



Prevalence of Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) Infections and their Co-infection among Blood Donors in Minia Governorate, Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. Author NAH put together the protocol of the study, carried out data analysis, real-time PCR for HBV and HCV and supervised the quality control of sample processing. Author SMAE supervised the quality control of the study and reviewed the article. Author ZMM collected the samples and assured quality control of samples and performed the EIA assay for HBV and HCV antibodies. Author MAH had input in the protocol of the study, supervised the quality control of the study and wrote the manuscript.

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ABSTRACT

Aim: Infections with Hepatitis B virus (HBV) and Hepatitis C virus (HCV) cause serious morbidity and mortality. This study was designed to determine the prevalence of Hepatitis B and C infections and their co-infections among blood donors in Minia governorate, Egypt.

Study Design: A cross-sectional study.

Place and Duration of Study: The study was conducted over a period of 6 months starting from May 2011 till December 2011 and it included 5410 samples from blood donors at the Regional Blood Transfusion Center in Minia governorate.

Methodology: Both HBsAg and antibodies to HCV were detected by EIA in 5410 blood samples from potentially healthy asymptomatic blood donors. Detection of HBV DNA and HCV RNA was

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carried out by real-time PCR (RT-PCR).

Results: Most individuals were males (4305; 79.6%) and were from rural areas (3695; 68.3%). The sero-prevalence of infections was 0.9% (48 cases) for HBV and 6% (322 cases) for HCV, and 0.1% (7 cases) for co-infection. Out of 7 samples with co-infection, only one (14.3%) was positive for HBV DNA and HCV RNA. The mean age of HCV-antibody positive donors (33.2 ± 9.41 years) was significantly higher than that of the HBV-positive donors (27.3 ± 6.06 years) and co-infection (29.9 ± 10.21 years) ($P < 0.05$). Prevalence of HBV and HCV was higher in males (1% and 6.6% respectively) while HCV was higher in rural areas (6.8%) unlike HBV which did not show any difference in residential distribution (0.9% for both rural and urban areas).

Conclusion: The prevalence of both viruses is low and that of the dual infection is lower than any of the two viruses alone.

Keywords: Hepatitis B; hepatitis C; co-infection; blood donors.

1. INTRODUCTION

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are two different hepatotropic viruses. HBV belongs to family Hepadnaviridae of hepatotropic DNA viruses, whereas HCV belongs to the hepacivirus genus within the Flaviviridae family. About 400 and 210 million people worldwide are infected with HBV and HCV, respectively [1], which means that one fifth of the world's population is either infected with one of the two viruses.

The infection of HBV can result in a broad spectrum of clinical diseases, varying from a low viraemic asymptomatic "inactive" carrier state, acute hepatitis or progresses to chronic hepatitis, which may lead to a cirrhosis rate of 2 to 5% per year in HBe-positive patients and hepatocellular carcinoma (HCC) with a cumulative 5-year incidence of 15 to 20% [2]. Both HBV-related end-stage liver disease and HCC are responsible for around 1 million deaths per year [3].

After being considered of minor importance when it was discovered in 1989, HCV proved to be of global importance, as it affects almost all countries and is now responsible for over 80% of cases of chronic hepatitis [4]. The 2008 Egyptian demographic health survey (EDHS) gathered information on the prevalence of HCV among a population between the ages of 15 and 59 years and found that 14.7% had positive anti HCV antibody and only 9.8% were positive for the viral RNA [5]. In another study carried out in Egypt, the annual seroprevalence of HBV and HCV infection have decreased from 2.3% to 0.9% and from 17.7% to 7.4% respectively [6].

HCV is considered as a national epidemic in Egypt due to the past mass vaccination with parenteral- antischistosomal-therapy (PAT) [7-8]

and due to other reasons as shown in a recent systematic review [9]. On the other hand, the prevalence of HBsAg in Egypt is of intermediate endemicity (2–8%) with about 2-3 million HBV chronic carriers among Egyptians [10-12].

The co-infection of both HBV and HCV is common due to shared modes of transmission. The natural history of HBV and HCV co-infection was difficult to categorize due to heterogeneous populations; however, a classification based on five categories of clinical features and immune profiles is now available. HBV and HCV co-infection is classified into: a) acute co-infection (acute hepatitis with simultaneous HBV and HCV infection), b) HCV superinfection (acute HCV on top of chronic hepatitis b infection), c) HBV superinfection (acute HBV on top of chronic hepatitis C), d) chronic HCV with occult HBV: (positive HCV RNA, negative hepatitis B surface antigen (HBsAg) and anti- HBs and positive HBV DNA), e) chronic co-infection: (chronic hepatitis with positive HBV DNA and positive HCV RNA) [13].

Both viruses are common serious complications of blood transfusion. The reduction of their transmission has been achieved in developed countries by reducing unnecessary transfusions, using only regular voluntary donors and by applying systematic screening of all donated blood for infection [14]. HBsAg is the hallmark of HBV infection and is the first serological marker to appear in acute hepatitis B. Most patients recovering from acute hepatitis B clear HBsAg within 4-6 months after onset of infection; however, persistence of HBsAg for more than 6 months indicates chronic HBV infection [15]. One of the criteria for CHB is the detection of HBV DNA in plasma or serum. A threshold of $\geq 20,000$ lu/ml (100,000 copies/ml) is specified for active viral replication during hepatitis B e antigen

(HBeAg)-positive CHB [16]. The detection of anti-HCV antibodies in plasma or serum is based on the use of third-generation enzyme immunoassays (EIAs) that detect antibodies directed against various HCV epitopes [17]. Qualitative detection assays are based on the principle of target amplification using classic polymerase chain reaction (PCR), real-time PCR or transcription-mediated amplification (TMA) [18].

In Egypt, distribution is geographically divided into; lower and upper Egypt as well as urban and rural settings. Minia is one of governorates of middle Egypt and it is considered to be an urban city surrounded by semi urban regions with rural areas extending 60 km north and south of Minia city. Our objective was to determine the prevalence of hepatitis B and C infections and co-infections among blood donors in this governorate.

2. MATERIALS AND METHODS

The study was conducted over a period of 6 months starting from May 2011 till December 2011 with 5410 samples from blood donors at the regional blood transfusion center in Minia governorate, which is the only blood bank in the governorate. Blood samples were collected after the patients had consented.

HBsAg enzyme immunoassay kit (ETI-MAK-4, DiaSorin) was used for qualitative detection of HBsAg, bioELISA HCV 4.0 (BIOKIT, S.A. - 08186) was used for qualitative detection of antibodies to HCV, QIAamp viral RNA mini kit (QIAGEN Inc, USA - 52906) was used for purification of viral RNA and QIAamp DNA mini kit (QIAamp DNA Mini Kit (QIAGEN, Santa Clarita, USA- 51106) was used for purification of viral DNA. HCV RNA detection was done by real-time PCR analysis on an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using fluorescent labeled probes. The HCV primers and probe sequences were directed against the 5'NCR (noncoding region) of the HCV genome. Each 25 µl PCR reaction contained forward and reverse HCV primers, HCV probe, IPC forward and reverse primers, IPC probe, 12.5 µl of 2X RT-PCR buffer and 1 µl of 25X RT-PCR Enzyme Mix (Applied Biosystems) and 8.5 µl of RNA. The PCR cycling conditions consisted of one 10 min cycle at 45°C and one 10 min cycle at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 45 sec. HBV DNA detection was carried

out by real-time PCR analysis (same device as for HCV RNA). Each 25 µl PCR reaction contained 15 µl of HBV TM Master Mix (Applied Biosystems) and 10 µl of DNA. The PCR cycling conditions consisted of one 10 min cycle at 95°C, followed by 45 cycles of 95°C for 15 sec and 60°C for 1 min.

Data were expressed as mean ± standard deviation (SD) or number and percent. T-student test and ANOVA test were used to compare independent groups of continuous data. Chi-square test was used to compare independent groups of categorical data. P-value <0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

The mean age was 29.4±8.2 years and it ranged from 19 to 58 years. There were 4305 (79.6%) males and 1105 (20.4%) females. There were 3695 (68.3%) from rural area and 1715 (31.7%) from urban area (Table 1).

Table 1. Demographic characteristics of the participants (n= 5410)

Variable	Result
Age (years)	
Range	19 – 58
Mean ± SD	29.4±8.2
Sex	
Males No (%)	4305 (79.6%)
Females No (%)	1105 (20.4%)
Residence	
Rural No (%)	3695 (68.3%)
Urban No (%)	1715 (31.7%)

Using EIA for 5410 samples of the blood donors; 377 (7%) were seropositive for HBsAg, HCV IgG or both of them. From the positive samples; 48 (0.9%) were positive for HBsAg, 322 (6%) were positive for HCV IgG, while 7 samples (0.1%) were positive for both viruses (Table 2).

Table 2. Seroprevalence of hepatitis B, hepatitis C and co-infection

EIA results	Number	Percent
Positive HBsAg	48	0.9%
Positive HCV IgG	322	6%
Co-infection	7	0.1%
Negative EIA	5033	93%
Total	5410	100%

The mean age of HCV-positive donors was significantly higher than that of the HBV-positive

donors ($p < 0.001$): 33.2 ± 9.41 years for positive HCV IgG versus 27.3 ± 6.06 years for positive HBsAg. The seroprevalence of HBV and HCV was higher among males (1%, 6.6% respectively) but that was only significant for HCV ($p < .05$) with odds of males being HCV positive nearly twice in comparison to females (Table 3). With regards to residence, HCV is significantly higher in rural areas (6.8%), which is not the case for HBV which has similar distribution in rural and urban areas (0.9% and 0.9%) (Table 3). For the seven co-infection cases, the mean age was 29.9 ± 10.21 years, 4 were males and 4 were from rural areas.

Using PCR for the seropositive co-infection samples (7 samples), there were 4 samples (57.1%) positive for HCV RNA, 2 samples positive for HBV DNA (28.6%) and only one had both HCV RNA and HBV DNA (co-infection) (14.3%) (Table 4). After including the results from the seropositive co-infection samples, the prevalence of HBV DNA was 0.92% (50 samples), the prevalence of HCV was 6.02% (326 samples) while that of co-infection was 0.018% (one sample). The only case with co-infection was a 21 years old female patient from an urban area.

The risk of transfusion-transmitted hepatitis remains very high in developing countries, so proper screening of donated blood is a priority. The current study demonstrates the prevalence of HBV, HCV and their co-infection among blood donors in Minia Blood Bank. Albeit blood donors may not reflect the general population, most of the studies are carried out in this group because

it could present a better understanding of the epidemiology of these diseases in the community [19-21].

The included samples are convenience samples and do not represent the prevalence in the community. In our study, the seroprevalence of HCV was higher in older age groups than in HBV, and in males from rural areas which is comparable to other studies in Egypt [22-27]. The relatively higher prevalence of HCV than HBV could be explained by the compulsory HBV vaccination in the first year of life. The higher prevalence in males could possibly be due to higher exposure to risk factors. Our results are also comparable to those obtained in a previous study performed in Minia (Upper Egypt) by Khattab and coworkers [28] and to a similar study carried out in Alexandria (Lower Egypt) by Wasfi and colleagues [29].

The difference in the results between the HBsAg and DNA levels, where out of the 7 HBsAg positive co-infection samples only two samples had HBV DNA, could be due to weak correlation between the DNA and HBsAg levels. This could possibly be ascribed to the difference in the pathways of HBV DNA synthesis and HBsAg synthesis which are affected variably by the host immune defense [30-32]. On the other hand, false positive results for HCV antibody may be found in patients with autoimmune diseases possibly due to high concentration of immunoglobulin components in their blood [33-34].

Table 3. Comparison of the demographic data of the individuals with positive HBsAg and positive HCV IgG

Demographic characteristics	Positive HBsAg		Positive HCV IgG	
Age				
(mean \pm SD)	27.3 ± 6.06		33.2 ± 9.41	
Sex				
Males, no (%)	43 (1%)	2.2 (0.877-5.617)‡	284 (6.6%)	1.9 (1.404-2.801)‡*
Females, no (%)	5 (0.5%)		38 (3.4%)	
Residence				
Rural, no (%)	33 (0.9%)	1 (0.553-1.885)‡	250 (6.8%)	1.7 (1.265-2.166)‡*
Urban, no (%)	15 (0.9%)		72 (4.2%)	

‡ OR (95% CI),
* Significant p value < .05

Table 4. Nucleic acid type in seropositive coinfection samples

Nucleic acid	Number	Percent
Positive HCV- negative HBV	4	57%
Negative HCV- positive HBV	2	28.6%
Positive HCV- positive HBV	1	14%
Total	7	100%

Given that HBV and HCV have the same transmission routes, co-infection may occur in an estimated 7- 20 million individuals infected with both viruses worldwide [35]. In the present study, 7 samples (0.1%) were seropositive for both HCV and HBV but only one sample (0.018%) was positive for both viruses using PCR. The exact prevalence of HBV and HCV co-infection is not known because most of the published reports focused on highly selected and limited populations. Multiple studies evaluated the rates of HCV co-infection among HBsAg carriers; the rates range from 1.3% to 18%, depending on the geographic centers and selected patients [36-39]. The prevalence of co-infection in neighboring countries is variable with 5% in Tunisia and less than 0.01% in Morocco while in Lybia the prevalence was 1.3%; however, the latter study was carried on a group of dialysis patients [40-42]. The low prevalence rate of co-infection in our study could likely be due to the reciprocal inhibition of either virus on the other's replication [43-44].

4. CONCLUSION

The prevalence of HBV and/or HCV infection(s) among blood donors in the study area is low. This could indicate an increase in public awareness or the absence of some risk factors. These factors should be investigated in areas with higher prevalence. Further studies are recommended among different population groups to determine the exact prevalence.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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