

# Prevalence of Hepatitis B Virus and Hepatitis C Virus in Egypt A Retrospective Study

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## Abstract

In Egypt, 8–10 million people are estimated to have viral hepatitis. By the age of 15, about 50% or more of Egyptians had probably been exposed to HAV infection. Also, throughout the first ten years of life, more than 60% of Egyptians tested seropositive for anti-HEV, a self-limiting hepatitis infection. In Egypt, the three most common viruses that cause hepatic cirrhosis, chronic hepatitis, and hepatic cancer (hepatocellular carcinoma [HCC]) are the hepatitis B virus (HBV), the hepatitis C virus (HCV), and the hepatitis D virus (HDV). From 1990 to 2017, Egypt exhibited the highest age-standardized cirrhosis mortality rate in the world. The national infantile immunization program has reduced the incidence rate of HBV from 1.3% to 1.5%. Because HDV antibodies (IgG) range from 8.3% to 43% among all HBV cases, coinfection of HBV cases with HDV is widespread in Egypt. HCV prevalence (7%) was recently reported. One of Egypt's significant public health problems, viral hepatitis, requires increased concentration and financing from health policymakers.

**Methods and material:** the study was done on 270,900 blood donors from the National Cancer Institute and Nasser Institute blood banks. Third generation ELISA technique was used to screen HBsAg and anti HCV antibodies. All seronegative blood has been further tested via NAT to detect viremia for both viruses. Seroprevalence of HBsAg and HCV antibodies was (1.8%), (and 5.3%) respectively with a P-value <0.01%. Coinfection by both viruses was (0.09%). Seronegative blood, which showed viremia was (0.003%), and (0.007%) for HBV and HCV, respectively. HBV and HCV prevalence in the studied group in the period between Jan (2005) - Jan (2018) was (1.8%) and (5.3%), respectively. Comparing both techniques showed a higher detection level using NAT than ELISA for both HBV & HCV by (0.003% and 0.006%), respectively which were statistically insignificant.

**Conclusion:** The recent study has demonstrated that the overall rate of infection in Egypt of both HBV and HCV has declined over previous years. The implementation of both immunization campaigns against the HBV and the new anti-HCV drug by the government, which was distributed in hepatitis centers over 26 governorates, has markedly reduced the previous percentages of both viruses all over Egypt.

This study aims to estimate the HBV & HCV prevalence in Egypt during the selected studied period & among blood donor. Coinfection of both viruses in the selected study population. Estimating the usefulness of NAT as a sensitive and specific tool in the diagnosis of active HCV and HBV viremia in seronegative Egyptian blood donors was also evaluated.

**Keywords:** HBV, HCV, Prevalence, Coinfection

## INTRODUCTION

Viral hepatitis is caused by a viral infection that causes liver inflammation (WHO, 2012). Hepatitis viruses of types A, B, C, D, and E have been identified as the viral etiologies of hepatitis-related epidemic jaundice. Viral hepatitis is a significant public health concern, infecting millions of people annually; some infections subsequently lead to hepatocellular carcinoma (HCC), liver cirrhosis, and many mortality, among a significant percentage of infected cases. The World Health Organization (WHO) estimated that 1 in 3 people worldwide had been infected by either HBV or HCV (WHO, 2012; Hajarizadeh et al., 2013). Infection with any one or several of the hepatitis viruses, such as HBV (2 billion), HCV (185 million), or HEV (20 million), infects approximately

2.3 billion individuals worldwide (Ott et al., 2011; Kamar et al., 2012). This results in about 1.4 million fatalities annually. Most of these deaths (approximately 90%) are caused by HBV and HCV, with other hepatitis viruses accounting for the remaining 10% (Jacobsen and Wiersma & 2010; WHO, 2010; Wiktor & Hutin, 2016).

The risk of HBV infection rises with age, with people aged 35 to 44 having the highest risk. Because adults have higher rates of viral elimination than children, roughly 30% of whom are between the ages of one and five, so they acquire chronic HBV infection after exposure (Chou et al., 2015; Wang & Li, 2015). The prevalence of infection in the elderly may be as high as 1.4%, leading to the implementation of the comprehensive vaccination campaign introduced in 1992. Infection with HBV was more common in Upper Egypt and urban areas. In contrast, HCV infection was more likely found in rural and Lower Egypt. The coinfection of HBV-HCV rate was (0.06%), which if compared to the seroprevalence rate of HCV, may show that the spread of both infections is through a different route of disease (WHO EMRO, 2018). According to the Egypt Demographic and Health Surveys (EDHS), HCV seroprevalence among adults aged 15–59 was 10.0% in 2015 and 14.7% in 2009, both of which were significantly greater compared to worldwide rates (Spradling et al., 2012; Smith et al., 2012; GBD, 2004; Micallef et al., 2006). The seroprevalence was 10% in the age categories 15–59 years (comparison to 14.7% in 2008 (DHS)) and 0.4% in the age category <15 years, with an overall rate of infection of 6.3% in the age category < 60 years. In the 15–59 years and <15 years, the viremia prevalence declined to 4.4% instead of 7.0% and 0.2%, respectively (WHO, 2016).

Despite this progress, Egypt formed a national HCV control strategy and launched HCV prevention and therapy programs to treat over 250,000 chronically affected patients annually. This strategy aimed to reach a level of infection of <2% by 2025 (Micallef et al., 2006; WHO, 2016; Chevalier, 2011).

Both viruses exhibit a high prevalence of infection, most commonly due to blood transfusions without effective screening and drug injection. These causes are considered medically and socially uncontrolled biohazards (Hullege et al., 2015). Most patients are unaware of their infection due to the inaccessibility of testing, accompanied by a significant increase in cost, which elevates the risk of experiencing advanced complications. Therefore, early identification of the infection is a crucial prevention method for complications and the most cost-effective approach (Hullege et al., 2015; Urbanus et al., 2014).

In 1970, new blood donation regulations were implemented, which required all blood donations to undergo screening against hepatitis B surface antigen & HCV antibodies. These regulations lowered HCV transmission to 7% and HBV transmission to 1-2% (Moftah, 2009). As a result of the discovery of molecular techniques and a better understanding of HCV biology in 1989, HCV-specific diagnostic assays could be developed. As a result, this development leads to a further decline in viral transmission of HCV through transfusion. Public health efforts focused on reducing the number of incident infections by continuing to develop recombinant immunoblot assays (RIBA) and HCV-specific enzyme immunoassays (EIAs) for confirmation. By the conclusion of the 1990s, this had reduced the prevalence of HCV associated with blood transfusions to less than 0.01% (Gupta et al., 2014).

The commonly used serological assay of HBsAg and HCV-Ab through a point-of-care rapid diagnostic test (RDT) or an enzyme immunoassay (EIA) is the standard routine method (Chu & Lok, 2002; Galli et al., 2008)]. In August 2017, the WHO authorized four HCV-Ab RDTs. However, the HBsAg RDTs still needed to meet the requirements needed for WHO prequalification (WHO EMRO, 2018). Nucleic acid testing (NAT) was authorized to confirm the viremia state of both HCV RNA and HBV DNA. HCV showed various genotypes, with genotype one being the most common because it accounted for 46% of all infections of HCV. Genotype 3 is the second most prevalent, with 22%, followed by genotypes 2 and 4, with 13% prevalence. One disadvantage of HCV antibody screening is the potential delay in diagnosis because HCV seropositivity, which can vary from weeks to months and is referred to as the "preseroconversion window" (interval during which HCV RNA is discovered without the existence of anti-HCV antibodies), is long, resulting in the transfusion of infected cases. As a result, in the late 1990s, blood banks implemented nucleic acid-based testing (NAT). As a result, the window duration was decreased from 13 weeks for EIA-based testing to three days for NAT-based evaluation. Consequently, the risk of infection is decreased to one per million units of transfusion (0.0001%) (Saldanha et al., 2001; Hyland et al., 2003).

As RIBA increased the first antibody enzyme-linked immunosorbent assay's specificity, it was initially used as the standard method of diagnosis for some HCV (Ag). Nevertheless, RIBA has been replaced with PCR and Transcription-Mediated Amplification (TMA), two technologies regularly utilized for qualitative and quantitative testing (Marwaha & Sachdev, 2014). Advancements in the treatment of HCV decrease its action in about 15% to 30% of cases within six to twelve months of first exposure. These patients are still anti-HCV antibody positive, but since they do not have viremia, they are considered cured and no longer at risk for viral complications (Omran et al., 2018).

## **SUBJECTS AND METHODS**

The research has been done on 270,900 blood donors between January 2005 and January 2018 at the microbiology lab and blood bank unit of the National Cancer Institute, Cairo University, and Nasser Institute blood bank. Nasser Institute supplied 203,000 participants, of whom 188,570 were males (93%) and 14,340 were females (7%) and were randomly selected from the age range of 20 to 50 years.

A written informed consent, approved by the Institutional Review Board (IRB) ethical committee of the NCI, which follows the rules of the Helsinki IRB, was obtained from each patient before starting the data collection. For the sake of patients' privacy, they were assigned code numbers.

The National Cancer Institute provided 67,900 participants, of whom 53,400 were males (79%), and 14,500 were females (21%), randomly selected from 18 to 50 years.

All cases were healthy individuals determined by:

- Full medical examination of blood pressure & weight.
- Normal hemoglobin concentration
- No previous blood transfusion, operation, or jaundice history was recorded.
- Blood samples of 10 ml each were obtained in a sterile vacutainer & left to clot. The supernatant was further divided into 2 parts one for serological testing & one for NAT testing.

## **SEROLOGICAL ESSAY:**

### **Enzyme-Linked Immunosorbent Essay**

Anti-HCV antibodies were estimated using the ORTHO® HCV 3.0 ELISA Test system for HCV antibodies using recombinant HCV antigens (C22-3, C200, and NS5).

Hepatitis B antibody has been tested utilizing the HbsAg Omega Diagnostic Kit against all 8 HbsAg subtypes.

### **Molecular Testing for HCV RNA & HBV DNA using NAT**

Using Procleix® Tigris® System

The Procleix® Ultrio® Plus Assay\* is a qualitative in vitro nucleic acid amplification test for diagnosing HIV-1 RNA, HCV RNA, and HBV DNA in serum and plasma samples from human donors evaluated in pools.

Principle: The PROCLEIX® ULTRIO® Plus Assay has three major phases that all happen in a single tube: specimen preparation, transcription-mediated amplification (TMA) to amplify the HCV RNA and HBV DNA targets, and hybridization protection assay (HPA) to detect the amplicons. Viral RNA and DNA are separated from samples employing target capture employing magnetic microparticles, which are subsequently isolated from the sample in a magnetic field, during the specimen preparation. TMA, a transcription-based nucleic acid amplification technique that uses the two enzymes MMLV reverse transcriptase and T7 RNA polymerase, amplifies the target. Reverse transcriptase produces a DNA copy of the target sequence, which includes a T7 RNA polymerase promoter sequence. Using the DNA copy template, T7 RNA polymerase creates several copies of the RNA amplicon.

The Procleix Ultrio Plus Assay uses the TMA technique to amplify specific HIV-1 RNA, HCV RNA, and HBV DNA areas. HPA detects amplicons by employing single-stranded nucleic acid probes with complementary chemiluminescent labels. Specific to the amplicon, the labelled nucleic acid probes hybridize. The hybridized probe's chemiluminescent signal is detected in a luminometer during and given as Relative Light Units (RLU) during the detection process the steps of sample processing, amplification, and detection, internal control is applied to every test sample. Due to the varied kinetics of light emission from probes with various labels, the internal control signal in every tube or assay reaction can be distinguished from the HCV/HBV signal.<sup>25</sup> A probe that emits light quickly (a "flasher signal") is used to detect internal control-specific amplicons. Internal control and combined HCV/HBV signals can be distinguished using the PROCLEIX ULTRIO Plus Assay, but single HCV and HBV signals cannot. This is done using the PROCLEIX® ULTRIO® Plus Assay Kit, which contains an internal control reagent kit, target capture, amplification, enzyme reagents, probes, target enhancers, and negative and positive calibrator reagent kits (for HBV, HCV) [PROCLEIX®ULTRIO®PLUS Assay Kit Developed by Gen-Probe in collaboration with Novartis]

NAT is performed using Procleix® Tigris® System

### **Statistical Analysis**

The Statistical Analysis System (SPSS) software was used to manage and analyze the data. There were two sides to each p-value. P-values  $\leq 0.05$  have been regarded as significant. The validity measures for the parameter under study were sensitivity, specificity, and diagnostic accuracy.

## **RESULTS**

We estimated the prevalence of HBV and HCV in blood donors in Egypt during the selected study period in this investigation. The prevalence of HCV infection among male and female ratios was estimated, and the predominance of infection was calculated. Coinfection by both viruses was estimated. The usefulness of NAT as a specific and sensitive tool in diagnosing active HCV and HBV viremia in seronegative Egyptian blood donors over conventional serological methods was analyzed. Comparison between serological techniques (ELISA) and molecular testing (NAT) in the detection of the viruses was estimated.

A total number of 270,900 samples were obtained using the following criteria of choice: normal blood pressure, hemoglobin concentration, and good physical condition

Donor consent was obtained on the time the sample was taken.

The first group constituted 67,900 participants from National Cancer Institute.

The second group contained 203,000 participants from Nasser Institute **Figure 1.**

Those in the first group were healthy volunteers of blood who were relatives of cases admitted to NCI from all over Egypt and were subjected to thorough medical examination

The second group contained 203,000 participants from Nasser institute. They were healthy blood donors from all governorate, blood donors of family & friends of patients undergoing operations, or bone marrow transplantation.

All participants were selected on basis of normal blood pressure, hemoglobin concentration, and good physical condition. Oral questionnaire was taken concerning history of jaundice, blood transfusion, chronic illness and any history of drug intake or operation.

The results found that total number of male participants was 242,100 (89.4%) & 28800 female participants (10.6%).

## Figure 2.

### Serological technique:

Results obtained by using the ELISA technique showed the following results of HbsAg:

- Total number of HbsAg antibody-positive cases was 4914 out of 270,900 total cases (1.8%). Seven hundred seventy-nine positive cases from 67,900 NCI donors were detected (1.1%). Whereas those among Nasser Institute showed 4135 infected cases out of 203,000 donor (2%) P-value<0,001. Showing a higher rate of seroprevalence among Nasser Institute donors.

### Figure3

Results of HCV antibody testing:

- Total number of donors showing seroprevalence of HCV Ab was 14,457 cases out of a total of 270,900 case (5.3%). Those who showed positivity from 203,000 at Nasser Institute were 11,657 cases (5.7%). Those who showed positivity among 67,900 donors from NCI were 2800 case (4%) P-value<0.001. Showing higher rates of infection among Nasser Institute

### Figure 4.

- Total number of infections among males was much higher than females (4.4% & 1.1% respectively) in HCV **Figure 5.**
- Coinfection of HBV and HCV were 260 cases (0.09%).

All seronegative cases submitted to NAT technique:

### HBV DNA testing using NAT

- The HBV DNA of 265,986 seronegative donors has been tested. The number of positive cases were 9 cases initially seronegative (0.003%,) which were either missed or were during the seroconversion window period. **Figure 6**

### HCV RNA testing using NAT

- A total of 256,443 seronegative blood specimens have been tested for HCV RNA, and 17 positive samples have been detected (0.007%), which were considered missed by ELISA or were during the seroconversion window period.

### According to our study, the prevalence of HBV in Egypt

- Total number of HBV +ve cases by both techniques was 4923; thus, HBV prevalence was reported to be around (1.8%) using both serological & molecular techniques.

### According to our study, the prevalence of HCV in Egypt

- Total number of HCV +ve cases was 14474; thus, prevalence was reported to be around (5.3%) using both serological & molecular techniques.

The efficiency in the detection of both viruses using both techniques was

- HBV infection by NAT was 9 cases out of 270,900 were missed (0.003%)  
While HVC infection by NAT showed 17 cases out of 270,900 were missed (0.006%). Indicating no significant statistical difference between both techniques.

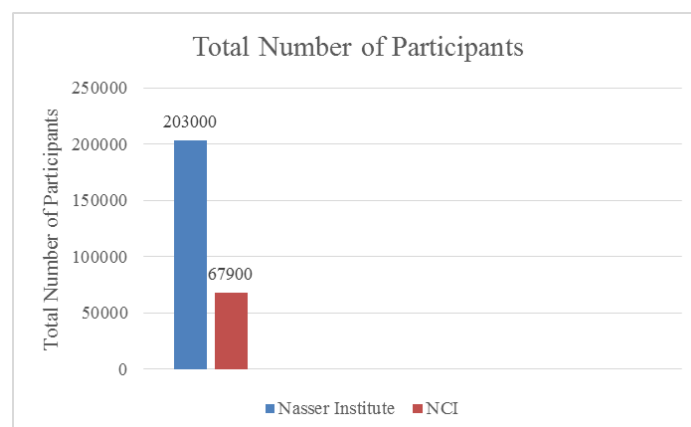


Figure 1: Total Number of Participants in Institutes

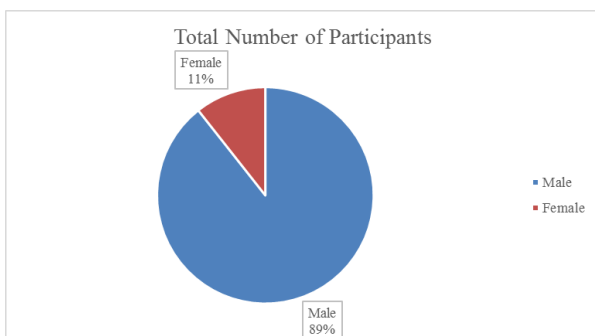


Figure 2: Total Number of Male and Female Participants

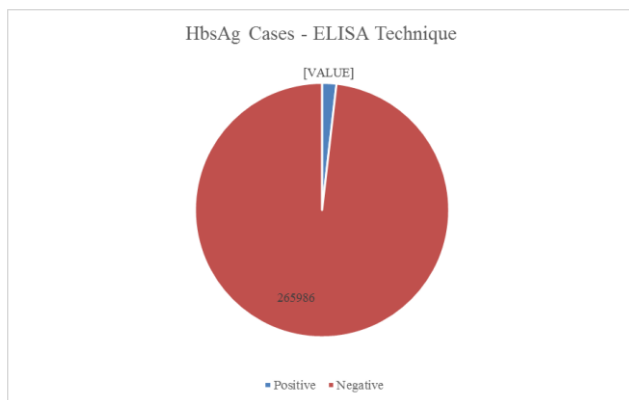


Figure 3: HbsAg positive Cases – ELISA Technique

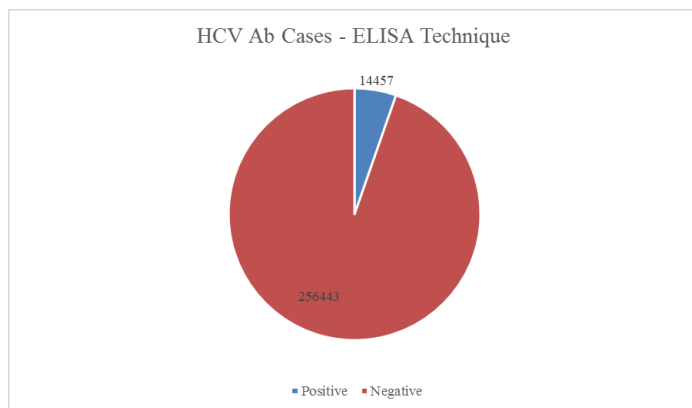


Figure 4: HCV Ab positive Cases – ELISA Technique

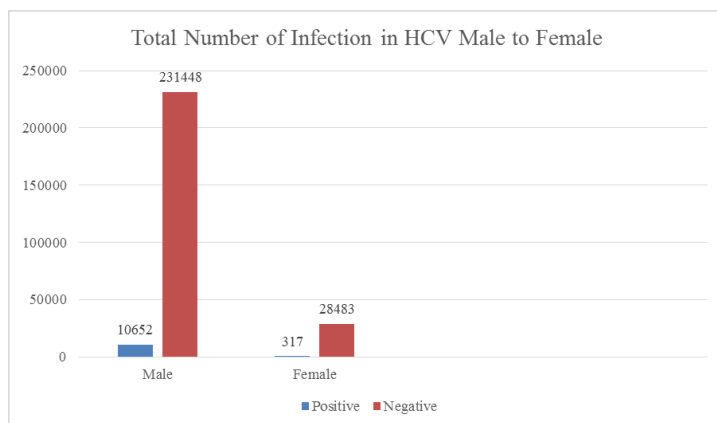


Figure 5: Total Number of Infections in HCV Male to Female

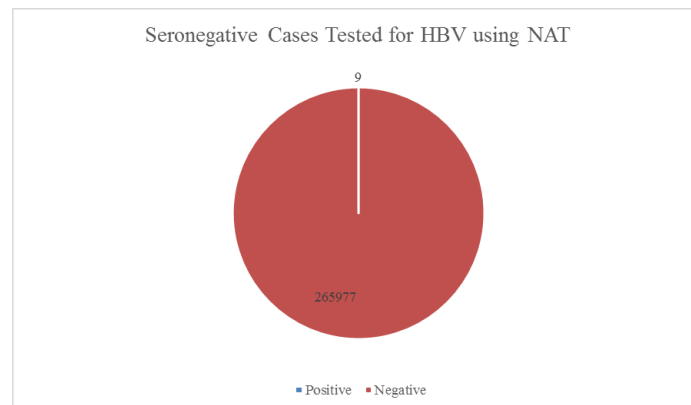


Figure 6: Seronegative Cases Tested for HBV DNA using NAT

## DISCUSSION

One of Egypt's most challenging health problems, viral hepatitis, is estimated to affect at least 8 to 10 million persons, or at least 10% of the population suffering from the disease with an increased risk of more being infected [WHO EMRO, 2018]. The consequences of liver cirrhosis and hepatocellular carcinoma represent an economic challenge that must be addressed [Abo-Amer et al., 2018]. The WHO supported a plan of action that consists of viral surveillance of blood bank products by NAT for early diagnosis of infected blood, the general immunization campaign against HBV, and recently the decision of the Egyptian government in October 2016 to implement the new treatment made available to 26 national liver treatment centers around the country against HCV (WHO, 2016).

According to Wasfi et al. (2011) was conducted in over 24 governorates in Egypt, and 119 donors (3.5%) and 47 (1.4%) tested positive for HBsAg and anti-HCV, respectively. These results were found to be by our study in the case of HbsAg that showed a seroprevalence of HbsAg to be (1.8%) but slightly lower in the case of anti-HCV antibody than our study (5.3%), which may be due to the small population size studied by Wasfi et al. (2011).

According to Sherif et al., in 2017, the seroprevalence of HbsAg (1.4%) and coinfection was (0.06%) which was found to be by our results that showed seroprevalence of HbsAg (1.8%) and coinfection to be (0.09%) (Ismail et al., 2017).

As per a 2017 study by Asmaa et al, the percentage of HCV antibodies was (6.3%) <60 yr, Viremia was (4.4%). The viremia level was much higher than our results, which corresponded to (0.007%) while the antibody titer was in accordance to our study (5.3%). This could be explained by the reality that they performed their research on all blood samples while ours was done on seronegative samples only. Their study was also carried out on a more extensive age range while ours was conducted on age between 18-45 who were eligible for donation. In their study, males showed higher rate of infection than females (4.4%, 1.1%) respectively. These results are under our study, which showed (4.4% & 1.1%) male and female infection ratio, respectively (Gomaa et al., 2017).

According to estimates from Zanati et al. (2015), males are more likely than females to have HCV (El-Zanaty, 2015). This conclusion is by the findings made by Vallab et al. (2014), who reported that males (0.7%) had a greater prevalence of HCV than females (0.66%). Our study showed male to female infection ratio (of 4.4% & 1.1%) respectively. This was explained to them by the fact that, due to their male lifestyles, they have a greater risk of being exposed to and transmitting HCV (Vallab et al., 2014). According to Abo Amer et al. (2018), the prevalence of HCV antibodies was 1%, and females (304/27,421; 1.1%) had a greater prevalence of infection than males (194/21,371; 0.9%). These findings did not follow our study that showed a prevalence rate of (5.3%) of HCV antibody and male to female ratio (4.4%, 1.1%) respectively. This may be explained by the size of population included in both studies as ours was much more extensive (270,900) compared to their study which included only 48,972. Moreover, another factor to consider was that our study was over a longer duration of 13 years (Abo-Amer et al., 2018). On the other hand, Abo Amer et al 2018 set al. HCV-RNA detected by PCR to be 355/48,972 (0.7%) higher than our study (0.007%). This may be due to the because study all 48,972 were tested for HCV RNA by PCR. At the same time, ours anti-HCV on anti HCV antibody negative samples, our study was also conducted on a larger population, and the volunteers were all from different areas around Egypt (Abo-Amer et al., 2018).

According to El Ekiaby et al. (2015), there were two blood centers in Egypt with rates of WP-NAT-yield donations ranging from 1:3100 to 1:9500 and total HCV infections ranging from 2.6% to 4.5%. This was not by our study, which showed total HCV infection (5.3%) and HCV WP-NAT (0.007%). This may be because, in El Ekiaby et al., the study was performed on all blood from donors in the two blood banks without excluding seropositive blood as was done in our study which WP-NAT was restricted to seronegative blood only (El Ekiaby et al., 2015).

EL Ekiaby et al. (2015) and Bruhn et al. (2015) stated that just two of the 175 donors who had probably resolved infections showed detectable RNA on replicate testing (calculated VLs of 0.5 and 1.8 copies/mL). Thus, it was reported that the additional safety provided by serological tests of blood that had undergone ID-NAT screening appeared to be small and that, in Egypt, the remaining risk associated with ID-NAT and serology testing has been assessed to be one in 250,000. This occurred in accordance to our study that demonstrated that only 17 cases out of 270,900 donors were missed during screening of blood donors by the ELISA technique (0.007) (El Ekiaby et al., 2015; Bruhn et al., 2015).

Lelie et al. (2017) also stated that the detection of HBV-DNA is superior to the detection of HBsAg in repeated donors, despite ID-NAT and serology being complementary in diagnosing HBV infection in first-time donors. Our study showed deficient levels of viremia using NAT in all seronegative blood samples of donors for both HBV and HCV (0.003%, 0.007%), respectively (Lelie et al., 2017).

## CONCLUSION

A recent study has demonstrated that HBV and HCV's overall infection rate in Egypt has declined over previous years. Serological techniques have benefited the detection and exclusion processes of infected blood donors, thus protecting most recipients. With the implementation of NAT, the window phase was also covered to prevent the misuse of seronegative blood with viremia, thus minimizing the risk of infection. Implementing the National immunization campaign against HBV has reduced the number of HBV-positive cases compared to past studies. With the new implantation of the new drug approved by the Egyptian Government, there are high expectations for further reduction in the number of patients with active viremia, thus decreasing the transmission rate and complications of HCV and its load on the economy.

### Acknowledgments

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### Conflicts of interest:

There are no declared conflicts of interest by the researchers.

### Declaration of Interest

The researchers declare that they do not have any known financial or interpersonal conflicts that could have influenced the research presented in this study.

### Author Contributions

D.Y.K., S.R. designed the research; D.Y.K. and S.R. carried out the research; D.Y.K., S.R. analyzed the data and wrote the manuscript. The manuscript was approved and reviewed by all researchers.

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