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Assessments of Sero-Detection of HEV 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, HEV virus has relation with recurrent miscarriage according to some previous study conducted in many countries. Objectives: The aim of present study to Sero-detection of HEV virus (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 (45women 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). HEV antibodies were analyzed in the sera of the entire participants using ELISA techniques. Results: Ninety women were enrolled in each arm of study. Miscarriage serum IgG sero-positivity for HEV (31.1% vs. 28.9%) and borderline (6.7%) vs. 4.4%) by ELISA. There were no significant difference inmiscarriage serum IgM Sero-positivity for HEV (4.4%vs. 2.2%) and borderline (2.2%) by ELISA. In logistic regression analysis of the predictors for miscarriage (OR=1.8, 95% CL=1.8-2.1, P value 0.000) IgG Sero-positivity were at risk for miscarriage, Other significant risk factors include micreocytichypochromicanaemia, vaginal bleeding, pre-eclampsia and family history. Conclusion: In the current study HEV IgG and IgM 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: HEV antibodies, recurrent miscarriage women, ELISA.***

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1. Introduction

Hepatitis E is a liver disease caused by the hepatitis E virus (HEV): as mall virus, non-enveloped RNA virus, with a positive-ense, single-stranded ribonucleic acid (RNA) genome. The only member of the Hepeviridae, is classified in the genus Hepevirus. The virus has at least 4 different types: genotypes 1, 2, 3 and 4. Genotypes 1 and 2 have been found only in humans. Genotype3 and 4 viruses circulate in several animals (including pigs, wid boars, and deer) without causing any disease. The virus is shed in the stool of infected person, and enters the human through the intestine. It is transmitted mainly through the faecal–oral route due to faecal contamination of drinking water. Other routes of transmission include: vertical transmission from pregnant woman to fetus, transfusion of infected blood products and ingestion of under cooked meat. Pregnancy appears to be potential risk factor for viral replication and leads extreme low immune status of Indian\Asian pregnant women. Mortality rates among pregnant women, those infected in the 3rd trimester, have ranged between 5% and25%, much higher that men and non pregnant women. (1) it has been reported that a significant proportion of pregnant women with acute hepatitis E (up to70%) progress to acute liver failure with a short pre-encephalopathy period, rapid development of cerebral edema and high occurrence of disseminated intravascular coagulation. (2) vertical transmission of HEV infection from mother to infant, although rare, has been reported. Babies born to HEV-RNA positive mother had evidence of hepatitis E infection. (2-3) fulminant HEV infection pregnant contributes to highest mortality rate of the fetus and mother. The maximum severity occurring during the third trimester (44.4). (1-4-5) Hepatitis E in pregnancy is also associated with high rates of spontaneous abortion, intrauterine death, and preterm labour. Information the incidence and prevalence of hepatitis E virus (HEV) infection in Indian pregnant women therefore studies the prevalence of HEV infection in pregnant women hepatitis and outcome of their pregnancy. HEV is endemic in several African countries with high mortality rate among pregnant women. The prevalence of antibodies to HEV in Ethiopian pregnant women is not known. However, rarely case control studies have been conduction Sudan according to knowledge will be brilliant and unique research to achieve these studies. The study was conducted to investigate to prevalence of anti-HEV IgG and anti-HEVIgM among pregnant women. (5)

Miscarriage, also known as spontaneous abortion and pregnancy loss, is the natural death of an embryo or fetus before it is able to survive independently (6). Some use the cutoff of 20 weeks of gestation after which fetal death is known as a stillbirth. The most common symptoms of a miscarriage is vaginal bleeding with or without pain) Sadness, anxiety and guilt may occur (7). Tissue. Miscarriage occurs in one in five pregnancies and can have considerable physiological and psychologycal implications for the patient. It is also associated with significant health care cost. Risk factors for miscarriage include an older parent, previous miscarriage, exposure to tobacco smoke, obesity, diabetes, and drug or alcohol use, among others (*8*). In those under the age of 35 the risk is about 10% while it is

about 45% in those over the age of 40 (6). Risk begins to increase around the age of 30 (*8*). About 80% of miscarriages occur in the first 12 weeks of pregnancy (the first trimester) (*6*). The association of systemic infections with Rubella, brucellosis, cytomegalovirus, Treponema palladium, influenza virus and vaginal infection with bacterial vaginosis, with increased risk of miscarriage has been demonstrated. Q fever adeno-associated virus, bacavirus, Hepatitis and Mycoplasmagenitalium infections do not appear to affect pregnancy outcome. The effect of *Chlamydia trachomatis*, *Toxoplasmagondi*, Human *pappilloma virus*, Herpes simplex virus, Parvovirus B19, Hepatitis B and Polyoma virus BK infections remain controversial, as some studies indicate increased miscarriage risk and others show no increased risk. (9)

2. Materials and Methods

A case- control study was conducted at Wad Madani teaching hospital Department of Obstetrics gynecological, AlGezira state, Sudan during the period of July-October 2018. A sample of 90 women in each arm of the study have over 80% power to detect a difference of 5% at $\alpha = 0.05$. 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 analyses at the Central Research lab. Complete blood count calculated by using hematological analyzer (Sysmex–XP 300) Manufacture Company, The three meain physical technology used in it, direct current impedance, advanced optical light scatter technology, flour-scen t flow cytometry and spectrophotometry. These were used in combination with chemical reagent that lyes or alter blood cell to extend the measurable parameters. Wide range of test is done by it.

The specimens were analyzed for detection of HEV IgG and IgM antibodies by commercially available enzyme–linked immunosorbent assay HEV IgG and IgM ELISA" kit Euro immune, I NC America

The tests were performed as instructed by the manufacturer. The reagents have positive and negative controls were already to be used solution that specific for HEV. Results of cut-off of HEV index more than 1.0 IU\ml considered as positive result and cut-off of HEV index less than 1.0IU/ml considered as negative result.

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

3. Statistics Analysis

The collected data were analyzed using SPSS version 24and double checked before analysis. Means and proportions of the socio-demographic and clinical characteristics were calculated for HEV sero-positive groups. Univariate and multivariate analysis were used for HEV IgG and IgMsero-positive 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 ± 0.5751vs. 25.66 ± 0.6089P=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 vs31.91 ± 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 ± 0.2821 p=0.0044 "0.4121 to 2.170"), RWDSD (44.98 ± 0.8974 vs52.48 ± 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.

Sero detection of IgG and IgM antibodies by using ELISA techniques, total of 45 miscarriage women (cases) for IgM 2 (4.4%) positive for HEV. 1 (2.2) Borderline for HEV and 42 (93.3%) negative for HEV by ELISA technique. A total of 45 non-miscarriage women (control) for IgM 1 (2.2%) positive, 44 (97.8%) negative by ELISA technique. And for IgG antibody of miscarriage 14 (31.1%) positive, Borderline 3 (6.7) and 28 (62.2%) negative by ELISA technique. And IgG for non-miscarriage 13 (28.9%) positive, 2 (4.4) Borderline and 30 (66.7%) negative by ELIAS technique. Table 2.

Predicators factors for miscarriage

Univariate and Multivariate analysis showed that preeclampsia, microcytic hypo- chromic anemia, vaginal bleeding, sero-positivity of anti-HEV IgG (ELISA technique), 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-Gaze era 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	AntiHEVIgG	"0.4769± 0.08307"	"0.4726± 0.08538"	0.8461"-"-0.2425 to 0.2944""
21	Anti HEVgM*	"0.1208± 0.04498"	"0.1503± 0.06650"	0.7022""-0.1952 to 0.1327""

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)

Table 2 Assessment of Sero-detection of IgM and IgG antibodies of by using ELISA

Item.bv4`A\	Number	IgM			IgG		
	Techniques	ELISA			ELISA		
		Positive	Borderline	Negative	Positive	Borderline	Negative
Miscarriage	45	2 (4.4%)	1 (2.2%)	42 (93.3%)	14 (31.1%)	3 (6.7%)	28 (62.2%)
No Miscarriage	45	1 (2.2%)	0 (0.0%)	44 (97.8%)	13 (28.9%)	2 (4.4%)	30 (66.7%)
Total	90	3 (3.3%)	1 (1.1%)	86 (95.6%)	27 (30.0%)	5 (5.6%)	58 (64.4%)

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	Microcytichypochromic anemia	11	1.086-110.2	0.04	2.9	1.3-6.7	.000
12	Sero-positivity of Anti-HEVIgG*	0.622	0.103-5.006	.657	0.288	0.023-3.567	0.332
13	Sero-positivity of Anti-HEVIgM*	1.8	1.6 -2.1	.000	6.9	5.2-9.2	.000
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

5. Discussion

Our result shown that significant association between IgG sero-positivity of HEV and miscarriage by using ELISA techniques (31.1%) and (6.7), while no association between IgM sero-positivity of HEV and miscarriage by using ELISA techniques (3.3%) and (1.1). In the previous study found very high overall frequency rates (61.2% (57/93) of HEV antibody among pregnant women, suggesting the possibility of subclinical infections. the overall frequency of HEV among pregnant women attending Khartoum Teaching Hospital is higher than that found in Darfur, Western-Sudan (31.1%), Khartoum hospitals (41.1%) and India (60%) and lower than that in Egypt (84.3%) (10, 11, 12).Hepatitis E in pregnancy is also associated with high rates of spontaneous abortion, intrauterine death, and preterm labour (13). Hepatitis E virus is associated with abortion during pregnancy, we found about (36.8% (21/57) of HEV positive patient was abortive. the high seroprevalence of HEV in pregnant women at Khartoum teaching hospital may suggest wide spread among pregnant women in this country. Furthermore, the high positive rates of seroprevalence of anti-HEV IgG among pregnant women in third trimesters were 59.6% (34/ 57) and this agreed with many different previous studies (11, 12, 13).

In addition to that, in the present work, the seroprevalence of anti-HEV IgG among pregnant female is higher with water supply than wells souces (71.9% (41/57) vs 28.1% (16/57)) respectively which imply that infected pregnant women by HEV are slightly more in rural area than urban area (50.9% (29/57) vs. 49.1% (28/57)) respectively (14). The virus is transmitted through the fecal-oral route an infected person getting into the mouth, referred to as the fecal-oral route, may be direct from person to person by contaminated finger or indirect through food or water. Also the incidence of acute viral Hepatitis E increases after floods as this allows sewerage contamination of piped and ground water as our study period coincides with rainy season and this agreed with previous study in Bangladesh and southwestern Vietnam (15, 16). All of women enrolled in this case control study have no symptoms of hepatitis.

In the current study there were predictors for miscarriage exhibited that women with preeclampsia, microcytichypochromic anemia, and sero-positivity of anti-HEV 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 represents the association between anti-HEV sero-positivity and miscarriage. The prevalence of IgG 30.0% and 5.6% by using ELISA techniques respectively, also show the prevalence of IgM 3.3% and 1.1 % by using ELISA techniques respectively. Cases should be retest to conform, HEV screening test and HEV vaccine 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. Funding

None received

Ethic approval as per university standard written approval committee has been and collected and preserved by the authors.

Availability of data and materials
Please contact authors for data requests.

10. Competing interests

Authors have declared that no competing interests exist

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