Hamsa A. Faraj et al /J. Pharm. Sci. & Res. Vol. 11(4), 2019, 1571-1576

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Prevalence of Human Cytomegalovirus Infection in Non-Married Diabetes type 2 Iraqi Women

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Abstract

Human cytomegalovirus (HCMV) is a member of the Beta-herpesviridae family, and represent the most common factor of intrauterine viral infections. The purpose of this study was to study the association between HCMV infection with glucose regulation and Dyslipidemia in non-married Iraqi women, during the reproductive age. Two hundred blood samples were obtained from non-married women with history of diabetes mellitus type 2 and one hundred as healthy control. Their aged ranged from 13– 45 year and they were admitted to Al-kidney Center of Diabetes and Endocrine Gland in Baghdad. The primary diagnosis of HCMV was carried out depending on the presence of antibodies against the virus in serum .Enzyme-linked Immunosorbent Assay (ELISA) was used for this purpose. In addition the sera samples were examined for FBS (Fasting Blood Sugar), HBAIC (Hemoglobin A1C), Lipid profile and CRP(C - reactive protein). Body Max Index (BMI) were calculated also. Depending on the serological detection of the virus, patients groups again were subdivided into subgroups as following: Neg IgG- Negative IgM 44 (22%), Positive IgG- Negative IgM 142 (71%) and Positive IgG- Positive IgM 14(7%). The 100 control samples groups of non -diabetic also sub-grouped to a Negative IgG- Negative IgM 77 (77%) and Positive IgG- Negative IgM 33 (33%). The results of the comparative statistical analysis between all sub grouped involved in this study showed a significant differences especially in positive IgG, negative IgM control and patient's subgroups, the result showed that all factor are non- significant except FBS, triglyceride and IgG were p-value 0.001 and in case of VLDL the p-value was 0.01 and in HBA1C p-value was 0.05. There was a significant correlation between IgG with HBA1C p-value was 0.01. IgG showed no correlation with any marker in the Negative IgG, IgM Patients subgroup while in Positive IgG, IgM patient's subgroup IgM was significantly correlated with age and FBS p-value was 0.01, and at 0.05 level with HBA1C, Triglyceride, VLDL and LDL. In conclusion, this study demonstrated that there was a significant association between Cytomegalovirus and type 2 diabetes mellitus in Iraqi non married women, as well the current search founded a possible association between Diabetic dyslipidemia and chronic CMV infection in our population because of the strong correlation between high HBAIC and Dyslipidemia in seropositive CMV. This finding suggests that CMV infection might be a risk factor for the development of diabetes type 2 in women.

Keywords: human cytomegalovirus, non-married women, Dyslipidemia, diabetes type 2.

INTRODUCTION

Human cytomegalovirus (HCMV) considered as a member of herpesviridae family, subfamily beta-herpesviral for HCMV and other animal species. The genome of HCMV is linear double-stranded DNA, with an envelope and the virus capsid is icosahedral symmetry (1).

The virus can be spread through saliva, Breastfeeding, placental transfer, blood transfusion, sexual contact, and solid organ transplantation. After primary infection, the virus has the ability to found in the host in a latent state, regularly reactivates the virus from the latent or dormant state after severe harm of the immune system by disease or medication, CMV supposed to be the most common pathogens to human because most of CMV infection remain asymptomatic (2).

Numerous types of viral infections have been related with enlarged risk of diabetes mellitus (3). The researchers found seropositivity of CMV is significantly associated with glucose organization or regulation indicators and thus infection by CMV could be a risk factor for diabetes disease progress (4).

The progressive prevalence in developing countries of diabetes among younger individuals is due to inactivity, obesity, and aging and it's associated with insulin resistance.

When islets of pancreatic gradually defeat to promote the production of insulin as indemnification for insulin resistance, chronic hyperglycemia, insulin deficiency, and eventual diabetes disease will develop. (4)

A chronic stress of the immune system is common with the herpes virus and HCMV establishes persistent with life-long infections and become reactivated sporadically or periodically, and consider seropositivity of HCMV is one parameter of the “Immune Risk Profile” and has been associated to syndromes and diseases with an inflammatory component including frailty, cardiovascular disease, cognitive impairment, functional impairment, cancer, and mortality (5). Therefore, activate or reactivation of latent state of CMV infection is considered as a potential cofactor for inflammatory diseases. Various findings suggest that continuous CMV infection may contribute to the pathogenesis of diabetes disease (6).

CMV may hasten immune deterioration by inducing the aggregation of late-differentiated CD4+ and CD8+ T-cells and production of pro-inflammatory cytokines and generate an additional pro-inflammatory environment (7).

Furthermore, reports revealed that pro-inflammatory cytokines have harmful effects on pancreatic β-cells which may lead to a deficient response to insulin resistance, resulting in the onset of diabetes disease. Moreover, asymptomatic CMV infection is recognized to be a risk factor for the development of new-onset post transplantation diabetes mellitus (PTDM).

One study, indicate that RNA of CMV was identified in the islets of Langerhans of the pancreas of diabetes patients, (8).

In Iraq we did not find any local study, according to our knowledge, concerned with the study of the prevalence of HCMV among unmarried women in general and among women with diabetes type II in particular, thus the current study was designed to reach the following aim:

Serological diagnosis for HCMV infection in non-married women with diabetes type II, by using Enzyme Linked Immunosorbent Assay (ELISA), to outline the percentage of virus infection. Determine the risk factors: Age, BMI (Body Max Index), FBS (Fasting Blood Sugar), HBAIC (Hemoglobin A1C), Lipid profile and CRP(C - reactive protein) associated with cytomegalovirus infection. And study the association between HCMV infection and an indicator of glucose regulation and Dyslipidemia in Iraqi women in reproduction age

MATERIALS AND METHODS

Blood samples were collected from 300 non-married women with age ranging from 13 to 45 years, 200 were diabetic patients (case group) and 100 are apparently healthy (control group) who attending to Al kidney Center of Diabetes and Endocrine Gland in Baghdad, during the period from January to March (2018)

The samples were tested for IgG, IgM anti cytomegalovirus by using enzyme-linked immunosorbent assay (ELISA) technique, BMI calculated in addition HBAIC, Lipid profile and CRP where measured by diagnostic kits for each.

Serological Assay for Primary Detection: Five milliters of blood were collected from each woman under sterile condition, who participated in the morning fasting sub study and had fasted for at least 8 hours. Serum anti-HCMV IgG and IgM antibody levels were assayed by used enzyme-linked immune-sorbent assay (ELISA) tests (Cytomegalovirus (CMV) IgG/IgM Enzyme Linked Immune Assay (ELISA)/BioCheck/German and seropositivity was determined by using the manufacturer's guidelines.

Procedures: Optical density values obtained from participants' samples were fitted to a standard curve. These concentrations were then compared with a cut-off value to compute CMV index scores. The index value where calculated to obtain qualitative specimen results as following:

1-Cut-off value where obtained by this equation:

Cut-off value = mean absorbance of calibrator 2– blank absorbance.

2-The index value where calculated by dividing the specimen absorbance by the cut-off value.

The normal value of CMV above 1.2 were considered Positive CMV and under 0.9 where considered Negative CMV and between them were considered border line.

Biochemical analysis

HBA1C, fasting glucose, Cholesterol, triacylglycerol, LDL, HDL and high-sensitivity C-reactive protein levels were measured by an accredited clinical laboratory according to standard laboratory procedures.(9)

HbA1c levels were measured using I-Chroma and fasting glucose levels were measured using the glucose hexokinase enzymatic assay, in accordance with the latest standard guidelines and recommendations for laboratory analysis in the diagnosis of diabetes. Cholesterol, triacylglycerol and HDL were measured by Spinreact Kits. These values were also used to calculate the LDL and VLDL Values. (10).

Diabetes and metabolic syndrome classification

Diabetes was classified according to the ADA guidelines in individuals with fasting glucose levels of >6.94 mmol/l and/or HbA1Clevels of ≥6.5% (48 mmol/mol) in the absence of known diabetes.

Those with self-reported, doctor diagnosed diabetes were also classified as diabetic. Prediabetes was classified as a fasting glucose level between 5.55 and 6.94 mmol/l and/or an HbA1C level between 5.7% (39 mol/mol) and 6.4% (46 mmol/mol)(11). The remaining normal glycaemic individuals, therefore, had fasting glucose and HbA1c levels of <5.55 mmol/l and <5.7%, respectively. The metabolic syndrome components were assessed as the following: (1) waist circumference >88 cm (women); (2) plasma triacylglycerol >1.70 mmol/l; (3) plasma HDL-C or <1.29 mmol/l (women);(4) plasma fasted glucose ≥5.55 mmol/l. Each of these components were dichotomized (yes or no) and added together to create a metabolic syndrome component score (range 0–5). Those with a score of ≥3 were classified as having the metabolic syndrome.

Statistical analysis: statistical package for the social science system version SPSS 20. P<0.05 was considered statistically significant.

RESULTS

This was descriptive, cross sectional study, aimed to detect cytomegalovirus among type 2 diabetic in non-married women and to determine the risk factors: Age, BMI (Body Max Index), FBS (Fasting Blood Sugar), HBAIC (Hemoglobin A1C), Lipid profile and CRP(C - reactive protein) associated with cytomegalovirus infection.

The primary diagnosis of HCMV was carried out depending on the presence of antibodies types and titer against the virus. Depending on these results the patients groups again where subdivided into subgroups as follows Negative IgG-Negative IgM 44 (22%), Positive IgG-Negative IgM 142 (71%) and Positive IgG- Positive IgM 14(7%). While 100 samples of control groups non diabetic also sub grouped to the Negative IgG-Negative IgM 77 (77%) and Positive IgG- Negative IgM 33 (33%) table (1).

The results of CRP detection were negative for all groups involved in this study, indicating that there is no relationship between it and CMV infection or other study marker.

The results of comparative statistical analysis between all subgrouped involved in this study showed significant differences as follows:

The comparison between the Negative IgG, Negative IgM control and Patients subgroups table (2) showed that all

factors non-significant except between FBS and HBA1C in the two subgroups, p-value was 0.001, compared between Positive IgG and Negative IgM of control and patient's subgroups, results showed a relation between BMI in two subgroups (P-value 0.05) and in FBS p-value were 0.001 and HBA1c p- value were 0.03 and in cholesterol, triglycerides, VLDL and IgG the p-value were 0.001 and un LDL p-value were 0.009 this results showed that a relationship between Diabetes, Lipid profile and IgG infection of CMV.

In Negative IgG, Negative IgM control sup groups and Positive IgG, Positive IgG, positive IgM patient's subgroups, a comparative study showed non-significant p-value in age, BMI, HDL, LDL and cholesterol between the two subgroups, While in HBA1C, triglyceride and VLDL, p- value were 0.001.

In positive IgG, negative IgM control subgroups and positive IgG, positive IgM patient's subgroups, the result showed that all agents are non- significant except FBS, triglyceride and IgG were p-value 0.001 and in case of VLDL the p-value was 0.01 and in HBA1C p-value was 0.05.

On the other hand, the correlation results between the same study groups showed large and varied differentiations especially with respect to HBA1C and lipid profile. IgG showed a significant correlation with FBS only p value was 0.01 in the Negative IgG, IgM control subgroup.

In Positive IgG, Negative IgM control subgroup there where a significant correlation between IgG and triglyceride on level 0.01 and with LDL on 0.05 level

The correlation results in patient's subgroup improved that there were a significant correlation with IgG, IgM and different analysis included in this study. In Positive IgG, Negative IgM patient's subgroup there was a significant correlation of IgG with HBA1C (p value 0.01). IgG showed no correlation with any study marker in the Negative IgG, IgM Patients subgroup while in Positive IgG, IgM patient's subgroup IgM was significantly correlated with age and FBS at 0.01level, and at 0.05 level with HBA1C, Triglyceride, VLDL and LDL.

Table (1): The divided of groups and sub groups of studies samples

Study groups number (%)		
Sub groups	**Control groups (Non Diabetic)**	**Patient's groups(Diabetic)**
Neg IgG/Neg IgM (-ve IgG/-ve IgM)	77(77)	44(22)
Pos.IgG/Neg IgM (+ve IgG/-ve IgM)	33(33)	142(71)
Pos.IgG/Pos. IgM (+ve IgG/(+ve IgM)	0(0)	14(7)
Total	**100(100)**	**200(100)**

Table (2): The comparative of statistics analysis results between Negative IgG Negative IgM control and patient subgroups

(Negative IgG/ Negative IgM) Control VS Patient			
Variables	**Control groups (Non Diabetics) Mean± SD(77)**	**Patient's groups (Diabetics) Mean± SD(44)**	**P- value**
Age	30.77±8.5	26.1±10.5	NS
BMI*	27.18±4.72	26.9±5.4	NS
FBS	93.4±12.4	227.5±89.8	0.001
GHB	5.2±0.46	8.58±1.7	0.001
TSC	186.8±18.6	188.2±25.9	NS
STrig	129.1±32.9	129.6±36.9	NS
SVLDL	25.83±6.42	25.93±7.7	NS
SHDL	48.78±5.84	49.54±6.58	NS
SLDL	112.2±20.3	112.5±22.6	NS
IgG	0.7±0.22	0.72±0.26	NS
IgM	0±0	0±0	0

*NV of BM1 (Body mass index): (Less than 18.5 kg/m2 mean decreases in Wight) (18.5-24.9 kg/m2 mean healthy Wight) (30 39.9 kg/m2 mean obesity)/*NV of FBS (Fasting blood sugar): 65 – 115 mg/dl./* NV of HBA1C (Hemoglobin A1C): 4.2 – 6.2 mg/dl. /* NV of TSC (Total serum cholesterol): up to 200 mg/dl./* NV of TG (Triglyceride): up to 150 mg/dl./* NV of TSC (Total serum cholesterol): up to 200 mg/dl./ * NV of HDL (High Density lipoprotein): up to 50 mg/dl. /* NV of LDL (Low Density lipoprotein): up to 180 mg/dl./* NV of VLDL (Very low Density lipoprotein): up to 35 mg/dl./* NV of CMV IgG: Negative 0.9 – 1.2 Positive./ * NV of CMV IgM: Negative 0.9 – 1.2 Positive. /* NV of CRP(C - reactive protein): Less than 12.0 mg/dl

Table (3): The comparative of statistics analysis results between Positive IgG Negative IgM control and patient subgroups

(+ve IgG/-ve IgM) C VS P			
Variables	**Control groups (Non Diabetics) Mean± SD(33)**	**Patient's groups (Diabetics) Mean± SD(142)**	**P- value**
Age	30.17±8.26	30.4±10.4	NS
BMI	25.6±4.5	27.79±4.65	0.05
FBS	102±7.43	231.5±91	0.001
GHB	5.28±0.34	9.34±8.69	0.03
TSC	172±11.2	216.7±45.3	0.001
STrig	122±33.1	185.4±54.6	0.001
SVLDL	24.4±6.62	37.1±10.9	0.001
SHDL	50±4.86	49.94±33.3	NS
SLDL	101.6±10.1	132.6±42.7	0.009
IgG	1.35±1.2	2.04±0.54	0.001
IgM	0±0	0±0	0

Table (4): The comparative of statistics analysis results between Negative IgG Negative IgM control and Positive IgG. Positive IgM patient subgroup

(-ve IgG/-ve IgM) VS (+ve IgG/+ve IgM) C VS P			
Variables	**Control groups (non-Diabetics) Mean± SD(77)**	**Patient's groups (Diabetics) Mean± SD (14)**	**P- value**
Age	30.77±8.5	29±10.4	NS
BMI	27.18±4.72	25.2±3.97	NS
FBS	93.4±12.4	189.3±64.1	0.000
GHB	5.2±0.46	7.84±1.37	0.001
TSC	186.8±18.6	192.7±27.4	NS
STrig	129.1±32.9	203±53.4	0.001
SVLDL	25.83±6.42	40.62±10.7	0.001
SHDL	48.78±5.84	48.89±10.6	NS
SLDL	112.2±20.3	103.7±28.3	NS
IgG	0.7±0.22	2.35±0.39	0.000
IgM	0±0	1.37±0.22	0.000

Table (5) the comparative of statistics analysis results between Positive IgG Negative IgM patient and Positive IgG Positive IgM patient subgroup

(+ve IgG/-ve IgM) VS (+ve IgG/+ve IgM) P VS P			
Variables	**Patient's groups (Diabetics) Mean± SD(142)**	**Patient's groups (Diabetics) Mean± SD(14)**	P- value
Age	30.4±10.4	29±10.4	NS
BMI	27.79±4.65	25.2±3.97	NS
FBS	231.5±91	189.3±64.1	NS
GHB	9.34±8.69	7.84±1.37	NS
TSC	216.7±45.3	192.7±27.4	NS
STrig	185.4±54.6	203±53.4	NS
SVLDL	37.1±10.9	40.62±10.7	NS
SHDL	49.94±33.3	48.89±10.6	NS
SLDL	132.6±42.7	103.7±28.3	NS
IgG	2.04±0.54	2.35±0.39	NS
IgM	0±0	1.37±0.22	0.000

Table (6) The comparative of statistics analysis results between Positive IgG Negative IgM control and Positive IgG Positive IgM patient subgroup

(+ve IgG/-ve IgM) VS (+ve IgG/+ve IgM) C VS P			
Variables	**Control groups (Non Diabetics) Mean± SD(77)**	**Patient's groups (Diabetics) Mean± SD(14)**	P- value
Age	30.77±8.5	29±10.4	NS
BMI	27.18±4.72	25.2±3.97	NS
FBS	93.4±12.4	189.3±64.1	0.001
GHB	5.2±0.46	7.84±1.37	0.05
TSC	186.8±18.6	192.7±27.4	NS
STrig	129.1±32.9	203±53.4	0.001
SVLDL	25.83±6.42	40.62±10.7	0.01
SHDL	48.78±5.84	48.89±10.6	NS
SLDL	112.2±20.3	103.7±28.3	NS
IgG	0.7±0.22	2.35±0.39	0.001
IgM	0±0	1.37±0.22	0.000

DISCUSSION

The positive cases of CMV IgG were detected among diabetic patients by using ELISA technique and the results were nearly in accordance with jerrald., *et al* (2015), and with Sijia chen *et al* (2012). CMV infection was significantly associated with dyslipidemia in female and this result is nearly in accordance with Shanon Fleck *et al* (2017). Locally there is no similar study about Diabetics, Dyslipidemia and infection with CMV in females.

The Infection of CMV indicated that the diabetic female patients had previously been infected with CMV. After CMV infection, IgG were remain in the body for long time and protects significantly against the next infection. Therefore negative result of CMV IgG test means that the female diabetic patients had not been infected with CMV.

In control group a significant correlation between CMV Negative IgG with FBS, this result showed the non-diabetic persons were have no infection CMV so that no effect from CMV on pancreatic cells, In control group positive IgG were a significant correlation between IgG and Triglyceride, this mean that IgG may promotes development of Diabetic and Dyslipidemia in the future.

In diabetic patients, positive IgG/negative IgM subgroup showed age correlation with lipid profile except HDL, HBA1C and LDL were a strong correlation with CMV IgG so this result support shanon *et al* (2017) study in which CMV infection was significantly associated with high HBA1C and dyslipidemia in females, while in negative IgG/ negative IgM, lipid profile were a significant correlation with age that is mean older women.

Where dyslipidemia on the contrary of younger women, and there is no correlation between IgG and any marker so this diabetic group causes by other reasons like environmental, bad habits or diabetic family history.

The last subgroup were positive IgG/ positive IgM of diabetic patients, had a strong correlation between dyslipidemia and older women, and positive IgM had a correlation with FBS, HBA1C, triglyceride, VLDL and LDL also this results agree with chen *et al*., (2012) which revealed that CMV infection significantly associated and might be a risk factor for the development of diabetes in females.

Our main finding was an association between CMV sero-positivity and high glycated hemoglobin and dyslipidemia in Iraqi female, elevated levels of HbA1c and non-fasting glucose in females infected with this virus for a long time. The suggested role for CMV infection in the pathogenesis of diabetes in females is that, CMV might be involved in accelerating pancreatic failure to compensate for insulin resistance via at least two possible mechanisms. First, it could influence the pancreatic cells directly, secondly, it might act indirectly by influencing the immune system which in turn affects the pancreas. Consistent with the first possibility is the report that CMV may infect and reside in pancreatic cells without causing cytopathic effects but nonetheless influencing insulin production directly after repeated reactivations (13).

Additionally, infection of human pancreatic β-cells with CMV induced the release of pro inflammatory cytokines and increased cellular immunogenicity (14).

The indirect effects of CMV could be exerted via infected monocyte production of IL-1β which induces TNF-α production in human pancreatic duct cells, driving cells into apoptosis and thus compromising β-cell function (15). Other components of the immune system, influenced by prolonged CMV infection, could hypothetically also contribute to a more pro-inflammatory environment, which is an important feature of diabetes (16).

Also the results of this analysis provided a novel insight into the relationship between common chronic sources of inflammation and metabolic abnormalities, previously reported, and obesity was significantly associated with Dyslipidemia and elevated HBA1C levels in females in this study.

This analysis is unique for its finding that CMV infection yielded significantly odds of Dyslipidemia in both females with normal weight and in those with extreme obesity (17).

One possible explanation for the significant magnitude of the association between CMV seropositivity and Dyslipidemia in normal-weight females is that the absence of obesity-induced inflammation provides a context for

which the pro inflammatory effects of CMV infection can become fully appreciable. In contrast, the inflammatory threshold required to trigger Dyslipidemia may already be met or even exceeded in females with overweight and obesity, where any additional sources of inflammation have minimal effect on influencing metabolic outcomes. (18)

Obesity is often accompanied by impaired immune responses, so it is possible that in the setting of excessive nutrient overload, CMV replication is significantly amplified, leading to substantial increases in cellular uptake of lipids necessary for the production of viral progeny, thereby resulting in lower amounts of circulating lipids available for measurement.

At the cellular level, CMV infection results in a dramatic increase in the number of glucose transporters present in the plasma membranes of infected cells in order to increase the uptake of glucose metabolites required to support intracellular viral activity and replication (19). Because of this, we initially anticipated the association between CMV and Dyslipidemia to be at least partly explained by alterations in fasting serum glucose levels.

In this analysis we found the younger diabetes females were less weight and less dyslipidemia than the older women and this finding are agree with (20),so there are no relation between weight and CMV seropositivity in females with diabetes, Interestingly, all these results given the significant associations between CMV and metabolic components in diabetic females.

In conclusion, this study demonstrated there was a significant association between cytomegalovirus and type 2 diabetes mellitus in Iraqi non married women, as well the current search founded a possible association between Diabetic dyslipidemia and chronic CMV infection in our population because of the strong correlation between high HBAIC and Dyslipidemia in seropositive CMV. This finding suggests that CMV infection might be a risk factor for the development of diabetes type 2 in women.

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