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RESEARCH ARTICLE

Association between HCMV and Some Procoaculations Factors in Aborted Women in AL- Najaf Province, Iraq.

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Abstract

This study has been conducted on seventy three patient's women suffering from abortion who have been checked Central Laboratory of Al-Hakeem Hospital in Al-Najaf city from November 2013 to April 2014. Their ages range from (14-44) years old .In addition, (20) healthy adults women, without any history of abortion or inflammatory diseases as control group. Blood samples have been withdrawn from abortion women a volume of 5 milliliters of venous blood, 0.9 milliliters have been anticoagulated with 0.1 milliliters sodium citrate and centrifuged at 3000 r.p.m for 15 minutes. The plasma has been aspirated to another test tube for storage in deep freeze (-20˚C) until analysis for precoagulate V factor, whereas serum sample was obtained from the rest of blood and stored at -20°C until processing. Serum concentration of anti-HCMV-IgG was measured by means of Enzyme Link Fluorescent Assay technique (ELFA). Serum concentration of procoagulant factor X activator (FXa) and factor V (F5) were measured through ELISA technique. The results have shown a significant relations of ($P<0.0001$) among anti-HCMV IgG antibody in the groups of patients women suffering from abortion compared to those of the healthy Control Group. HCMV-IgG is correlated positively with factor V (F5) ($p< 0.0001$), while CMV-IgG has shown a negative correlation with factor Xa (FXa) ($P< 0.0001$). It has been concluded that the serum level of anti HCMV-IgG antibody plays a major role in the diagnosis of patient with HCMV-aborted women also the changes in level of both factor V and factor Xa plays a major role in cases of abortion.

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INTRODUCTION

An abortion is the spontaneous or induced loss of early pregnancy. Early pregnancy refers to any pregnancy less than twenty weeks of gestation and can be defined as the inability of the fetus to survive outside of the uterus. The term miscarriage is often used to denote spontaneous abortion [1].

Human cytomegalovirus is important as it is one of the causes of abortion and in more cases congenital damage causes fetal death and mental retardation. The most common intrauterine infection associated with congenital CMV [2].

The common virus that can infect people of all ages is Human Cytomegalovirus (HCMV). When CMV enters the body it stays for life, but a healthy immune system keeps this virus in check. People who are infected with this virus will never have any symptoms

CMV can cause severe diseases in immunocompromised patients, either via reactivation of latent CMV infection or via gaining of primary CMV infection [3]. When women are infected with CMV, one of the smallest risks is miscarriage [4]. The virus is excreted through body fluids, and the most common modes of transmission are via the oro-pharyngeal and genital tract, taken in to consideration transmission can also occur through breast milk,

[5, 6]. One of the important factors in coagulation process is factor X activator (FXa) and Factor V (F5) [7]. The hypercoagulability may be due to counteract to instability associated with hemochorial placentation. Certain acquired thrombophilic factors may predispose to arterial and/or venous thrombosis and have a possible relation to pregnancy complications, including recurrent miscarriage (RM) [8].

Aims of Study: This study is aimed to investigate the role of HCMV in abortion through:

1-Investigating the role of CMV infection in aborted women throughout levels of CMV specific IgG antibody.

2- Finding out the relationship between CMV infections and coagulation factor X activated (FXa) and Coagulation factor V in pregnancy women.

3-Assessing the link between CMV infections with some variables such as: age, residency, number of abortions and some blood parameters.

2. Materials and Methods

2.1 Subjects and clinical assessment: Seventy five patients women suffering from abortion have been received by the Central Laboratory of Al-Hakeem hospital in Al-Najaf city from November 2013 to April 2014, their ages range from (14-44) years old. Control group is comprised of fifteen (20) healthy women without any history of abortion or inflammatory diseases. Blood samples were withdrawn from aborted women a volume of 5ml of venous blood, 0.9 ml was anticoagulated with 0.1 ml sodium citrate and centrifuged at 3000 r.p.m for 15 minutes, the plasma were aspirated to another test tube for storage in deep freeze (-20˚C) until analysis for precoagulate factor. The remaining of venous blood without anticoagulate was left to coagulated and the serum were aspirated to another glass test tube and centrifuged at 3000 r.p.m for 15 minutes and separated in glass tube in deep freeze (-20˚C) until analysis .

2.2 Study Design: The patient women classified according to the following criteria:

A- Ages of patient women:

A1: The patient women with ages (< 20) years. A2: The patient women with ages (21-25) years. A3: The patient women with ages (26-30) years. A4: The patient women with ages (31-35) years.

B- Numbers of Abortion:

N1: The patients with one abortion.

N2: The patients with more than one abortion.

C- Residency:

R: Rural. U: Urban

2.3 Methods

2.3.1 Measurement of serum VIDAS CMV IgG:

VIDAS is an automated quantitative enzyme immunoassay for use on the VIDAS family instruments for the quantitative measurement, of anti –cytomegalovirus IgG (CMVG) in human serum, using the technique ELFA (Enzyme Link Fluorescent Assay) kit (Assaypro, USA). The required reagents only were removed from the refrigerator and exposed them to the room temperature for at least 30 minutes. One "CMVG" strip and one SPR were used for each sample, control or calibrator to be tested the storage pouch has been carefully resealed after the required SPRs have been removed. The test is identified by the code "CMVG" on the instrument. The calibrator was identified by "S1", and tested in duplicate. The positive control was tested, it's identified by C1, the negative control was tested and it's identified by C2. The calibrator controls and samples were mixed by using vortex- type mixer (for serum separated from the pellet). The test portion was 100 µl from the calibrator, controls and samples. CMVG SPRs and CMVG strips were inserted into the instrument. The color labels were checked with the assay code on the SPRs and the reagent strips were matched. The entire assay steps are performed automatically by the instrument. The assay was completed within approximately 40 minutes. After the assay is completed, the SPRs and strips were removed from the instrument. SPRs and strips were disposed in to an appropriated recipient. Once the assay completed, results were analyzed automatically by the computer. Fluorescence was measured twice in the reagent strip's reading cuvette for each sample tested. The first reading was background reading of the substrate cuvette before the SPR was introduced in to the substrate. The second reading was taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) was calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

2-3-2 Serum Human Activated Coagulation Factor X (FXa) Estimation:

This assay executed with specific kit for test,was supplied by (Cusabio Biotech co., Ltd. USA-catalog NO.CSB-E12696H).

One hundred µl of standard, Blank, or was added per assigned well. Wells were covered with adhesive strip provided. Incubated for 2 hours at 37 ˚C. The liquid of each well was removed without wash. One hundred µl

of Biotin-antibody working solution was added to each well. Incubated for 1 hour at 37 ˚C. Biotin-antibody working solution may appear cloudy. It was warmed up to room temperature and mixed gently until solution appears uniform. Each well and wash was aspirated, repeating the process two times for a total of three washes. Wash: each well was filled with wash buffer (200 µl) and lifted it for 2 minutes, then the liquid was removed by flicking the plate over a sink. The remaining drops were removed by patting the plate on a paper towel. Complete removal of liquid at each step is essential to good performance. After the last wash, any remaining wash buffer was removed by aspirated or decanted. The plate was inverted and blotted it against clean paper towels. One hundred µl of HRP-avidin working solution was added to each well. The micro titer plate was covered with a new adhesive strip and Incubated for 1 hour at 37 ˚C. The aspiration and wash were repeated five times as step above ninety µl of TMB substrate was added to each well and incubated for 15-30 minutes at 37 ˚C. The plate was kept away from drafts and other temperature fluctuations in the dark. Fifty µl of stop solution was added to each well when the first four well containing the highest concentration of standard develop obvious blue color. If color change does not appear uniform, the plate is gently tapped to ensure thorough mixing. The optical density of each well was determined within 30 minutes,by using a microplate reader set at 450 nm.

2-3-3 Plasma Coagulation Factor V (F5) BioAssay ELISA Kit:

Wells were determined for diluted standard, blank and samples. Seven wells were prepared for standards, 1 well for the positive control and 1 well for blank. 100 µl of dilutions of standard, blank and samples were added into the appropriate wells, respectively,covered with a plate sealer and incubated for 2 hours at 37˚C. The liquid from each well was removed but not washed. One hundred µl of detection reagent A working solution was added to each well. Incubated for 1 hour at 37˚C after covered it with the plate sealer. The solution aspirated and washed each well for 1-2 minutes with 350 µl of 1x wash buffer to each well using a squirt bottle, multi-channel pipette, manifold dispenser or auto washer. The remaining liquid was completly removed from all wells completely by snapping the plate on to absorbent paper and washed a total of 3 times. After the last wash, any remaining wash buffer was removed by aspirating or decanting. The plate inverted and blots it against absorbent paper . One hundred µl of detection reagent B working solution added to each well and incubated for 30 minutes at 37˚C after covering it with the plate sealer . The aspiration/wash process repeated for total 5 times as conducted in step above. Ninety µl of substrate solution was added to each well. Covered with a new plate sealer. Incubated for 15-25 minutes at 37 ˚C (not exceeded 30 minutes). Protected from light. The liquid turned blue by adding Substrate Solution. Fifty µl of sop solution was added to each well. The liquid turned yellow on addition of stop solution. The liquid was mixed by tapping the side of the plate gently tapping the plate to ensure through mixing incase the color does not appear. Any drop of water and fingerprint on the bottom of the plate was removed to confirm there are no bubbles on the surface of the liquid. Read the absorbance at 450 nm immediately.

2-4 Statistical analysis:

Data analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). All of the descriptive variables were expressed as the mean ±SE (standard error). The correlations between the concentrations of HCMV-IgG and Coagulation Factors were tested by using Pearson's correlation test.

The group analyses were performed by using one-way ANOVA and Tukey's post-hoc analyses. For all tests, a p value less than 0.001 was considered statistically significant.

3- Results

3-1 Detection of CMV- IgG antibody:

The results revealed significant increase ($P< 0.0001$) in the specific anti-CMV IgG-antibody in the groups of patient's women suffering from abortion compared to healthy control group. However it's less significant in A4 and N1 (Figure 1).

3-2 Coagulate Factors:

3-2-1 Factor V: The study showed a higher significant increase ($p< 0.0001$) in the level of FV in all groups of patients suffering from abortion than the healthy control group (Figure 2).

3-2-2 Factor X: Statistically significant decrease in Factor X was found in the groups of patients suffering from abortion comparison to healthy control group ($P< 0.0001$) (Figure 3).

3-3 Relationships of CMV-IgG Levels to Coagulation Factors: CMV-IgG was correlated positively with factor V (F5) ($r = 0.917$, $p< 0.0001$), while CMV-IgG had negative correlation with factor Xa (FXa) ($r= - 0.494$, $P< 0.0001$) (Figure 4).

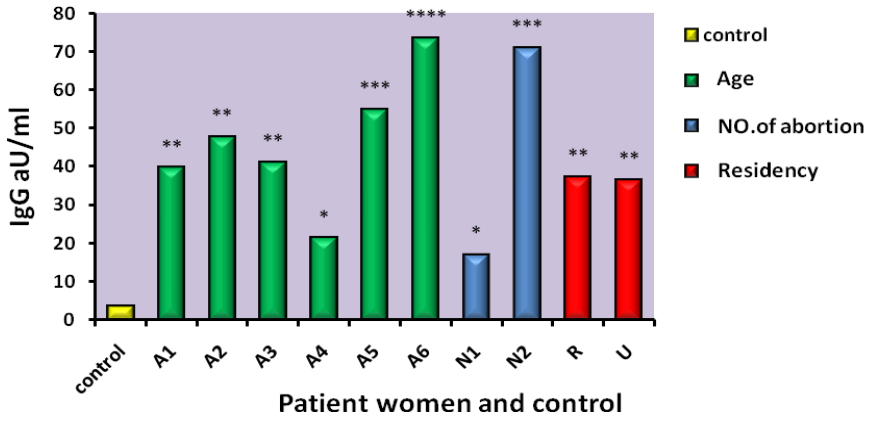

Figure 1: Anti CMV IgG antibody in all groups compared with healthy control group.
Data are expressed as means ± standard error (SE).
*** indicate significant difference based on Tukey's multiple comparison tests.
P< 0.0001.

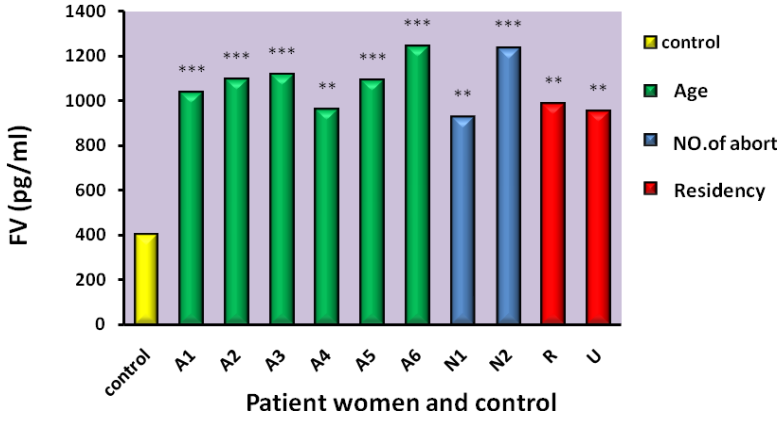

Figure 2: Level of FV in all studied groups of the patients compared with healthy control group.
Data are expressed as means ± standard error (SE).
*** indicate significant difference based on Tukey's multiple comparison tests.
P< 0.0001.

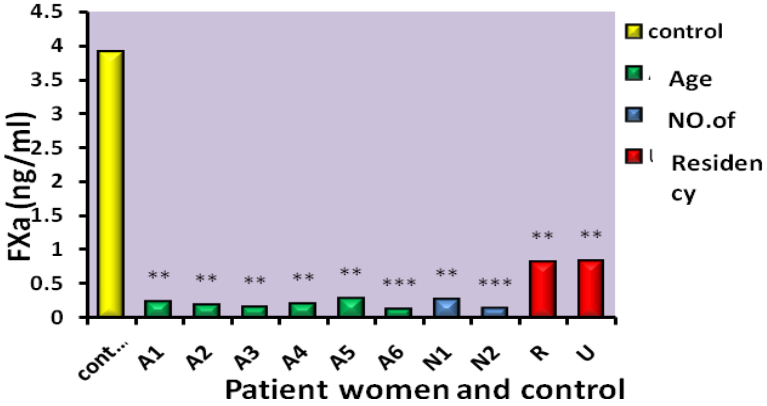

Figure 3: Level of FXa in all studied groups of the patients compared with healthy control group.
Data are expressed as means ± standard error (SE).
*** indicate significant difference based on Tukey's multiple comparison tests. P< 0.0001.

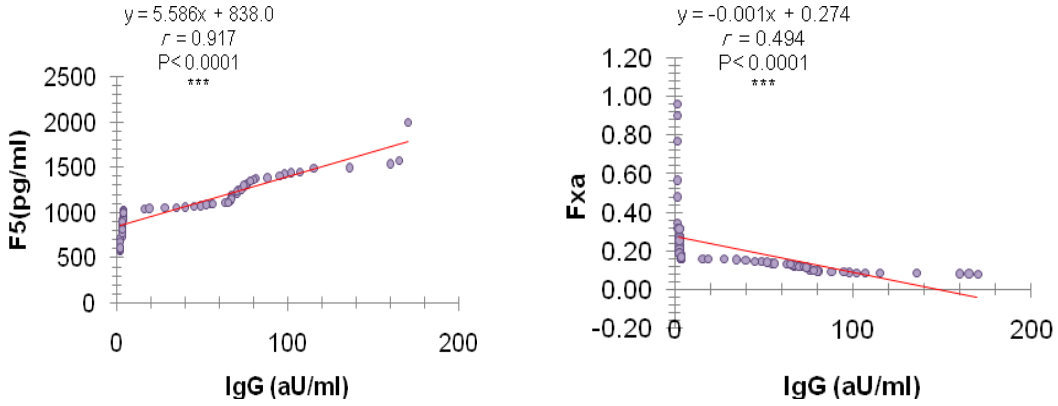

Figure 4: The correlation between CMV-IgG and coagulate factors (F5, FXa).

4-Discussion

In the present study of abortion, several surrogate markers were used for identifying the causing agents. Laboratory approach depends on detection of specific anti- HCMV-IgG by techniques, and evaluates the coagulation factors, Factor V (F5) and Factor X activator (FXa).

Dealing with some coagulation factors to indicate that the coagulation Factors (F5) and (FXa) play a major role in the increase or decrease in both possible lead to abortions because of the presence of the HCMV.

The disparities between the data of this study and the results previously reported may be explained by a number of factors, including numbers of abortion, ages, residence and coagulation factors (FXa and F5).

4-1 Detection of Specific HCMV-IgG: In conclusion diagnosis of primary maternal HCMV infection in pregnancy should be based on presence of virus-specific IgG in the serum of a pregnant woman who was previously seronegative, the diagnosis of secondary infection should be based on a significant increase of IgG antibody titer with or without the presence of IgM and high IgG avidity [9].

HCMV IgG antibodies among the patients which studied were significantly higher than control group. The high prevalence of IgG seropositive was probably due to cumulative effect of previous infection; reactivation or new infection lead to high seropositivly. A positive test for CMV IgG indicates that a person may be was infected with CMV at some time during their life but the IgG test cannot determine when a person was infected [10].

The present study reveals a significant increase of specific anti HCMV-IgG antibody in patients groups compared with healthy control group, and this result agrees with study of [11,] that referred to high levels of specific IgG-HCMV. The anti-specific HCMV-IgG antibody showed statistically significant increase between groups (A-1, 2, 3, 4, 5, and 6) and showed statistically significant increase between number of abortion groups (N1, N2). The titer of IgG is higher at age more than thirty and this indicates that cytomegalovirus infection is usually latent with potential for reactivation. HCMV may be transmitted horizontally as a result of organ donation, blood transfusion, sexual contact, and contact with infected saliva and urine. The high prevalence of IgG seropositive was probably due to cumulative effect of previous infection; reactivation or new infection lead to high percentage of seropositivity, a positive test for CMV IgG indicates that a person may be infected with CMV at some time during their life but the IgG test cannot determine when a person was infected [10].

The study performed by [12], find the highest percentage rates of specific HCMV- IgG seropositivity as the observation in his study may indicate the previous exposure of the tested women and now they are have immune against HCMV. Similar conclusions were reported by other researches in 2006 who noticed that most of the tested women were had immune against primary CMV infection and these results suggested that latent CMV infection predisposes to adverse pregnancy outcomes [13].

The results of the current study complies with [14] when they show specific HCMV-IgG positive results which were considered to be possibly infected with HCMV during the current pregnancy or a chronic infection

which can be confirmed by IgG avidity test because antibody binds to the antigen with less avidity during acute infection than chronic infection.

In this study, a significant association was found between the age of pregnant women and HCMV seropositivity. Majority of HCMV positive women were above 36 years of age they became more susceptible to perhaps due to a weakened immune system. According to this finding, pregnant woman above 36 years of age were at higher risk of HCMV infection, the present study agreed with study by Ross and her colleagues as they noted that HCMV is the most common viral infection worldwide and among different age groups including both sexes [15].

As for the residence group noted there are significant differences in HCMV- IgG compared with control group This study is similar to the observed by [16] those found that the specific HCMV IgG among tested women according to their residence from 132 women from urban residency 120 (91%) were positive for specific HCMV-IgG antibodies and 53 women from rural residency 47(89%) were positive for CMV-IgG antibodies.

4-2 Levels of Factor V and Xa: Serum levels of factor V were significantly elevated in all groups of patient with aborted women compared with healthy control group while level of factor Xa were significantly high decreased in all groups compared with control.

Cytomegalovirus is associated with hypercoagulability, reported to increase the risk of venous thrombosis, and may result in more morbidity, such as reactivation of cytomegalovirus. Our aim was to determine whether procoagulant abnormalities were associated with active CMV infection, these findings agree with study of [17] that reported the congenital FXII deficiency is strongly associated with recurrent spontaneous abortion.

Reported [18] That the deficiency of XII lead to deficiency FXa in the intrinsic pathway of coagulation process ,and that the Factor XII (Hageman Factor) deficiency indicator risk factor for pregnancy loss, in woman with a history of recurrent miscarriage, ,

Reported [19] that the procoagulant abnormalities were associated with active cytomegalovirus infection in these experiments nearly l09 virions /ml were found to decrease the FXa clotting times from 92 seconds ,and found that the active cytomegalovirus infection was associated with a procoagulant state. Thus active cytomegalovirus infection was more commonly found in patients with abortion.

It also corresponds well with the findings of another study showing that active cytomegalovirus infection is associated with enhanced factor V [20].

Reported [21] that the severe bleeding disorder abortion is caused by an absence of functional coagulation factor X and demonstrated that the injection mice and dogs, by using the cytomegalovirus (CMV) immediate early (IE) inhibited the levels of factor X expression in muscle (for at least 2 years).

4-3 Relation of HCMV-IgG Antibody to FV and FXa:

1- Factor V (F5): The present study revealed significant positive correlation between factor V and both specific HCMV-IgG.

Herpesviruses, particularly human cytomegalovirus (HCMV), can infect endothelial cells and directly damage intact vascular endothelium, altering its thrombo resistant surface as a result of procoagulant activity mediated by specific viral antigen, and necessary for the coagulation enzyme complex assembly that leads to thrombin generation [22].

Demonstrated [20] that the factor V and fibrinogen levels can be seen to be significantly higher in patients with active HCMV infection, also observed higher levels of anticoagulant factors at the time of active cytomegalovirus infection.

2- Factor X Activator (FXa): The present study showed negative significant correlation between factor X and HCMV-IgG as in figures (4-11, 4-14) is indicated.

The present study complies with [17] that reported a negative correlation between FXII activity and the number of abortions in the recurrent spontaneous abortion group.

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