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Reconsiderations with basic viral tests in Wasit blood bank: CMV and EBV as an examples

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Abstract---Background: Blood donation is a procedure of collecting blood from a screened person who must follow the strict guidelines in the country where peoples lived. Aim of the study: To increase the safety of blood given to patients by evaluating the addition of new investigations to detect CMV and EBV infection of donated blood in the blood bank in Wasit Governorate. Patients and Methods: This study included 150 samples of blood taken from blood bags of donors from blood bank /Wasit Province. In this study used ELISA used only for detect infection with CMV and Real-Time PCR were used to detect infections with CMV and EBV. Results: The results of ELISA from 150 analyzed samples only 30/150 (20%) of the patients were diagnosed infection by CMV. While the results of RT-PCR 50/150 (33.3%) for the same virus, and the results of RT-PCR 13/150 (8.7%) for EBV. Conclusion: The guideline of blood and blood product donation should be reviewed to keep up the updated guideline of WHO to provide more suitable medical services to community.

Keywords---Cytomegalovirus, Real-Time PCR, and Enzyme -linked immuno sorbent assay, Polymerase chain reaction, Epstein-Barr virus.

Introduction

Blood donation is a procedure of collecting blood from a screened person who must follow the strict guidelines in the country where peoples lived. There are several types of blood donation: whole blood, platelets, plasma, and double red cells. The main cell in an individual's blood is red blood cell, one of many

advantages of donating blood is that blood donation can reduce the risk of cancer. Donating blood will decrease the excessive amount of stored iron, which is considered to be a high risk of cancer (Urgesa *et al.*, 2017) . Donated blood is an essential component in the management of many diseases. It is the main lifesaving for an individual with loss of large volumes of blood from hemorrhages or surgery (Tadesse *et al.*, 2018). Blood donation is the process of drawing blood from a healthy volunteer and collecting and storing it for transfusion into a patient with a matched blood group. Blood transfusion plays an important role in saving patient lives and stabilizing their medical conditions (Karnsakul and Schwarz., 2017). Cytomegalovirus is the most common congenital viral infection in many populations throughout the world. It does not yet have a vaccine and can be vertically transmitted to the fetus during both primary infections and recurrent infections (Jill Hutton., 2018). This virus is a major cause of non-hereditary adverse birth outcomes, including hearing and visual loss, neurologic deficits, and intrauterine growth retardation, and may contribute to outcomes such as stillbirth and preterm delivery (Njue *et al.*, 2021). Epstein–Barr virus is highly ubiquitous viruse, is a member of the herpesvirus family, is also known as human herpesvirus 4 and belongs to the gamma-herpesvirus subfamily (Fujiwara and Nakamura ., 2020), it infects more than 90% of the world’s adult population. EBV infection can affect a person’s blood and bone marrow. This virus is wide spread in all human population as lifelong a symptomatic infection of the B-lymphocyte pool, this virus can cause the body to produce an excessive number of white blood cells called lymphocytes (lympho-cytosis), and can also weaken the immune system, making it more difficult for the body to fight infection (CDC., 2020). 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 amplification 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 (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. 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 used to detect infection with CMV and RT-PCR technique were used to detect infections with CMV & EBV.

Results

In this study (150) blood samples were being collected and tested for detection CMV & EBV. 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 samples in this study were ELISA kit checked for detect CMV IgM. Results show that (30) samples found be positive for CMV out from 150 samples with percent (20%). And (120) samples was negative for CMV with percent (80%). Also ELISA results of CMV were twenty seven males and three females from (30) samples that detected out from all 150 samples. In this study, CMV has tested also by using RT-PCR technique. Results that showed in the table (2) CMV test results indicate a wide spread of this virus 50 (33.3%) since it not being tested within the tests that done in all Iraqi governorates, while EBV being represented only 13 (8.7%). RT-PCR results that done in this study we show CMV was found in 50 persons, 47 males and 3 females and for EBV was found only in 13 persons and all of them were males from all 150 donors persons.

Table 2: RT-PCR test results for blood which donated in Al-Kut transfusion Center

PCR	Frequency	Percent
CMV	50	33.3
EBV	13	8.7

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

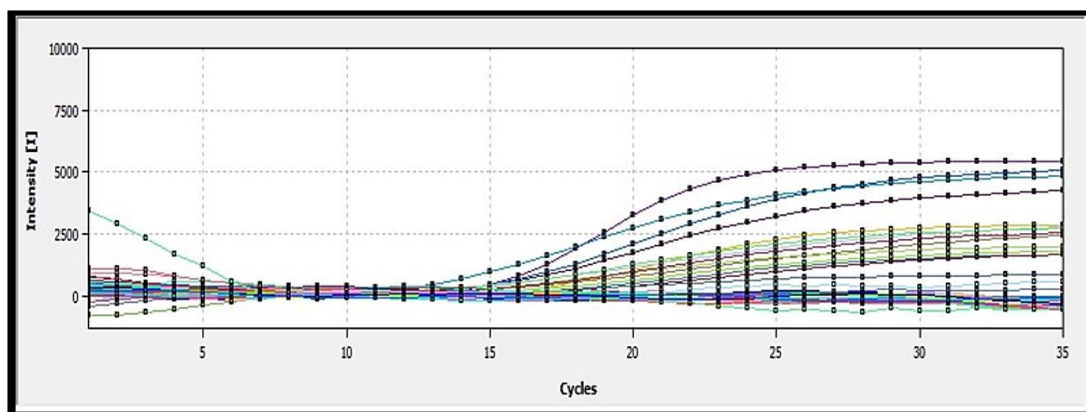


Figure (1) : RT-PCR amplification curve of Cytomegalovirus.

The figure (2) represented Real-Time PCR amplification plots of EBV. All raised curves above threshold consider positive. (FAM dye for detection of EBV, CY5 for positive control).

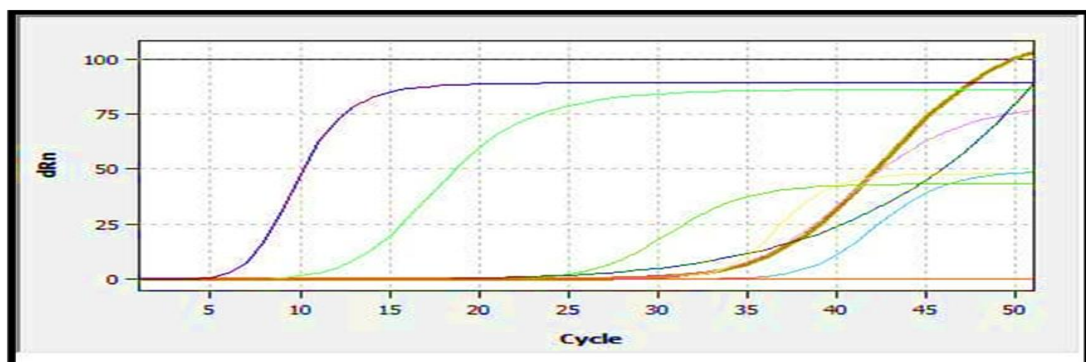


Figure (2): RT-PCR amplification curve of Epstein-Barr virus .

Table (3) shows correlation between age of blood donors and CMV PCR test. Results shows the equal between ages that less than 30 years and more than 40 years with percent 34% for each groups of ages which is represented the majority about positive percent for CMV PCR. While group age 30-40 years which is represented the minority about positive percent for CMV PCR. P value was 0.06 which means there is statistical significant between them.

Table 3: Correlation between CMV PCR test and age of blood donated person

Age	Negative CMV PCR	Positive CMV PCR	P. value
less than 30 years	26 (26)	17 (34)	0.06
30-40 years	52 (52)	16 (32)	
more than 40 years	22 (22)	17 (34)	
Total	100 (100)	50 (100)	

Table (4) shows the correlation between CMV PCR test and type of donation of donors. Results shows the elective donors which represented the majority of them with present 60% for positive CMV PCR . But urgent donors was found with present 40% . p. value was 0.1% there is no statistical significant in this tables.

Table 4: Correlation between CMV PCR test and type of donation of blood donated person.

Type of donation	Negative CMV PCR	Positive CMV PCR	P. value
elective	51 (51)	30 (60)	0.1
urgent	49 (49)	20 (40)	
Total	100 (100)	50 (100)	

Table (5) shows the correlation between CMV PCR test and address of blood donors. Results shows the majority of donors from Al-Kut were found with 68% . P. value was 0.3 there is no statistical significant.

Table 5 : Correlation between CMV PCR test and address of blood donated person

Address	Negative CMV PCR	Positive CMV PCR	P. value
Al-Kut	78 (78)	34 (68)	0.3
others	22 (22)	16 (32)	
Total	100 (100)	50 (100)	

Table (6) shows the correlation between EBV PCR test and age of blood donors . The result shows donors group ages less than 30 years were found with percent 23.1% . While the two group ages 30-40 years and more than 40 years both of them have the same percent 38.5% the positive result of PCR for EBV. P. value were 0.5% there is no statistical significant between them.

Table 6 : Correlation between EBV PCR test and age of blood donated person

Age	Negative EBV PCR	Positive EBV PCR	P. value
less than 30 years	40 (29.2)	3 (23.1)	0.06
30-40 years	63 (46)	5 (38.5)	
more than 40 years	34(24.8)	5 (38.5)	
Total	100 (100)	50 (100)	

Table (7) shows the correlation between address of blood donors and EBV PCR test. The result shows that elective donors were found with percent 46.2% , and for urgent donors were found with percent 53.2% for positive PCR results of EBV. P. value were 0.3% there is no statistical significant between all of them.

Table 7: Correlation between EBV PCR test and address of blood donated person

Type of donation	Negative EBV PCR	Positive EBV PCR	P. value
elective	75 (54.7)	6 (46.2)	0.3
urgent	62 (45.3)	7 (53.2)	
Total	137 (100)	13 (100)	

Discussion

In blood bank to ensure the safety of blood donation for donors and 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). Blood transfusion carries the risk of transmitting major infections that called transfusion- transmissible infections (TTIs). TTIs are the most severe compli- cations that may arise throughout blood transfusion (Al Hroob *et al.*, 2020). TTIs threaten the safety of recipients and the community as a whole and are a subject of real concern worldwide, blood transfusion still significant mode of transmission of TTIs pathogens, which including CMV one of these viral infection may be transmitted (Al-Hamadany *et al.*, 2020). Cytomegalovirus is considered one of the main viruses associated with blood transfusion. The virus has a worldwide distribution. It is more common in developing countries where most adults have been shown to be infected with CMV (Ziemann and Hennig., 2014) ,CMV infections have significant medical risks in immunocompromised patients and have caused significant fatality in patients undergoing bone marrow transplant (Ibrahim Saber and Mohammed., 2019). Individuals acquire CMV through infected blood products or direct contact with infected people. Transfusion of sero-negative blood products for immunosuppressed patients has vital importance in medical management (Al-Omari *et al.*, 2016). CMV is a recognized cause of morbidity and mortality in congenitally infected newborns and immunocompromised patients, most notably transplant recipients and HIV-infected persons. CMV is a virus of paradoxes and can be a potential killer or a silent companion lifelong. It is probably one of the most common infections known to humans and is characterized by a self-limiting infection in healthy individuals (Chakravarti *et al.*, 2009). This virus establishes latency and persists for the life of the individual. Infection with HCMV is common throughout the global (Zuhair *et al.*, 2019). CMVs prefer monocytes and other haematopoietic progenitor cells as a good site to survive and stimulate important changes in immune gene expression (Staras *et al.*, 2006). CMV usually infect the patients whom suffer from other chronic diseases that cause weakness in immune system such as cancer, dialysis, thyroid gland and thalassemia (Perotti and Perz., 2020).This virus is a human Beta-herpes infection which, after essential contamination, stays in an idle state for the whole life time of the host (Mocarski and Shenk., 2007). Additionally, is a vital viral reason for fetal conta- mination which may prompt intense clinical difficulties in the infant youngster,

for example, encephalitis, chorioretinitis, pneumonia, microcephaly and losing the hearing sense, beside weakening intellectual advancement(Ferrari de Freitas *et al.*, 2021).

Most healthy people who acquire CMV after birth experience few or no symptoms and no long-term sequelae (Chaemsupaphan *et al.*, 2020). Also CMV disease is usually acquired by adolescence and follows a benign course, while it might reactivate in patients with immune suppression and associated with high mortality and morbidity (Chiereghin *et al.*, 2021). CMV might play important role in the progression of chronic hematological diseases, such as hemoglobinopathies, lymphomas, myelomas, hemophilia, and aplastic and sickle cell anemia (Jean de Melo Silva *et al.*, 2020). In this study we used ELIZA IgM antibody to detected Cytomegalovirus in the serum samples of blood donors. This test is used to determine if the person is infected or not by CMV. IgM test detected antibodies in the blood (in human serum or plasma) that are produced in response to CMV infection, IgM antibodies are produced by the body first in response to a CMV infection, we can detected in the blood within a one or two week after the initial exposure (CDC., 2020). The presence of anti-CMV antibodies (IgM) among blood donors is a sign of potentially infectious virus in transfused blood products (Fares and Al-Barzinji, 2016). This test will help determine if the CMV infection is current or past. IgM levels rise for a short time, then decline and usually detectable levels after a few months, also IgM antibody levels rise again when latent CMV is reactivated (WHO., 2017; Britt., 2020).

In this study the prevalence of cytomegalovirus in blood donors in Wasit Province was 20% this result was significantly higher than that reported by other Iraqi researchers like Mohammed D. Khalid in the 2012 who reported that the seroprevalence of cytomegalo virus among blood donors was 3% in Nineveh governorate. Nanakaly and Hussien (2019) was 1.48% detected the seroprevalence of CMV among blood donors in Erbil governo-rate. Also this result was comparatively higher than other countries like, Al-Sabri *et al* (2017) who reported that the seroprevalence of CMV among blood donors in Yemen was 5.5% , Musa Kalamullah Bawa *et al* (2019) detected the seroprevalence of CMV among blood donors was 2.6% in Nigeria , Adane and Getawa (2021) reported the seroprevalence among blood donors was 13.77% in Brazil (in these studies detected IgM antibody by using ELISA technique). Positive IgM results to Cytomegalovirus are indicated of a primitive or repeated infection. IgM antibodies to CMV can continue for (2-9 months) after the initial infection. Not all patients with reactivated CMV infection will have noticeable levels of IgM antibodies (Ahmed *et al.*, 2014). In the present study CMV is endemic in our population. The high prevalence proved that CMV is simply transmitted than a some other infections like as measles specific care and appropriate vaccination program are needed to prevent the transmission of CMV(Shams *et al.*, 2011). In this study the results of RT- PCR technique for Cytomegalovirus and Epstein-Barr Virus of blood donors bags in Wasit province was 33.3% and 8.7% respectively. This result of Cytomegalovirus was considerable higher than that reported by other Iraqi researchers like, Al-Roubaey (2018) who detected CMV by using RT-PCR was 13.3% in Al-Najaf governorate, Mahmood and Al-Ghazal (2020)

they detected CMV by using RT-PCR was 8.5% in Nineveh governorate. Also this result was comparatively higher than other countries like El Sanousi *et al* (2016) who detected Cytomegalovirus by using the same method in Sudan was 29.7% , Elahe Lakzayi *et al* (2020) detected CMV by using RT-PCR was 8.2% in Iran. In this study, ELISA assay was used for the detection of CMV IgM and real time-PCR for detection of CMV DNA. The real time PCR was used as the gold standard for infection diagnosis. This was because a positive RT-PCR test signifies viral replication and detects high risk of CMV infection and transmission. The difference between the results is attributed to the fact that cytomegalovirus went to latent stage at certain cells quickly and the PCR which was adapted and used depended on the major immediate early gene, which is only shows for a short period of time during the infective cycle (El Sanousi *et al.*, 2016). RT-PCR has been developed to detect HCMV because of its time-saving feature and high sensitivity and specificity (Liu *et al.*, 2015).

EBV is a double-stranded DNA virus and the primary cause of infectious mononucleosis (IM), a common infection worldwide with a lifetime prevalence of 90% of humans without any symptoms in most cases, but has an oncogenic potential, especially in immunocompromised individuals (Venturini *et al.*, 2021). This virus is the first identified human oncogenic virus and it alone causes ~200,000 cancer cases and ~1.8% of total cancer-related death annually (Becnel *et al.*, 2021), common cause of infectious disease in immunocompromised patients, such as HIV-infected individuals and organ transplant recipients (Kliszczewska *et al.*, 2017). This virus can establish latency in the host cell, which represents a risk for the quality, safety, and efficiency requirements of transfusions or transplantation, EBV considering that can keep persistence in B-lymphocytes, after primary infection, Also can establish lifelong persistent infection in the host through the latent state, which may be reverted into productive infection under circumstances of immunosuppression this virus can remain latent in the host cells and be reactivated when the host immune system is compromised (Bakkalci *et al.*, 2020). EBV is linked to various hematological and autoimmune disorders (Cao *et al.*, 2021). It is an infectious agent used to immortalize and induce polyclonal activation of B cells. It has been widely described that this virus produces changes in the cells it infects and in the immune response, also stimulates the development of autoimmune diseases (Chabay *et al.*, 2020). The merit of this study is that the RT-PCR targeting EBV helped in the early diagnosis of infection caused by EBV and this study proved that PCR could differentially diagnose the infectious agents in patients having antibodies to EBV.

The PCR test result of Epstein Barr virus was 8.7% which was comparatively lower than that reported by other Iraqi researchers like Al-Bayati *et al* in the 2020 who detected EBV by using RT-PCR in Diyala was 17.65%, Al-Shuwaikh *et al* in the 2021 detected EBV by using the same technique in Babylon was 32.5% . Also this result was nearly lower than other countries like, Metwally *et al* (2021) who detected EBV by using RT-PCR was 37.5% in Egypt and Asouri *et al* (2020) also detected EBV by using Real time PCR was 9.37% in Iran. We can conclude the guideline of blood and blood product donation should be reviewed to keep up the updated guideline of WHO to provide more suitable medical services to community

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