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Applying RT-PCR standard detection procedures of HBV and HCV in Wasit blood bank

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***Abstract*---**Background: Blood and blood products are a unique and precious resource because they can be obtained only from individuals who donate blood or its components. Aim of the study: To increase the safety of blood given to patients by evaluating the addition of new investigations to detect HBV and HCV of donated blood in the blood bank. Patients and Methods: this study included 150 samples of blood taken from the blood bags of donors from the blood bank /Wasit Province. In this study used ELISA (as serological method) & RT-PCR (as molecular method) to detect infection with hepatitis B virus and hepatitis C virus. Results: The results of ELISA test, from 150 analyzed samples, only 6/150 (4.0%) of the patients were diagnosed infection by HCV and 1/150 (0.6%) infection by HBV. The results of PCR test, (4.0%) and (5.3%) for HCV C and HBV respectively. Conclusion: Diagnosis of hepatitis virus (B, C) by ELISA is still sufficient.

***Keywords*---**Hepatitis B virus, Hepatitis C virus, Enzyme –linked immuno sorbent assay, Polymerase chain reaction, and Real-Time PCR.

Introduction

Blood and blood products are a unique and precious resource because they can be obtained only from individuals who donate blood or its components. About 92 million blood donations are being done annually throughout the world (WHO., 2013; Chopra and Jauhari., 2015; Aslami *et al.*, 2015). WHO estimates that blood donation by 1% of the population is generally the minimum needed to meet

a nation's most basic require- ments for blood (Agrawal *et al.*, 2013; Dubey *et al.*, 2014). Safe blood is a necessity for improving health care and to prevent the transmission of infectious disease. Developing countries still have issues regarding the safety and quality of blood and blood products used for transfusion (Agravat *et al.*, 2014; Bilal *et al.*, 2016). Blood is an important requirement in different medical and surgical conditions, blood and its products are considered as a major source for transmission of Hepatitis C, Hepatitis B, HIV and a number of other infections (Valerian *et al.*, 2018; Guglielmetti Mugion *et al.*, 2021). Viral hepatitis is an important health and socioeconomic problem all over the world, despite all the advances in their prevention and management. Globalization has faded the borders which increased the threat of viral infections and put them on the agenda of governments, especially in Asia & Africa (Aljarbou., 2012; Ali A. Ramadhan., 2018). Hepatitis C is one of the most dangerous and potent hepatotropic viruses that cause human infection with HCV (Holz and Rehermann., 2015; Bang *et al.*, 2016). Which causes an inflammation of the liver; however, a chronic HCV infection can lead to liver failure, liver cirrhosis, and hepatocellular carcinoma with HCV (Rehermann., 2015;Yoon *et al.*, 2016). 60-80% of patients with acute hepatitis C develop to the chronic form when the virus overcomes host innate and adaptive immune defenses (Chigbu *et al.*, 2019; A.A. Rabaan *et al.*, 2020). Hepatitis B viral infection is an infectious- inflammatory disease of the liver caused by the hepatitis B virus (Obeagu *et al.*, 2016; Lo AOS., 2015). HBV is a highly contagious pathogen that has infected humans for more than 1,500 years. HBV causes acute and chronic liver disease and is endemic in many areas of the world. The virus is transmitted through contact with blood or other body fluids from an infected person (Guvenir and Arikan., 2020; Prifti *et al.*, 2021). Polymerase chain reaction is one of the most powerful technologies in the molecular biology, was invented by Mullis in 1983 and patented in1985. PCR is a very sensitive technique that allows rapid amplifi- cation of a specific segment of DNA, the principle of this method is based on the use of DNA polymerase which is an in vitro replication of specific DNA sequences. PCR method can generate tens of billions of copies of a particular DNA fragment (target DNA) from a DNA template, which allows to detection and identification of gene sequences using visual techniques based on the size and charge (Garibyan and Avashia., 2013; Kadri ., 2019) .

Patients and Methods

This cross-sectional study included (150) samples of blood taken from the blood bags of donors from the blood bank /Wasit Province /Iraq. It is taken one month only to collect the samples. These samples were collected randomly. All information of donors were collected from file system from blood transfusion bank center, the number of males was (147), with age range (16-61) years and numbers of females was (3), with age range (24-30) years. It carried out from February 2020 to August 2021. Eliza test and RT-PCR technique were used to detect infection with hepatitis B and C viruses.

Results

In this study used (150) blood samples being collected and tested for detection HBV and HCV. Table (1) shows the demographic data and information about blood donors that used in this research.

Table (1): The demographic data of the person who donate blood

Items	Frequency	Percentage
Gender		
Males	147	98.0
Females	3	2.0
Address		
Al-Kut	112	74.7
Others	38	25.3
Type of donation		
Elective	81	54.0
Urgent	69	46.0
Age in years		
less than 30 years	43	28.7
30-40 years	68	45.3
more than 40 years	39	26.0
Blood Groups		
A	32	21.3
B	42	28.0
A	13	8.7
AB	63	42.0
Total	150	100.0

All blood donors must be subjected HBV and HCV to ELISA test. Also all samples in this study were checked again with ELISA kit for detect HCV core Antibody, and HBV surface Antigen. Results of table (2) showed 6 out from 150 samples (4.0%) are positive for HCV, while just 1(0.6%) sample being positive for HBV. ELIZA results demonstrated only one female infected with HBV.

Table 2: ELISA test results of hepatitis for person who donate blood

Hepatitis	Positive (%)	Negative (%)
C	6 (4.0)	144 (96.0)
B	1 (0.6)	149 (99.6)

In table (3) RT-PCR results are shown six persons with HCV and all of them were males While HBV was found in eight persons, the majority of them were males but only one was female.

Table **3**: RT-PCR test results for blood which donated in Al-Kut transfusion Center

PCR	Frequency	Percent
HCV	6	4.0
HBV	8	5.3

Figures (1) represented Real-Time PCR amplification plots of HBV. All raised curves above threshold consider positive. (FAM dye for detection of HBV, ROX for positive control).

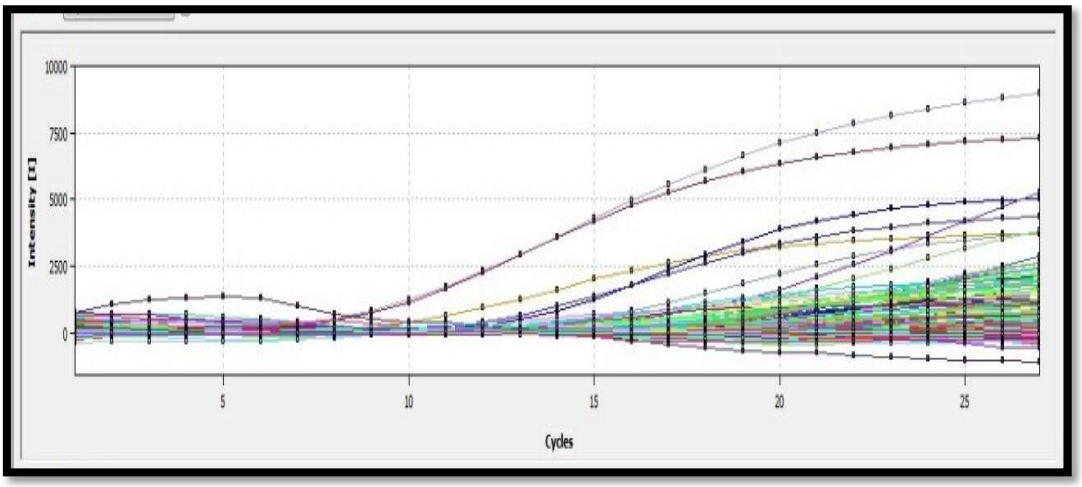

Figure (1):Real-Time PCR amplification curve of Hepatitis B virus

Figures (2) represented Real-Time PCR amplification plots of HCV. All raised curves above threshold consider positive. (FAM dye for detection of HCV, ROX for positive control).

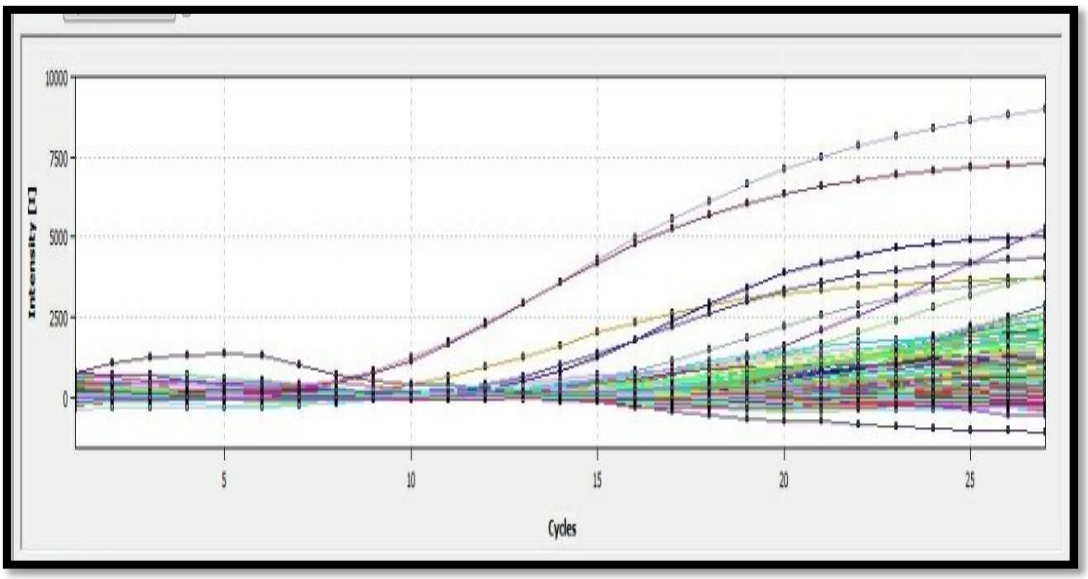

Figure (2): Real-Time PCR amplification curve of Hepatitis C virus

Discussion

In blood bank to ensure the safety of blood donation for both donors recipients, all volunteer blood donors must be evaluated to determine their eligibility to give blood (Refgha., 2018). Blood transfusion is an integral part of medical treatment, and trans- mission of infectious diseases through donated blood bags in the blood bank is an alarming situation (Naher *et al.*, 2021). Blood transfusion is considered to be a potential risk factor for the transmission of blood-borne viral infections (Eboumbou *et al.*, 2014). Hepatitis B and hepatitis C Virus infections are a significant global public health concern (Police *et al.*, 2020). Many men, 40-80% live with chronic hepatitis B or C are unaware of their serostatus, and remain infectious to others (Ayele *et al.*, 2020). Worldwide, the spread HBV and HCV increase at an alarming rate, this lead to a dramatic impact upon some countries such as Iraq. Also hepatitis B and C viruses are responsible for causing about 80% of liver cancer cases worldwide, and it could be the major cause of morbidity and mortality (Dehghani *et al.*, 2019). In Iraq there is no protocol for blood banking, we followed Word Health Organization recommendations that all donates should be screened for these viruses (HBV, HCV, HIV, Syphilis), because these viruses have greater risk for percipients (WHO., 2017). But all Iraqi governorates screening only HBV, HCV and HIV.WHO recommended to detect HBsAg for HBV and HCV Ab for HCV (WHO., 2017). HBs Ag is a protein on the surface of hepatitis B virus; it can be detected in high levels in serum during acute or chronic hepatitis B virus infection. The presence of HBsAg indicates that the person is infectious. HBsAg appear the first during acute HBV infection, HBsAg is the most critical serological marker to detect and remains detectable up to six months (Yucel and Akcael., 2018). The body normally produces antibodies to HBsAg as part of the normal immune response to infection. HBsAg is the antigen use to make hepatitis B vaccine (Kurdi *et al.*, 2014). HBsAg is the first serological marker of HBV infection to appear in course of infection, window period between HBV infection and detection of HBsAg estimated to be around 38 days, but depends on analytical sensitivity of assay used, immunocompetence of host and individual virus kinetics (WHO., 2017). HBsAg can be detected in the serum from (1-3weeks) before the onset of symptoms, the permanence of HBsAg for more than 24weeks indicates chronicity of HBV (WHO., 2017). The World Health Organization recommends that this test be performed to detect hepatitis B in all donated blood to ensure the safety of blood and to avoid accidental transmission of infection to recipients of blood products (WHO., 2021).

While Anti-HCV are proteins that human body make when HCV find in the blood. Anti-HCV is the serological marker of HCV infection which used to detects the presence of antibodies of this virus that body produces in response to an infection (WHO., 2016). The mandate for serological screening is important in reducing the risk of transfusion transmitted infections and improving donation selection. In Iraq, serological screening for blood donors is a standard dependable method for blood screening assay without application of NAT (Ghinwa *et al.*, 2020). In this study the prevalence of hepatitis B virus in blood donors in Wasit Province was 0.6% , the small number of registered donors of hepatitis B virus can be explained by several explanations, which may be one of them:

- People know that they have the disease, which makes them avoid the process of donating blood, as this process can cause societal embarrassment, since viral hepatitis can be linked to other diseases.
- The effectiveness of investigations and identification processes conducted by health departments in identifying people with this disease and informing them that they should not be included in blood donation operations.
- The effectiveness of immunizations with the vaccine for this disease, which constituted an important factor in reducing the number of people infected with this disease. Wide spectrum application of hepatitis B vaccination (the effective vaccination for HBV that given) in my province and all Iraqi governorates could have a critical role in the reduction hepatitis B prevalence, especially in young adults.
- The effect of continuous public health awareness given to the masses about the dynamics of HBV infection as well as about the preventive measures including HBV vaccination.

This result 0.6% was significantly lower than that reported by other Iraqi researcher like Hussein *et al* (2017) reported that the prevalence of hepatitis B virus among blood donors was 0.78% in Duhok governorate. And Al-Nafakh *et al* (2019) reported that the prevalence of hepatitis B virus among blood donors was 3% in Al-Najaf governorate. Also this result was comparatively lower than other countries like, Abdelaziz (2020) detected that the prevalence of HBV among blood donors in Sudan was 1.3%. And Ehsan *et al* (2020) detected that the prevalence of HBV among blood donors in Pakistan was 2.04% (using ELISA technique detected HBsAg). While the result of HCV in blood donors in Wasit Province was 4.0%, the present study demonstrated an increase in anti-HCV with time which is considerable higher than that reported by other Iraqi researcher like Al-Sadi and Abdul-Jalil in 2016 reported that the prevalence of hepatitis C virus among blood donors was 2.8% in Basra governorate. And Saleh and Taher(2020) they detected the prevalence of this virus among blood donors was 0.06% in Erbil governorate . The recent review reported significant differences in prevalence and epidemiology of hepatitis C virus infection exist among the Middle East countries or even inside the countries regions (Fallahian and Najafi., 2011). However, most of the included studies in this review were performed in risk group rather than general population. Depending on the available data it may be suggested that HCV transmission in the Eastern Mediterranean Region occurs in health care setting and primarily associated with unsafe injections (WHO., 2009). Global HCV prevalence are mostly based on hepatitis C virus Seroprevalence studies data (Shepard *et al.*, 2005), and data submitted from different countries and WHO regions (WHO., 2013). Also this percent was considerable higher than other countries like, Al Hroob *et al* (2020) reported that the prevalence of HCV among blood donors was 0.13% in Jordan. And Alqahtani *et al* (2021) who detected that the prevalence of hepatitis C virus in Saudi Arabi was 0.32% (in these studies detected anti-HCV by using ELISA technique). In this study when compared ELIZA results between HCV and HBV we shows HCV is 4% which is greater than HBV is 0.6%. HCV is increasingly recognized as major health care problem in the whole world. Despite of strenuous efforts from scientists, antiviral approaches could not completely eradicate it due to the fact that HCV is extremely heterogeneous. HCV is an RNA virus has a highly mutation rates and lacks effective proofreading ability after its replication, that's why it introduces several

mutations and keeps on evolution with respect to time. Mutations are not randomly distributed in the whole genome, instead these exist at hypervariable regions of the genome which encode for envelop proteins; hence enable the virus to escape from host immune surveillance (Umar Saeed *et al.*, 2014). HCV generates more severely (aggressive) infection, and the severity of the disease ranges from a mild disease that lasts a few weeks to a dangerous disease that lasts a lifetime, and the reason for its spread is a defect in the given blood test. There is currently no vaccine are a viable and effective to prevent HCV infection, because IgGs are not effective for post-exposure prophylaxis (WHO., 2020; WHO., 2021).

In the present study the results of Real-Time PCR test for hepatitis B virus and hepatitis C virus (that used to detection HBV DNA and HCV RNA) of blood donors bags in Wasit province was 5.3% and 4.0% respectively. PCR test result of hepatitis B virus was 5.3% was relatively higher than that reported by other Iraqi researcher like Saleh and Hadi (2016) who detected that the result of HBV among blood donors was 0.29% by using RT-PCR technique in Babylon governorate. And Ghinwa et al in the 2020 who reported that the result of HBV among blood donors was 3% by using the same technique in Baghdad governorate. Also this percent was considerable higher than other countries like Alqahtani *et al* (2021) reported that the hepatitis B virus among blood donors by using real time -PCR technique in Saudi Arabi was 0.27%. And Heloise Skiavine Maderia *et al* (2021) who detected that the hepatitis B virus among blood donors by using the same technique in Brazil was 0.17%. While RT-PCR result of hepatitis C virus 5.3% was significant higher than that reported by other Iraqi researcher like Khalaf and Hussein (2021) reported that the HCV among blood donors was 7.8% in Diyala governorate. And Rostam and Smail (2021) detected that HCV among blood donors was 6.93% in Erbil governorate. Also this percent was relatively lower than that other countries like Aida *et al* (2018) who reported that the HCV among blood donors by using RT-PCR technique in Egypt was 8%. According to WHO guidelines for blood transfusion 2017, NAT screening method reduces the window period 3-5 days for HCV and17-27 days for HBV while the viral window periods were 9-80 days for HCV and 42-55 days for HBV with serological tests (WHO., 2017). When compared between results of ELISA test and Real-Time PCR method for HCV and HBV shows greater differences especially in the HBV ELIZA 0.6% but by using RT-PCR 5.3% because RT-PCR method has high sensitivity and accuracy, also these technique was reliable, direct and rapid test (Obaid., 2014), and the other advantages are Wide dynamic assay range, allowing maximum sensitivity, Objective, quantitative results, High degree of reproducibility, and Minimizing contamination risk (Lobert *et al.*, 2010).

Also I found through my present study that all the results of ELISA for hepatitis B and C viruses that were conducted by the blood bank and which I detected again using the same method are not the same as the results that appeared when it was detected by using RT-PCR method, this reflects Real-Time PCR method was highly sensitivity (more sensitivity) and accuracy from ELIZA method. Despite NAT has the ability to detect the HBV, HCV during the window periods too earlier than serological screening assay the blood screening should be done by serological and NAT assay because both NAT and serological assays can complement each other (Antonella Esposito *et al.*, 2017).we can conclude infected blood by hepatitis B

and C viruses by ELISA test are little in percentage which is semi-similar to what detected by PCR test.

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