Clinical and Immunological Study of Human Herpes Simplex Virus; CD 56 in Female Suffering from Recurrent Abortion

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Abstract

Background: Recurrent abortion (R A) is repeated termination of pre gnancy by elimination of products of conception and embryo with immunologic causes. Natural killer (NK) cells may responsible for immune-modulation in maternal decidua and the trophoblast. Herpes simplex virus 2(HSV-2) is the major sexual transmitted viruses cause of recurrent abortion.

Objective: Investigate the association between CD 56 and theti risk of recurrent abortion among Iraqi women patients.

Materials and Methods: Polymerase chain reaction was performed to detect HSV2 and evaluation of serumCD56 concentration by ELISA kit for all specimen (one hundred-fifty) from healthy and aborted woman

Results: Significant variation detected in distribution frequencies of serum CD56 concentration between case and healthy groups.

Conclusion: As results of current study, CD56 may be considered as a protected factor from recurrent abortion among Iraqi women.

Keywords: Recurrent abortion, CD56, uterine natural killer cells, ELISA, HSV2.

1. introduction

Modern trends in clinical medicine are determined by progression of infectious diseases where pregnancy can induces immunologic alterations which play a key role in susceptibility to danger of intrauterine viral infections with HSV-2 of pregnant women that may lead to recurrent abortion [1].

The primary infection by HSV-2 of woman with pregnancy may be associated with abortion and reactivation through immuno-depression with progesterone that lead to repeated HSV2 infections in the genital tract and cause recurrent abortion [2].

The CD56 is a homophilic binding glycoprotein that has role in cellular interaction between natural killer cells, CD4+ and CD8+ T lymphocytes as well as on dendritic cells [3].

In normal pregnancy CD56+ dNK cells increase the expression of IFNγ and mRNAs trophoblast cells and unbalanced activation of decidual NK might cause impairment of interaction between decidual NK and trophoblast cells during recurrent abortion [4].

NK cell CD56 could be used as a special predictive biomarker of RA [5].

2. Subjects and Methods

Polymerase chain reaction was performed to detect HSV2 and evaluation of serumCD56 concentration by ELISA kit for all specimen (one hundred-fifty) from healthy and aborted woman from hospitals of Middle Euphrates -Iraq. The collection of sample from February 2020 to September 2021.

Herpes simplex virus -2 detection by PCR

By using Patho Gene-spinTM DNA Extraction Kit (Intron /Korea); genome was purifying and migrated using gel (agarose) from the endometrium; swab from cervix;fetal fluid for amplify the Herpes virus DNA.

Program of PCR: Denaturation 95°C- 3 mi; Denaturation 95°C - 30 sec;Annealing 58.3 °C -30 sec; Extension 72°C - 20 sec; Final extension 72 °C for 5 min: for 35 cycles

HSV2-gpG migrating on agarose gel at (75V) for 1h and visualized by special dye; gel was photographed with (Cleaver- Scientific - UK).

Evaluation of CD56 Concentration in Blood Serum of Patients and AHC

The concentration of CD56 in the serum of female patients with recurrent abortion were evaluated by enzyme linked immunosorbent assay (ELISA)with Human CD56 ELISA kit after preparing of reagents, standard solutions and samples according to the guideline information, all reagents were keeping at room temperature before usage because the assay was performed at same temperature. The number of the strips were determined then inserted in the frame. The unused strips should be stored at 2-8°C. 3. Add Fifty µl standard to standard well. And 40µl sample to sample wells and then add 10µl anti-IL-10 antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells .Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately. Then determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Statistical analyses were performed using GraphPad Prism version 3.06. Standard deviations are plotted as error bars for the data points on all figures Two way ANOVA, One-way ANOVA, and Chi square (X2) were done to establish relationships of expression immunological variable levels according to the ELISA test results between women with and without clinical abortion. The correlation matrix between the selected variable and HSV2 infection in current study was estimated by using Spearman's correlation coefficient analysis. Correlation coefficients were considered significant at P values less than 0.05 by using using GraphPad Prism version 3.06. Asterisk (*) indicates that the differences was statistically significant

when compared with control group with patient groups. Chi square test (X2)was used to compare the selected groups ** p *** ,p<0.001

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RESULTS:

Detection of HHV-2 –DNA By PCR:

The P C R for the 40 samples with HSV—2 ,the (45 %) of the samples with herpes simplex virus -2 infection while 55% as not infected , (T able 1 ; Figures 1) .

Table-1 Percentage of HHV-2 Positive Signals in Women Patients with RPL by Using PCR Technique.

Total Viral genome	No.	%	Chi-Square (P-value)
Positive	18	45	*P=0.02 sign. (P>0.05)*
Negative	22	55	
Total	40	100	

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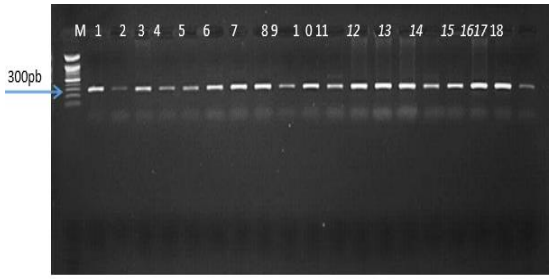

Figure 1: Detection of HSV-2 by PCR.

Evaluation of Serum CD56 concentration By ELISA among Study Population:

Table (2) was showed the concentration of serum CD56 were detected by ELISA technique. The mean of serum CD56 concentration in aborted women and apparently healthy control were 15.78 ±7.02 pg/ml and 10.74± 4.5 pg./ml, respectively.Significant difference (p=.03) was found by comparing the mean of serum CD56 concentration among AHC and women with RA groups

Table 2: Results of serum CD56 concentration by ELISA for AHC and women patients with RA

Immune Variables	AHC (Normal pregnancy) (pg/ml)	Women with RPL (pg/ml)
CD56	10.74± 4.5	15.78 ±7.02
P value	*P<0.05*	

Spearman's rho statistical testing to evaluate studied molecular markers in relation with HSV2 infections in women with clinical abortion

There is a strong positive relationship (with highly significant correlation) between HSV-2 and CD56 {r = 0. 422, P = 0.007 (p <0.01)}

Table 3 : Spearman's rho statistical testing to evaluate studied molecular markers in relation with HSV-2 infections in women with clinical abortion

Spearman's rho		HSV-2
CD56	r	.422**
	P-value	.007

**Correlation is highly significant (P<0.01).

3. Discussion

Among samples taken from endometrium; fetal fluids and Blood specimens of spontaneous aborted female patients were found to have 45 % (18 out of 40 cases) with HSV-2, while 55% (22 out of 40 cases) as negative of control healthy group.

The present results are consistent with those reported world-wide where as in India with high percentage 30.1% of HSV2 infection in woman with repeated pregnancy loss [6]

In other recent studies in Iraq: Babylon done by Borhani et al. [7] which revealed that the high rate 37.1% of HSV2 infection in aborted woman.

Moreover

our results were higher in comparison to study has showed a percentage of 2.6% [8].

In other recent survaey in Iraq: Baghdad done by which found that the percentage of HSV percentage (18.9 %) in repeated pregnancy loss woman, as well as in Iraq Al-Kufa the HSV-2 rate only15 % in aborted woman patients [10].

Our finding are inconsistent with those reported world-wide where as in Iran study showed DNA only in 2-.8% by PCR technique of Abortions. This could no significant variation among case and control groups [7].

Moreover, previous survey with HSV-2 in (decidua) samples with percentage (9.0%) and only (3.5%) on the fetal side were positive for HSV-2. [11].

4. Conclusions

Herpes simplex virus -2 was detected from

In woman with recurrent pregnancy loss and pregnant women (HSV—2) in noticeable level. The presence HSV--2 in women suffering repeated abortion was higher than woman with pregnant, this indicate of this infectious virus as cause recurrent abortion.

5. References

1. Shi T-l, Huang L-J, Xiong Y-Q, Zhong Y-Y, Yang J-J, Fu T, Lei X-F, Chen Q. The risk of herpes simplex virus and human cytomegalovirus infection during pregnancy upon adverse pregnancy outcomes: A meta-analysis. Journal of Clinical Virology. 2018;104:48-55. https://doi.org/10.1016/j.jcv.2018.04.016

2. Crimi S, Fiorillo L, Bianchi A, D'Amico C, Amoroso G, Gorassini F, Mastroieni R, Marino S, Scoglio C, Catalano F. Herpes virus, oral clinical signs and QoL: systematic review of recent data. Viruses. 2019;11(5):463. https://doi.org/10.3390/v11050463

3. Van Acker H, Capsomidis A, Smits E, Van Tendeloo V. CD56 in the immune system: more than a marker for cytotoxicity? Front Immunol. 2017; 8: 892. 2017.

4. D'Ippolito S, Ticconi C, Tersigni C, Garofalo S, Martino C, Lanzone A, Scambia G, Di Simone N. The pathogenic role of autoantibodies in recurrent pregnancy loss. American Journal of Reproductive Immunology. 2020;83(1):e13200. https://doi.org/10.1111/aji.13200

5. Yang J, Chen M, Ye X, Chen F, Li Y, Li N, Wu W, Sun J. A cross-sectional survey of pregnant women's knowledge of chromosomal aneuploidy and microdeletion and microduplication syndromes. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2021;256:82-90. https://doi.org/10.1016/j.ejogrb.2020.10.001

6. Tiwari S, Arora BS, Diwan R. TORCH IgM

seroprevalence in women with abortions as adverse reproductive outcome in current pregnancy. International Journal of Research in Medical Sciences. 2016;4(3):784-8.
7. Borhani MS, Hosseini SM, Tabrizi LC, Bagheri R, Kamali K, Aarabi M, Habibi M, Kamalgharibi A, Borhani MS. PCR Detection of Herpes Simplex Virus in Human Placenta and Aborted Materials in Patients with Spontaneous Abortion. Archives of Clinical Infectious Diseases. 2011;6(Suppl):17-20. Available from: https://brieflands.com/articles/archcid-76652.html
8. Obaid HM, Juma SA. TORCH screening test in pregnant women of Kirkuk city. Al-Mustansiriyah Journal of Science. 2016;27(5):17-25. https://doi.org/10.23851/mjs.v27i5.162
9. Hassan JS, Hana DB, Hassan FG, Al-Marsome HT. PCR Detection of Herpes Simplex-2 Virus in Human ssss Placenta in Patients with Spontaneous Abortion. Available from: https://www.uomus.edu.iq/lecture/ART20162246 %20 %20مع20%مشترك20%بحث20%ج.pdf
10. Mezher MN, Dakhil AS, Abdul-Jawad DH. Role of Epstein-Barr virus (EBV) in human females with breast cancer. Journal of Pharmaceutical Sciences and Research. 2017;9(7):1173. Available from: https://www.researchgate.net/publication/318780450
11. Finger-Jardim F, Teixeira LO, De Oliveira GR, Barral MFM, Da Hora VP, Gonçalves CV, Soares MA, De Martinez AMB. Herpes simplex virus: prevalence in placental tissue and incidence in neonatal cord blood samples. Journal of Medical Virology. 2014;86(3):519-24. https://doi.org/10.1002/jmv.23817