



Serological Study of Human Cytomegalovirus (CMV) in Diabetic Patients in Kirkuk Governorate

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Received date: 16 / 11 / 2014

Accepted date: 7 / 1 / 2015

ABSTRACT

The aim of this study was to know the relationship between the human cytomegalovirus and diabetes mellitus through detection of human cytomegalovirus (HCMV) infection in diabetic patients in Kirkuk governorate by screening of anti-human cytomegalovirus IgM and IgG antibodies in the serum of diabetic patients by using of Immunochromatography and detection of IgG by ELISA technique in blood samples . Blood samples collected from 82 diabetic women patients and 10 samples from healthy looking with out diabetic women .The results of this study by Immunochromatography method detected in 22 samples (26.82%) IgM only ,25(30.48%) samples IgG only and 2(2.43%) samples showed positive results for both IgM and IgG for HCMV . In contrast, the results of control group were negative for anti-HCMV IgM and IgG antibodies in both Immunochromatography and ELISA technique. ELISA technique was used to know the presence IgG of HCMV for detection the sensitivity and specificity of Immunochromatography compared with ELISA technique .

KeyWords : HCMV, Immunochromatography ,Diabetics,ELISA,

دراسة مصلية لفايروس المضخم للخلايا البشري بين مرضى الداء السكري في

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تاريخ قبول البحث: 2015 / 1 / 7

تاريخ استلام البحث: 2014 / 11 / 16

الملخص

لقد كان هدف الدراسة معرفة العلاقة بين الفايروس المضخم للخلايا البشري ومرضى السكري في مدينة كركوك من خلال التحري عن وجود الاجسام المضادة للفايروس في مصول مرضى السكري . تم جمع 82 عينة دم من مرضى السكري بالإضافة الى 10 عينات من اشخاص لا يعانون من السكري .لقد استخدمت تقنية **Imminochromatography** في التحري عن وجود الاجسام المضادة من نوع (IgM) و (IgG) واستخدام تقنية الاليزا للتحري عن (IgG) . وظهرت النتائج باستخدام تقنية **Imminochromatography** وجود (IgM) و (IgG) وكلاهما في :26,82% و 30,48% و 2,43% على التوالي . لقد اظهرت الدراسة ان انتشار فايروس المضخم للخلايا البشري في مجموعة الدراسة كانت اعلى بالمقارنة بمجموعة السيطرة مما يعني ان مرضى السكري اكثر عرضة للاصابة بفايروس المضخم للخلايا البشري. نتائج دراسة الحساسية و الخصوصية لفحص **Imminochromatography** بالمقارنة مع فحص ELISA كانت : 85,1% و 96,3% على التوالي . الكلمات الدالة: الاليزا ، داء السكري ، فحص **Imminochromatography** ، فايروس المضخم للخلايا البشري.



1.INTRODUCTION

Cytomegalovirus (from the Greek cyto-, "cell", and -megal-, "large") is a viral genus of the Herpesviruses group: in humans it is commonly known as HCMV or Human Herpesvirus 5 (HHV-5) [1]. Human Cytomegalovirus (CMV) is a herpes virus and the most common cause of congenital viral infection and malformation in the developed countries resulting from viral intrauterine infection [2,3,4]. Cytomegalovirus like all herpes viruses undergoes latency and reactivation in the host. Although HCMV has been shown to infect a broad-spectrum of cells in vivo and has been isolated from saliva, urine, blood and human milk [5,6]. However, in immune compromised individuals owing to the lack of immunologic control, the virus is able to reactivate and to cause severe CMV disease. Viral activity can be observed in all organs, including the pancreas [7] demonstrating that the virus has a broad cellular tropism. This broad cellular tropism is because widely spread receptors, such as integrin's and the epidermal growth factor receptor, serve as entry receptors [8,9]. These are also found on pancreatic cells making them putative targets for CMV infection [10]. Human Cytomegalovirus is ubiquitous herpes virus that leads to lifelong persistent infection. The frequency of infection ranges from 50% to 90% in general adult population, and varies with socio-economic level and to some extent, geographic location[11]. Human Cytomegalovirus (CMV) is the most common cause of congenital malformation in developed countries its clinical manifestations range from asymptomatic infection to severe fetal damage[12]. Up to 15% of intrauterine CMV infections result in symptomatic congenital disease at birth and 10 to 15% of those born with asymptomatic congenital CMV will develop significant clinical sequelae in infancy [13]. The presence of CMV-specific Immunoglobulin M (IgM) may not be indicative of primary infection, since it is also produced during reactivation and re infection[14]. Some researchers showed significant relation between CMV infection and spontaneous abortion [15]. Ninety percent of infants with congenital CMV infection display no clinical manifestations at birth, the remaining 10% of intrauterine CMV infections resulting into signs at birth and they are at serious risk of long-term neurological sequelae. The risk of any sequelae in infants with symptomatic congenital CMV at birth is 90% [16,17,18,19]. All pregnant women blood specimens were taken to be investigated regarding the prevalence of anti-cytomegalovirus infection antibodies M, and G (IgM, IgG). All pregnant women who are complaining from other causes of abortion were excluded from study.



After separation of blood specimens, their sera were tested to determine the concentration of CMV-IgM and CMV-IgG using Elisa technique[19]. In the immunocompetent adult, primary CMV infection is usually asymptomatic but can result in a mononucleosis syndrome[20]. CMV infection in immunocompetent hosts may rarely be able to lead to severe organ specific complications. But some serious complications have been reported. Severe hepatitis is a frequent presentation[21,22,23] . Human CMV has been isolated from saliva, urine, blood, human milk, cervical secretions, various tissue specimens, and even from human semen[24]. Human cytomegalovirus (HCMV) is the most common pathogen in uterus during pregnancy, which may lead to some serious results such as miscarriage, stillbirth, cerebellar malformation, fetus developmental retardation, but its pathogenesis has not been fully explained [25]. Tests for IgM antibody to CMV often lack specificity for primary infection because of false positive test results or because patients with past infection may have IgM antibody to CMV. The avidity of IgG antibody increases with time after initial infection and demonstration of low CMV-IgG avidity can improve the accuracy of identification of recent primary infection[26,27] .

2.MATERIALS AND METHODS

In the present study 82 of women diabetics patients and 10 healthy (non-diabetics) samples as (control) included for detection HCMV: using a plain tubes the blood was aspirated, labeled and allow samples to clot for 30 minutes before a centrifugation for 15minutes ,by using Immunochromatography method for detection IgM and IgG of HCMV (CTK Bioteck InC : USA) and using ELISA technique for detection IgG of HCMV according to the manufacturer's instructions (Biotech ,Inc USA)

3.RESULTS

Out of 82 DM samples samples positive results by Immunochromatography method detected in 22 samples (26.82%) IgM only ,25(30.48%) samples IgG only and 2(2.43%) samples showed positive results for both IgM and IgG for HCMV. **Table.(1)**. In this study ,serum samples from 82 patients with DM , were tested by Immunochromatography for HCMV detection . for IgG of HCMV was detected in 32.92% of specimens .

These specimens were retested with ELISA which revealed 28.04 IgG for were positive of HCMV with . **Table.(2)** . Out of 27 positive cases by Immunochromatography test , 23 patients were positive by ELISA ,so the sensitivity , specificity, positive predictive value , accuracy rate, positive concordance rate, negative concordance rate, total concordance rate and total disconcordance rate of Immunochromatography test was 85.1% , 96.3 % , 92%, 92.6%, 28% , 64.6% , 92.68% and 7.3% respectively. **Table.(3)**.

Table.(1): Results of HCMV among DM patients .

Type of tested samples	Results of Immunochromatography											
	IgM				IgG				IgM and IgG			
	Positive		Negative		Positive		Negative		Positive		Negative	
	No.	%	No	%	No	%	No	%	No	%	No	%
Type II DM samples	22	26.82	60	73.17	25	30.48	58	70.73	2	2.43	80	97.56
Control samples	0	0	10	100	10	100	10	100	0	0	10	100
Total	22	26.82	87	73.17	35	38.04	68	73.91	2	2.17	90	97.82

Table.(2) :Comparison of Immunochromatography and ELISA Tests to Detect HCMV in 82 Specimens .

Test	Tested Cases	+ve		-ve	
		No.	%	No.	%
ImCh Test	82	27	32.92	55	67.07
ELISA Test	82	23	28.04	59	71.95

Table.(3) : Evaluation of Immunochromatography Test Validity as Compared with ELISA Test for Detection of HCMV.

ELISA \ IM. Ch	Positive results	Negative results	Total
Positive results	23	2	25
Negative results	4	53	57
Total	27	23	82



23

Sensitivity of latex test = ----- × 100 = 85.1 %

27

53

Specificity of latex test = ----- × 100 = 96.3 %

55

23

Positive predictive value of latex test = ----- × 100 = 92 %

25

23+53

Accuracy rate = ----- × 100 = 92.6 %

82

23

Positive concordance rate = agreement in positivity of PCR and latex = ----- × 100 = 28%

82

53

Negative concordance rate = ----- × 100 = 64.6 %

82

23+53

Total concordance rate = ----- × 100 = 92.68%

82

2+4

Total discordance rate = ----- × 100 = 7.3 %

82



4.DISCUSSION

HCMV is a significant opportunistic pathogen in immunocompromised patients. Primary infection, reactivation of latent virus, and reinfection are possible and are often clinically silent. The onset of infection is marked by spiking pyrexia, which may resolve in a few days, Cytomegalovirus (CMV) can cause severe disease in immunocompromised patients, either via reactivation of latent CMV infection or via acquisition of primary CMV infection. [28,29] In the present study antibodies of HCMV IgM only , detected in 26.82 % detected in DM patients .Previous Studies regarding IgM of HCMV by Al-Kifaji[30] in Thi-Qar reported 35 % were positive among DM patients ,Khalf reportet 15.7% ,Hussan 115.23% ,Salman in Kirkuk 17.6%.Anmar reported that IgM in Mosul found in 12%,while study by Saad in Karbala reported 35.38 % were positive . Other study by Bertram et al. [31] found an association between seropositivity for CMV and mellitus, this susceptibility to assorted infections in a population with compromised immunity would partially explain the possibility of increased CMV seropositivity in persons with type- 2 diabetes, such as seen in our study. This infection might be latent and not clinically manifested. It is possible that molecular mimicry may be involved in some cases of CMV – induced diabetes, in this situation immune response against similar epitopes shared by antigenic determinants of CMV and islet-cell specific proteins may lead to islet–cell specific autoimmunity. Evidence for this is the finding that human CMV can induce an islet-cell antibody that reacts with a 38 kD autoantigen expressed in human pancreatic islet [32]Other syudy by Albaitushi [33] who concluded that the higher prevalence of seropositivity for human CMV in diabetic patientscomparing with normal individuals which means that cytomegalovirus patients with diabetic were at highrisk for CMV infections. A higher prevalence of CMV antibodies was observed in diabetic patients of all age-groups as compared with control group.Type-1-diabetes (T1D) also known as insulin-dependent diabetes mellitus (IDDM) or juvenile onset diabetes results from the progressive destruction of pancreatic beta cells resulting in insulin deficiency [34,35]. Genetic factors are thought to be a major component for the development of T1D [36], however studies on the risk of developing T1D using identical twins have shown that the concordance rate for the disease approaches only 40% [37], suggesting that the environmental factors including climate, exposure to pathogens, particularly viruses [38] and beta cells toxins may be involved in the initiation and/or progression of beta cells destruction



leading to T1D [39]. The earliest observations for the suggestion of virus contribution to T1D is that the onset of T1D sometimes follow acute infections and occurs with greater frequencies at certain times of the year which often indicate a viral cause, recent studies showed the presence of virus-specific IgM antibodies in recent- onset T1D patients. The most convincing evidence comes from studies in which viruses isolated from the pancreas of patients who died from acute T1D [40].

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