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The Seropositivity Rate of Human Cytomegalovirus [Hcmv] Among Hepatitis B Virus [Hbv] Patients, Hepatitis C Virus [Hcv] Patients, and Blood Donors in Diyala Province

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Abstract

The total number of samples was 112 that collected from 31 patients with hepatitis B virus [11 females and 20 males], 31 patients with hepatitis C virus [10 females and 21 males], and 50 males of blood donors during the period from the 1_{st} of January, 2022, to the 1_{st} of May, 2022. The samples were screened routinely for hepatitis C, hepatitis B virus, HIV, and Syphilis but not for HCMV. The goal of this research was to assess the incidence of HCMV IgG and HCMV IgM seropositivity in blood donors, HCV patients, and HBV patients in Diyala province using an enzyme-linked immunosorbent assay [ELISA]. The results revealed that the seropositivity of HCMV IgM was 3.2% in each of the HCV and HBV patients and 0.0% in blood donors. In contrast, the seropositivity of HCMV IgG was high among all study groups which were 96.8% among HCV patients, 100% among HBV patients, and 92% among blood donors. Based on the lifestyle, the percentage of HCMV IgM seropositivity for urban participants was 1.6%, and for rural participants, 2.0%. Meanwhile, the seropositivity of HCMV IgG was 96.7% in urban participants and 94.1% in rural participants.

Keywords: CMV, HCV, HBV, Blood donors, CMV IgM, CMV IgG, Lifestyle

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معدل الايجابية المصلية للفيروس المضخم للخلايا بين مرضى التهاب الكبد الفايروسيB و C و بين المتبرعين بالدم في محافظة ديالى

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الخلاصة

كان العدد الكلي للعينات هو 112عينة جمعت من 31 مريض بالتهاب الكبد الفيروســي نمط[C [HCV [10اناث و 21 ذكور] و31 مريض بالتهاب الكبد الفيروســي نمط[B [HBV] 11اناث و 20 ذكور] و 50 عينة من المتبرعين بالدم جميعهم ذكورجمعت خلال الفترة من 1 كانون الثاني2022 الى 1 مايس 2022 . كانت العينات مفحوصــة روتينيا بواسـطة الاليزا للتحري عن التهاب الكبد الفيروسي نوعي ال B و C وكذلك عن HIV ومرض Syphilis ولكن لم تفحص للتحري عن الفيروس المضــخم للخلايا HCMV. الغرض من هذا البحث هو تقييم حدوث الايجابية المصــلية ل HCMV IgM و HCMV IgG باستخدام تقنية الاليزا في المتبرعين بالدم ومرضى التهاب الكبد الفيروسي B وC في محافظة ديالى. بينت النتائج بان الانتشار المصلي ل HCMV IgM كان بنسبة 3.2% في كل من المرضى المصابين بالتهاب الكبد الفيروسي B والمرضى المصابين بالتهاب الكبد الفيروسي C و0.0% في المتبرعين بالدم. بينما كان الانتشار المصلي ل HCMV IgG عالي في جميع مجاميع الدراسة حيث كانت النسبة 96.8% في مرضى التهاب الكبد C و100% في مرضى التهاب الكبد B بينما كانت النسـبة في المتبرعين بالدم هي 92%. كذلك نسـبة الايجابية المصـلية ل HCMV IgM بين المرضــى والمتبرعين من سكنة الحضر كانت 1.6% بينما كانت 2.0% بين المرضـى والمتبرعين من سكنة الريف فيما كانت نسبة الايجابية المصلية ل HCMV IgG 96.7% بين المرضى والمتبرعين من سكنة الحضر و 94.1% بين سكنة الريف.

الكلمات المفتاحية : CMV، HBV، CMV IgM، نمط الحياة.

Introduction

Human cytomegalovirus (HCMV) is a Herpesviridae-family member, linear double-stranded DNA betaherpesvirus [1]. It is a common pathogen that causes a latent infection in its host. In healthy individuals, HCMV infection is usually asymptomatic or causes only minor symptoms [2]. In immunocompromised individuals, neonates, transplant patients, and pregnant women, infection with HCMV can lead to serious illness and even death [3]. HCMV is the most common congenital infection, causing hearing loss and neurodevelopmental disabilities in infected infants [4]. In immunocompromised people, HCMV reactivation is a serious health

issue. Reactivation from latency represents an important public health danger in immunocompromised individuals [5].

Monocytes and hematopoietic progenitor cells are believed to be the main reservoirs of HCMV latency in humans [5]. HCMV spread horizontally by body fluids that contain HCMV (such as urine, tears, sperm, vaginal or cervical excretions, breast milk, saliva, and blood) and organ transplantation and spread vertically from mother to child. Transfusion-transmitted cytomegalovirus can cause primary infection in recipients who are HCMV-seronegative or reinfection in recipients who are HCMV-seropositive and obtain blood or one of his products from HCMV-seropositive donors [6]. Transfusion-transmitted cytomegalovirus (TT-CMV) can have serious consequences for immunocompromised patients, who constitute a significant proportion of transfusion recipients [7]. The overall prevalence of transfusion-transmitted infections (TTIs) in apparently healthy blood donors in a region can be used to assess their prevalence in the general population of that region [8].

DaPalma and his colleagues (2010) recently proposed a system for classifying virus interactions when more than one virus is present in the same host into three major groups.: (i) direct interactions of viral genes or gene products, (ii) indirect interactions caused by the host environment changes, and (iii) immunological interactions that are special to organisms with an adaptive immunity [9]. HCMV mainly infects hepatocytes and macrophages in the liver [10]. Multiple research studies have looked into the role of coinfection with HCMV in the developing and incidence of hepatocellular carcinoma and speeding up the rate of liver fibrosis progression after liver transplantation [11]. Bader El Din and his colleagues (2011) concluded that, in addition to liver fibrosis staging, HCMV co-infection should be considered when developing predictive models for HCV response to interferon therapies [12]. Both HCMV and HBV can infect the liver and produce mild or severe diseases, depending on the host's immune system. In a stable chronic hepatitis B patient with a hepatitis flare, physicians must be aware of the possibility of acute infection with HCMV or reactivation. The pathogenesis of HCMV and HBV co-infections is still poorly understood [13]. At the same time, several studies have shown that HCMV has an inhibitory effect on HBV replication, such as Hu and his colleagues (2018), who

discovered that lower HBV replication levels were linked to an increased likelihood of HCMV infection. HCMV infection may inhibit HBV replication and decrease liver injury. In managing HCMV and HBV co-infection, there is an unmet need; research initiatives devoted to understanding HCMV and HBV interactions are desperately needed [14].

Materials and Methods

This cross-sectional study was conducted from the 1st of January, 2022, to the 1st of May, 2022 at Baqubah Teaching Hospital, Baqubah Public Health Laboratory, and the Main Blood Bank in Baqubah, which included 31 HBV-infected patients, 31 HCV-infected patients, and 50 blood donors. Because the number of female blood donors was very limited during the study period, there were no female participants in the current study.

Blood collection:

After sanitizing the aspiration site with 70 percent ethyl alcohol, 3 ml of blood was collected with 5-milliliter sterile disposable plastic syringes and placed in gel tubes. The blood samples in gel tubes were left upright in a rack for 20–30 minutes at room temperature to clot; Then samples were centrifuged for 5 minutes at 3000 RPM using a bench centrifuge to separate sera. Serum samples were aspirated and placed in Eppendorf tubes, kept at -20 ℃ until use.

Detection of anti-HCMV IgM and IgG antibodies:

Using an ELISA, all samples were examined for anti-HCMV IgG and IgM antibodies. Anti-HCMV IgM antibody levels were measured using the Foresight HCMV IgM kit (made in the USA). In brief, microwell plate wells were filled with 100 microliters of positive control (two wells), cut-off calibrator (two wells), negative control (two wells), and sample diluent (the rest of the wells according to the number of samples), followed by adding 5 microliters of each samples according to the sequence and one well is left without any additions as blank well. The color of the sample diluent changed from green to blue after the sample was added. The microwell plate was then sealed with a plate sealer and incubated at 37 ℃ for thirty minutes. After removing the plate sealer and washing the wells, all the wells received 100 microliters of the conjugate, but the blank well received none, and the plate was sealed and incubated at 37

℃ for 30 minutes. Then the microwell plate was washed after the removal of the plate sealer. Fifty microliters of substrates B and A were added to each well until the blank well, and the plate was sealed and incubated for 10 minutes at 37 ℃. After removing the plate sealer, 50 microliters of stop solution were added to each well. Finally, at 450 nm, the microwell plate was readable.

Also, the foresight HCMV IgG kit was used to measure HCMV IgG antibodies. The procedure for measuring IgG antibodies against HCMV was the same as for measuring anti-HCMV IgM antibodies, except for the first step, in which 100 microliters of calibrator 4, calibrator 3, calibrator 2, calibrator 1, and sample diluent were added to assigned wells.

Statistical analysis:

For statistical analysis, the SPSS version 25 software program was used. The percentages and frequency distributions for each variable were computed. *P* values above 0.05 were considered non-significant.

Results

The study included 31 HBV-infected and 31 HCV-infected patients, and 50 blood donors. The results revealed that the HCMV IgM positivity rate in each of the HCV and HBV patients was 3.2% and 0.0% among blood donors. The differences between study groups in HCMV IgM positivity rate were insignificant, with a *P*-value higher than 0.05 (table 1).

Table 1: The prevalence of HCMV IgM among the study groups**.**

						Total	*P*-value
			HCV	HBV	Blood donors		
CMV IgM	Positive	N	1	1	0	2	0.440
		%	3.2%	3.2%	0.0%	1.8%	
	Negative	N	30	30	50	110	
		%	96.8%	96.8%	100.0%	98.2%	
Total		N	31	31	50	112	
		%	100.0%	100.0%	100.0%	100.0%	

On the other hand, HCMV IgG positivity was elevated in all study groups; it was 96.8% in HCV patients, 100% in HBV patients, and 92% in blood donors. The differences in HCMV IgG positivity rates between study groups were also insignificant, with a *P*-value higher than 0.05 (table 2).

Table 2: The prevalence of HCMV IgG among the study groups.

			HCV	HBV	Blood donors	Total	*P*-value
CMV IgG	Positive	N	30	31	46	107	0.220
		%	96.8%	100.0%	92.0%	95.5%	
	Negative	N	1	0	4	5	
		%	3.2%	0.0%	8.0%	4.5%	
Total		N	31	31	50	112	
		%	100.0%	100.0%	100.0%	100.0%	

As shown in Table 3, the prevalence of HCMV IgM was not affected by participants lifestyle; it was 1.6% in the urban population and 2.0% in the rural population, with no signifi cant differences among the groups [*P*-value > 0.05].

Table 3: The prevalence of HCMV IgM according to living.

			Living		Total	*P*-value
			Urban	Rural		
CMVIgM	Positive	N	1	1	2	0.898
		%	1.6%	2.0%	1.8%	
	Negative	N	60	50	110	
		%	98.4%	98.0%	98.2%	
Total		N	61	51	112	
		%	100.0%	100.0%	100.0%	

The study's findings, however, revealed that the prevalence of HCMV IgG was unaffected by lifestyle; HCMV IgG positivity was extremely high, reaching 96.7% in the urban population and 94.1% in the rural population, with no significant differences [*P*-value higher than 0.05]. Table 4.

Table 4: The prevalence of HCMV IgG according to living

			Living		Total	*P*-value
			Urban	Rural		
CMVIgG	Positive	N	59	48	107	0.506
		%	96.7%	94.1%	95.5%	
	Negative	N	2	3	5	
		%	3.3%	5.9%	4.5%	
Total		N	61	51	112	
		%	100.0%	100.0%	100.0%	

Discussion

Blood transfusions are a routine procedure for some hospitalized patients.; they can save a patient's life [15]. However, blood transfusions from HCMV-infected donors are a major source of infection for high-risk groups. Improved donor selection can decrease the likelihood of HCMV transmission through blood products [16]. HCMV infects a wide range of body cells, such as neuronal, endothelial, fibroblasts, and hepatocytes, as well as monocytes in the blood and macrophages inside tissues (which aid in infection dissemination and serve as latent infection sites) [11]. Although specific HCMV IgM antibodies in the blood have long served as a diagnostic marker for primary infection, IgM can also be present during reinfection or viral reactivation and therefore is not restricted to primary infection. Contrary to IgM, low-avidity HCMV IgG is usually only present during primary infection and gradually increases to high avidity over 3-5 months [17]. As a result, the presence of low-avidity IgG and IgM antibodies suggests a recent primary infection. The results of HCMV IgG avidity tests can be misleading when used on sera that lack HCMV IgM antibodies and have low levels of IgG antibodies (the infection is not recent) [18].

Table (1) shows that the percentage of HCMV IgM positive results was (3.2%) for each of HBV and HCV patients. While it was (0%) for blood donors. The patients who were HCMV IgM positive were also HCMV IgG positive.

Khalid (2012) reported that 3% of blood donors in Mosul/Iraq were HCMV IgM positive [19]. According to Khudir and Molan (2014), 1.6% of healthy students at Diyala University tested positive for HCMV IgM [20]; this difference may be due to sample size. AL-Khilkhali and his colleagues (2015) reported that in Al-Najaf city, 5.26% of HBV patients were HCMV IgM positive [21]. In other countries, for example, a study in the Madinah Region, Saudi Arabia reported that 1.6% of blood donors were positive for both HCMV IgG and HCMV IgM antibodies [22]. According to Safabakhsh and his colleagues (2013), 1.6% of blood donors in Mashhad, Iran, tested positive for CMV IgM [23]. Furthermore, anti-HCMV IgM antibodies were not detected in serum samples from patients [chronic HBV and chronic HCV patients] or control groups, according to a study published in Turkey [24].While a study in Egypt found that 28% of chronic HCV patients tested positive for HCMV IgM, compared to 16.7% of controls (apparently healthy people) [25].

HCMV IgG positivity was found in 96.8% of HCV patients, 100% of HBV patients, and 92% of blood donors, as shown in Table (2). Our findings agreed with a study on pregnant women in Zakho City, Kurdistan Region, Iraq, which found high HCMV IgG seropositivity rates of 95% [26]. However, in Alexandria, Egypt, Gawad and his colleagues (2016) reported that 96.6 percent of blood donors tested positive for HCMV IgG [27]. Whereas, Turkey study indicated, 86.4% of chronic HBV patients and 84% of chronic HCV patients had anti-HCMV IgG antibodies [24].

On the other hand, our results are relatively high compared to other Iraqi studies, such as Abbas and Zaman (2019), who estimated that 37.77% of blood donors in Kirkuk City were seropositive for HCMV IgG [28]. Anti-HCMV IgG positivity was reported to be low in other countries, according to Kowalzik and his colleagues (2020), who estimated that 37.5% of blood donors in Germany were positive for anti-HCMV IgG [29].

Table(3) revealed that the prevalence of HCMV IgM was 1.6% in the study participants of urban population and 2.0% in the study participants of rural population. These results agreed with Ahmed (2017), where the HCMV IgM positivity rate in study participants of rural populations was higher than in urban ones [30].

Table (4) indicated that the prevalence of HCMV IgG was 96.7% in study participants of urban population and 94.1% in study participants of rural population. The percentage of HCMV IgG seropositivity was very high in both urban and rural populations but was a little higher in urban population than rural ones. These results did not agree with Ahmed (2017), who indicated that the positivity of HCMV IgG was higher in rural population than in urban population [30].

Conclusion

The high seroprevalence of HCMV in blood donors demonstrates that the virus is common in Diyala Province and can pose a high risk during blood transfused to immunocompromised individuals, who make up a significant proportion of transfusion recipients. There is also a high seroprevalence of HCMV in HBV and HCV patients, who may be at increased risk of accelerating and developing liver diseases when co-infected with HCMV.

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