature for 24 hours, and this might contribute to the transmission of nosocomial infection (30). On the other hand, our study demonstrated that about 18.2% of the cases (LiaTek-III positive anti-HCV in both groups) had negative HCV-RNA

in their sera. This is in line with the findings of a study conducted by Tahan *et al.* (2005) who mentioned that during the first 3–6 months, only 15-30% of the patients with positive HCV-RNA may become negative, while in others, HCV-RNA remained positive (31).

Identification of HCV genotypes is important for diagnosis, treatment and epidemiological analysis. Variation of HCV genotypes according to geographical distribution is complex, with HCV genotype 1 being the most prevalent worldwide (9). HCV genotypes such as 1a, 1b, 2a, 2b, 2c had a broad global distribution, while genotypes 5 or 6a were found in very specific areas, and genotype 4 is predominantly detected in the Middle East and Central Africa (32).

In contrast to the study of Sanaullah *et al.* (2011) who reported that the most predominant HCV genotypes was 3a 1 and 2a, , our study found that HCV-4 and 1b were the most predominant HCV genotypes among Iraqi HCWs. This is almost similar to the findings of Alfaleh and Ramia (1997) that HCV-4 is the most predominant genotype followed by HCV-1b and 1a among Saudi patients (33). Predominance of HCV-4 among the Iraqi population usually presented as a mixed infection which may indicate the presence of HCV nosocomial infection/transmission.

## Conclusions

HCWs in Iraq were significantly at a risk of an infective and infected state of HCV infection, having a significantly higher risk of acquiring HCV infection. This indicates that occupational risk and nosocomial transmission does exist among the population. HCV-4 is the most predominant genotype among the HCWs.

Currently, there is no HCV vaccine available. Thus, health education, implementation of universal precautions in handling blood and body fluid among HCWs, pre-employment HCV screening and annual HCV antibody and RNA screening among HCWs are recommended as preventive measures to control HCV infection.

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## Competing interests

It is hereby declared that there is no conflict of interest pertaining to this paper.

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