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Assessments of Sero-Detection of Herpes Simplex *virus*-1 and 2 Antibodies IgG and IgM among Spontaneous Recurrent Miscarriage Women; Case Control Study in Gezira State 2018

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Abstract: ***Background: Recurrent miscarriage is a critical problem and it takes to increase during current decade, herpes simplex virus-1 and 2 have relation with recurrent miscarriage according to some previous study conducted in many countries. Objectives: The aim of present study to Sero-detection of herpes simplex virus -1 and 2 (IgG and IgM antibodies) by using ELISA techniques among women with recurrent miscarriage in Gezira state and assessment of other risk factors according to questionnaire. Method: Analytic - case control study (45 women in each arm) was conducted at Wad Madani teaching hospital Department of Obstetrics gynecological, AlGezira state, Sudan. The cases were women with recurrent miscarriage and controls were healthy pregnant women (non-miscarriage).Herpes simplex virus1and 2antibodies were analyzed in the sera of the entire participant using ELISA techniques. Results: Ninety women were enrolled in each arm of study. Miscarriage and non miscarriage serum IgG sero-positivity for HSV-1 44 (97.1%) vs 41 (91.1%) and HSV-2 is 13 (28.9%) vs 4 (8.9%) by ELISA. There was no significant difference in miscarriage serum IgM sero-positivity for HSV-1 16 (35.6%) vs 6 (13.3%) and borderline 2 (2.2%) vs 2 (4.4%) by ELISA. In logistic regression analysis of the predictors for miscarriage (OR=2.047, 95%CI=.179-23.4.57, P.value 0.04).IgM sero-positivity were at risk for miscarriage. Other significant risk factors include microcytic hypochromic anemia, vaginal bleeding, pre-eclampsia and family history. Conclusion: In the current study herpes simplex virus -1 and 2 IgG sero-positivity is associated with miscarriage. Using ELISA techniques are presumptive tools to confirm the results. Preventive measure should be implemented. Further research in needed.***

Keywords: HSV-1 and 2, antibodies, recurrent miscarriage women, ELISA.

1. Introduction

Miscarriages or Spontaneous abortion (SA) is defined as the loss of fetal product before 20 weeks of gestation [1]. Miscarriage is common occurring in about 25 % of pregnancies, usually in the first 12 week of pregnancy .Ten percent to 15% of clinically recognized pregnancies end Recurrent in SA and total pregnancy loss is estimated to 30% to 50% of all conception [2]. Various effectors associated with spontaneous abortion such as Genetic and uterine anomalies, Endocrinopathy, immunological dysfunctions, infectious agents, environmental contaminants, psychogenetic elements and endometriosis. Maternal infections considered the main reason of pregnancy wastage in females with Bad Obstetric History [3]. Risk factors for miscarriage include an older parent previous miscarriage exposure to tobacco smoke, obesity, diabetes, and drug or alcohol use among others. In those under the age of 35 the risk is about 10% while it is about 45% in those over the age of 40 [4].

Herpes simplex virus (HSV) is an ubiquitous enveloped and double stranded DNA virus, belonging to the family of Herpesviridae transmitted across mucosal membranes and no intact skin that migrate to nerve tissues where they persist in a latent state. HSV-1 predominates in orofacial lesions, and it is typically found in the trigeminal ganglia [5].Whereas HSV-2 spread across epithelial mucosa, skin interruptions. Then, it transfers to nervous tissues indeed lumbosacral ganglia to initiate a latent infection [6]**.** High percentage of genital herpes infections are causing by HSV-2.Females during reproductive age become under high risk for exposure to occurrence of HSV-2 infection with a possible of transmission to the embryo in pregnancies. Prior to the 20th weeks of gestation, intrauterine transmission of HSV-2 lead to abortion, stillbirth and congenital abnormalities in live fetus [**7**]. HSV-1 is the main cause of oral herpes, including acute herpetic gingivostomatitis (mostly as primary infection) and herpes labialis (recurrent or secondary infection). However HSV-1 has emerged increasingly frequently as an agent in genital herpes in some populations and this may have an impact on acquisition of HSV infection in pregnancy [8]. Both primary and secondary genital HSV-1 and 2 infections may develop during pregnancy with potentially severe consequences to the fetus or neonate if transmitted to the embryo or fetus these infectious agents may cause rarely early embryonic or fetal damage with or without miscarriage or major congenital or developmental anomalies [9]. Some study that indicate HSV-1 gingivostomatitis in the first and second and third trimester of pregnancy is associated with adverse fetal effects.[10].Turkey asymptomatic pregnant women who were admitted to Gynecology and Obstetrics clinics of Izmir Ataturk Research and Training Hospital for routine control were investigated for IgG and IgM antibodies specific for HSV-1 and HSV-2 were screened by commercial ELISA Total IgG seropositivity rates for HSV-1 were found as

94.7% (108/114), while IgM seropositivities were 0 (0/114) [11].

2. Materials and Methods

A case- control study was conducted at Wad Madani teaching hospital Department of Obstetrics gynecological, Al-Gezira state, Sudan during the period of July-October 2018. A sample of 90 women in each arm of the study has over 80% power to detect a difference of 5% at α = 0.05. We assumed that 10% of the women might have incomplete data or samples. A volume of 5 ml blood samples were collected from each patient through venipuncture technique then displaced into plain container, allowed to clot, centrifuged and kept at -20 until serological analysesa tthe Central Research laboratory (the place where have done your work).

Complete blood count calculated by using hematological analyzer (Sysmex–XP 300) Manfacture Company, The three main physical technology used in it, direct current impedance, advanced optical light scatter technology, flourscent flow cytometry and spectrophotometry. These are used in combination with chemical reagent that lyes or alter blood cell to extend the measurable parameters. Wide range of test was done by it.

The specimens will be analyzed for detection of HSV-1 IgG and IgM and HSV-2 IgG antibodies by commercially available enzyme–linked immune sorbent assay HSV-1 IgG and IgM and HVS-2 IgG ELISA" kit chemux Bioscience, INC America this company can used or Euro immune. The tests were performed as instructed by the manufacturer. The reagents have positive and negative controls were already used solution that specific for HSV-1 and 2. Results Of cut-off of HSV-1and 2 index more than 1.0 IU\ml considered as positive result and cut-off of HSV-1 and 2 indexes less than 1.0 IU\ml considered as negative result.

Compact automated immunoassay system based on the Enzyme linked. Reagent for the assay is ready to use and predispensed in sealed reagent strips. All of the assay steps are preformed automatically by the instrument.

3. Statistics Analysis

The collected data were analysed using SPSS and double checked before analysis. Means and proportions of the socio-demographic and clinical characteristics were calculated for HSV-1and 2 sero-positive groups. Univariate and multivariate analysis were used for HSV-1 and 2 IgG and IgM seropositive groups as dependent variable and, socio-demographic and obstetrics variables as independent variables. Odds ratio OR with 95% confidence interval was calculated and statistical significance was defined as P value <0.05.

4. Results

Forty five women were enrolled in each arm of study. Socio-demographical and clinical characteristic of case and control in Al-Gaze era Hospital with P value (95% confidence interval) that was significant difference in the age (30.89 ± 0.9504 vs. 26.02 ± 0.8531 P=0.0003"-7.409 to -2.324"), biomax index (27.85 ± 0.5751 vs. 25.66 ± 0.6089 P=0.0104"-3.860 to -0.5250"), MCV (84.22 ± 1.010 vs 90.72 ± 1.057 p= 0.0001 "3.590 to 9.410"), MCHC (33.16 ± 0.3316 vs 31.91 ± 0.3579 p= 0.0125 "-2.216 to -0.2733"), MPV (9.593 ± 0.2327 vs 8.687 ± 0.1015 p= 0.0006 "-1.412 to -0.4012"), RDWCV (14.59 ± 0.3397 vs 15.88 ± 0.2821p= 0.0044 "0.4121 to 2.170"), RWDSD (44.98 ± 0.8974 vs 52.48 ± 0.8195 p= 0.0001 "5.078 to 9.917") while there was no significant difference between case and control include RBCs (3.843 ± 0.1349 vs 10.65 ± 6.849 p= 0.3235 "-6.836 to 20.44") as shown in Table 1.

Forty five women were enrolled in each arm of study. Sero-detection of IgG and IgM antibodies by using ELISA techniques, total of 45 miscarriage women (cases) for IgM 16 (35.6%) positive and 27 (60.0%) negative and 2 (4.4%) border line for HSV-1 by using ELISA techniques. A total of 45 non-miscarriage women (control) for IgM 6 (13.3%) positive and, 37 (82.2%) negative, 2 (4.4%) Borderline For HSV-1 by using ELISA techniques. Total of 45 miscarriage women (cases) for IgG 44 (97.7%) positive, 1 (2.2%) negative .And IgG for non-miscarriage 41 (91.1%) positive, 4 (8.9%) negative by ELIAS techniques.

Total of 45 miscarriage women (cases) for IgG of HSV-2 13 (28.9) positive and 32 (71.1%) negative.And IgG for non-miscarriage 4 (8.9%) positive, 41 (91.1%) negative HSV-2 by using ELISA techniques) Table1

Predicator's factors for miscarriage

Univariate and Multivariate analysis showed that preeclampsia, microcytic hypo- chromic anemia, vaginal bleeding, and menstruation cycle and biomass index were significantly associated with miscarriage in both univariate and multivariate. While diabetic patient, age, and family history were significant associated with miscarriage in univariate analysis table (3)

Table 1: Socio-demographical and clinical characteristic of case and control in Al-Gazeera Hospital

No	Items	Control N=45 Mean ± SEM	Case N=45 Mean ± SEM	P value (95% confidence interval)
1	Age	26.02 ± 0.8531	30.89 ± 0.9504	0.0003"-7.409 to -2.324"
2	Biomass index	25.66 ± 0.6089	27.85 ± 0.5751	0.0104"-3.860 to -0.5250"
3	RBCs	10.65 ± 6.849	3.843 ± 0.1349	0.3235 "-6.836 to 20.44"
4	Hb	10.93 ± 0.2420	10.58 ± 0.3481	0.4187 "-0.4995 to 1.188"
5	TWBCs	9.109 ± 0.4661	7.907 ± 1.214	0.3577 "-1.386 to 3.790"
6	Platelates	251.7 ± 12.61	243.8 ± 14.61	0.6803 "-30.43 to 46.39"
7	PCV	33.94 ± 0.6871	31.84 ± 1.053	0.0984 "-0.4025 to 4.602"
8	MCV	90.72 ± 1.057	84.22 ± 1.010	0.0001 "3.590 to 9.410"
9	MCH	29.00 ± 0.5027	28.11 ± 0.5391	0.2311 "-0.5784 to 2.356"

10	MCHC	31.91 ± 0.3579	33.16 ± 0.3316	0.0125 "-2.216 to -0.2733"
11	MPV	8.687 ± 0.1015	9.593 ± 0.2327	0.0006 "-1.412 to -0.4012"
12	PCT	0.2115 ± 0.01113	0.2579 ± 0.03219	0.1762 "-0.1143 to 0.02136"
13	RDWCV	15.88 ± 0.2821	14.59 ± 0.3397	0.0044 "0.4121 to 2.170"
14	RWDSD	52.48 ± 0.8195	44.98 ± 0.8974	0.0001 "5.078 to 9.917"
15	Neutrophil	65.34 ± 1.864	66.43 ± 1.908	0.6829 "-6.403 to 4.216"
16	Lymphocyte	27.42 ± 1.617	32.19 ± 4.928	0.3599"-15.10 to 5.551"
17	Monocyte	4.627 ± 0.3153	5.324 ± 0.3098	0.1180 "-1.578 to 0.1822"
18	Eosinophil	2.553 ± 0.1767	2.267 ± 0.14	0.2108 "-0.1660 to 0.7394"
19	Basophil	00.00	00.00	Constant
20	Anti HSV-1IgG	"1.956± 0.03871"	"2.435± 0.03791"	0.0001"-0.5822 to -0.3754"
21	Anti HSV-1IgM*	"0.08998± 0.006309"	"0.1157± 0.01427"	0.3134""-0.05806 to 0.006592"

Table 2: Assessment of Sero-detection of HSV 1and 2 IgM **dna**IgG antibodies of by using ELISA

Item	Types of the viruses	IgM ELISA			IgG ELISA	
	HSV1	Positive	Borderline	Negative	Positive	Negative
Miscarriage	45	16 (35.6%	2 (4.4%)	27 (60.0%)	44 (97.7%)	1 (2.2%)
No Miscarriage	45	6 (13.3%)	2 (4.4%)	37 (82.2%)	41 (91.1%)	4 (8.9%)
Total	90	22 (24.4%)	4 (4.4%)	64 (71.1%)	84 (93.3%)	5 (5.6%)
	HSV2					
Miscarriage	45	Nil	Nil	Nil	13 (28.9%)	32 (71.1%)
No Miscarriage	45	Nil	Nil	Nil	4 (8.9%)	41 (91.1%)
Total	90				17 (18.9%)	73 (81.1%)

Table 3: Logistic regression analyses of the predictors for Miscarriage

		Univariate			Multivariate		
NO	Variable	OR	95% CI	P value	OR	95% CI	P value
1	Tribes	0.93	.865-1.004	0.065	1.000	0.000-1.000	1.000
2	Education	1.107	.698-1.756	0.667	2.639	0.369-18.859	0.333
3	Jobs	.712	.420-1.206	0.206	1.000	0.000-1.000	1.000
4	Rate of Miscarriage	.000	0.000-0.000	0.993	1.000E-013	0.000-1.000	0.998734
5	Family history	2.94	2.946-948	.000	1.000	0.000-1.000	1.000
6	Menstruation Cycle	3.775	1.2-11.5	0.02	2.59	0.078-8.61	0.028
7	Vaginal disease	0.230	0.211-1.453	0.230	0.689	0.239-1.987	.491
8	Vaginal Bleeding	6.353	2.1-19.2	.001	1.39	.043-4.47	0.001
9	normochromic anemia	0.29	0.030-2.723	0.1	0.554	0170-1.801	.326
10	macrocytic anemia	2.1	0.723-5.846	0.176	0.554	0.170-1.801	.326
11	Microcytic hypochromic anemia	11	1.086-110.2	0.04	2.9	1.3-6.7	.000
12	Sero-positivity of Anti-HSV1IgG *	1.333	0.214- 8.288	0.03	0.288	0.023-3.567	0.332
13	Sero-positivity of Anti-HSV1IgM *	2.047	0.179-23.4	0.04	0.609	0.067-5.525	0.660
16	MMR vaccine	0.389	0.130-1.166	0.1	3.919	0.758-20.268	0.103
17	Tetanus vaccine	9.649E8	0.000-1.166	1.1	1.7	.000-.000	0.997
18	all the vaccine MMR+TT	0.339.	0.109-1.058	0.1	3.375	0.845-13.473	.085
19	Diabetic patient	11.1	11-11.38	.000	10	0.10-10.3	0.476
1	Thyroid	8.9	8.1-8.9	000	8.739E-008	8.739E-8.739E-	0.476
16	Hypertension	1.08	0.065-17.8	0.96	0.972	0.057-15.741	0.951
17	Preeclampsia	16.1	1.9-131.1	0.01	2.983E-009	1.314E-010-6.776E-008	0.000
18	Blood group	0.000	.010-1.722	0.1	7.2	5.4-8.42	0.997
19	Age	5	2-13	0.001	.336	.090-1.250	.104
20	Biomass index	5	2-12	0.001	1.73	0.062-4.79	0.001
21	HB	1.3	0.56-3.1	0.4	0.574	0.126-2.615	.473
22	RBCs	2.3	0.85-6.2	0.1	0.494	0.156-1.564	0.230
23	Platelets	0.7	0.21-2.2	0.52	1.208	0.343-4.251	.768
24	TWBCS	0.7	0.254-1.97	0.5	1.928	0.635-5.855	.246
25	Vaccination	0.6	0.23-1.4	0.23	1.277	0.280-5.831	0.753
26	PCV	.432	0.162-1.157	.095	.594	0.180-1.959	.392
27	MCV	1.000	0.234-4.271	1.000	1.571	.327-7.549	.573
28	MCH	.577	0.248-1.343	.202	0.442	0.165-1.188	0.106
29	MCHC	1.545	0.616-3.878	.354	2.112	0.759-5.881	.152
30	MPV	.302	.058-1.587	.157	0.677	0.085-5.401	0.713
31	PCT	1.000	0.269-3.724	1.000	0.811	0.144-4.576	0.813
32	RDWC	4.375	1.750-10.9	.002	3.531	1.190-10.472	.023
33	RDWSD	19.158	5.158-71.1	.000	17.019	4.187-69.179	.000
34	Neutrophil	0.518	0.044-6.037	0.599	.309	.012-4.033	.309
35	Monocyte	2.098	0.364- 12.1	0.407	4.718	0.256-87.032	0.297
36	Eosinophil	1.400	0.295-6.651	0.672	0.633	0.043-9.426	0.740

37	Basophil	1.400	0.295-6.651	0.672	0.356	0.054-2.322	0.280
38	Lymphocyte	1.680	0.405-6.962	0.474	1.036	0.154-6.989	0.971
39	HSV2IgG	1.2	0.7-1.8	0.02	1.3	0.07-1.9	0.003

5. Discussion

Our result showed that significant association between IgG sero-positivity of HSV-1 and miscarriage by using techniques ELISA (97.7%), while no association between IgM sero-positivity of HSV-1 and miscarriage by using techniques ELISA (35.6%).Our result showed that significant between IgG sero-positive of HSV-2 and miscarriages by using techniquesELSA1 13 (28.9%).While Non Miscarriage IgG sero-positive 4 (8.9%).The present studies compared with other study carried out in Gynecology and Obstetrics clinics of Izmir Ataturk Research and Training (2009) it found to be higher than their results was 0 (0%) HSV-1 IgM positive while IgG seropositivity rates for HSV-1 were found as (108/114) 94.7% [11].Other study carried out in Nigeria (2013) it found to be highest than other results was 0 (0%) HSV-1 IgM positive while IgG seropositivity rates for HSV-1 were found as (255/264) 96.6% [12]. In Zair the seroprevalence of HSV1 and HSV-2 antibodies inpregnant women were 85% and 32% respectively. The difference in the HSV2 positivity between our studied population and the Zaire study could well is due to the assay used. The ELISA used in our report is highly specifictoHSV2.The seropositivity for HSV-1IgG antibodies in our study (90.5%) confirms the findings of previous investigators (33, 38) [13]. In the present study the relationship between seropositivity and trimester demonstrated that seropositivity of HSV IgG was the highest for third trimester 22 (24.4%) ; while it was the highest for third 6 (6.7%) trimester for HSV IgM. In Allahabad (India) Seropositivity of HSV IgG was the highest for third trimester and it was followed by second and first trimester; while it was the highest for first trimester for HSV IgM and it was followed by third and second trimester. Seroprevalence of HSV IgG IgM was 32 (53.3%), 61 (66.3%), and 27 (69.2%) for first, second and third trimester, respectively. Sero-prevalence of HSV IgG IgM group was 2 (3.3%), 1 (1.1%) and 1 (2.6%) for first, second and third trimester, respectively. Seroprevalence of HSV IgG, group were 26 (43.3%), 30 (32.6%), and 11 (28.2%) for first, secondhand third trimester, respectively [14].In the present study the relationship between seropositivity and abortion demonstrated that seropositive of HSV-1 IgM were 1 (1.1%), IgG were 4 (4.4%)) among pregnant women were had history of abortion while HSV-1 IgM were7 (7.8%), IgG were 28 (31.1%) had not , when compared with study carried out in Tehran, Iran (2014) demonstrated that There were no significant relationship between abortion and serologic results of HSV-1 among pregnant women had history of abortion whilie 146 had not [15].

In the current study there were predictors for miscarriage exhibited that women with preeclampsia, microcytic hypochromic anemia, and sero-positivity of anti-HSV 1 and 2 IgG have high risk for miscarriage as univariate multivariate factor significant effects. While women with thyroid Diabetic patient, vaginal bleeding, menstruation cycle, and family history have reasonable as univariate risk for miscarriage. These factors may increase the risk of miscarriage.

In this study also the socio-demographical and clinical characteristics of case and control have association with miscarriage age (P value 0.0003), biomass index (P 0.0104), PCV (P 0.098), MCV (P 0.0001), MPV (P 0.0006), RDW-CV (P 0.0044), and RDW-SD (P 0.0001) as remarkable signs.

Limitation of this study like using Pcr to confirm the result of borderline of EliSA

6. Conclusion

This study shows the prevalence of HSV1 IgG 991.1% by using ELISA techniques respectively, also showed the prevalence of IgM 35.6% by using ELISA techniques respectively. And show the prevalence of HSV2 IgG (28.9%) by using ELISA techniques respectively-VSH. 1and 2vaccine is recommended for childbearing age of women. More research is needed.

7. Abbreviation

Cl: Confidence interval , OR: Odds ration RBCs (Red blood cells), Hb (Hemoglobin), PCV (Packed Cell Volume), MCV (Mean Cell Volume), MCH (Mean cell hemoglobin), TWBCs (Total White blood cells), MPV (Mean Platelet Volume), PCT (Plateletcrit), RDW-CV (Red Blood Cell Distribution Width), RDW-SD (Red Cell distribution width it measures the width of red cells size distribution)

8. Consent to participate

The specimens and information have been collected from patients have not been used for any purposes rather than this study and preserved by authors.

9. Ethic approval

This study was approved form Department of medical microbiology, Medical Laboratory, Al-Neelain University, Khartoum, Sudan.

10. Availability of data and materials

Please contact authors for data requests.

11. Competing interests

Authors have declared that no competing interests exist.

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