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Sero-Detection of Hepatitis C Virus Antibodies among Spontaneous, Recurrent, Miscarriage Women in Gezira State (Sudan)

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Abstract

Background: Recurrent miscarriage is a big problem that is increasing nowadays. Hepatitis C virus (HCV) has an association with recurrent miscarriage according to reports from many countries. Miscarriage, also known as spontaneous abortion, occurs when an embryo or fetus dies naturally before it is able to survive independently. Some authors use the cutoff of 20 weeks of gestation after which fetal death is known as a stillbirth. 80% of miscarriages occur in the first 12 weeks of gestation.

Objective: To sero-detect HCV antibodies among spontaneous, recurrent-miscarriage women in Gezira State (Sudan).

Materials and methods: This was an analytical, case control study conducted at Wad Madani Teaching Hospital (Al Gezira State, Sudan) among 45 women with recurrent miscarriage (test group) and 45 healthy, non-miscarriage, pregnant women (control group). All study population were investigated for HCV IgG and IgM antibodies using the enzyme immunosorbent assay (ELISA) technique.

Results: All 90 specimens investigated shoed negative results for HCV IgG antibody. However, there were significant differences between the miscarriage and non-miscarriage HCV IgM sero-positivity: 19 (21.01%) vs 6 (6.7%), and borderline 17 (18.9%) vs 31 (34.4%). In logistic regression analysis of the predictors for miscarriage (OR = 2.047, 95% Cl = 0.750-10.57, p = 0.04), HCV IgM sero-positive women were at risk for miscarriage. Other significant risk factors detected in the study were microcytic hypochromic anemia, vaginal bleeding, pre-eclampsia, and miscarriage family history.

Conclusion: Sero-positive hepatitis C virus IgM antibody was associated with miscarriage in pregnant women.

Keywords: HCV antibodies, Miscarriage, Pregnant women, ELISA, Gezira State (Sudan).

Introduction

Risk factors for miscarriage include chromosomal abnormalities (most common cause), previous miscarriage, an old parent, drugs, obesity, thyroid problems, tobacco smoking, genital tract infections, diabetes mellitus, alcoholism, and autoimmune disorders. The most common

symptoms of a miscarriage is vaginal bleeding with or without pain, sadness, anxiety, and feeling of guilt. The underlying cause in about half of cases involves chromosomal abnormalities. Other conditions that can produce similar symptoms include an ectopic pregnancy and implantation bleeding[1].

Tissue miscarriage occurs in one of five pregnancies and can have considerable physiological and psychological implications for the patient. In those under the age of 35 years, the risk is about 10% while it is about 45% in those over the age of 40. Systemic infections resulting from rubella, brucellosis, cytomegalovirus, *Treponema palladium*, influenza virus, and bacterial vaginosis were found associated with increased risk of miscarriage. Q-fever, adenovirus, Baca virus, hepatitis and *Mycoplasma genitalium* infections do not appear to affect pregnancy outcome. The effect of *Chlamydia trachomatis, Toxoplasma gondii,* human papillomavirus, herpes simplex virus, parvovirus B19, hepatitis B virus, and polyomavirus infections remain controversial, as some studies indicate increased miscarriage risk and others show no increased risk[2].

HCV is a liver disease caused by the hepatitis C virus. This virus can cause acute and chronic hepatitis ranging in severity from mild to life long illness; and it is a major cause of liver cancer. HCV occurs most commonly in Africa and central and east Asia. Globally, an estimated 71 million people have chronic HCV infection. WHO estimated that in 2016: nearly about 399,000 people died from HCV mostly from cirrhosis and liver cancer. The few studies on HCV infection in Sudan demonstrated a low seroprevalence rate, nearly 2.2% in Gezira State[3].

HCV may spread by blood to blood contact and associated with blood transfusion, intravenous drug addiction, poor sterilized medical equipment, body tattooing, shared personal items, vertical mother to child transmission during pregnancy, and during child birth. However the biological mechanisms that are involved in this process are incompletely understood. HCV is a small enveloped single-stranded positive sense RNA virus. It is a member of the genus *Hepacivirus* in the family *Flaviviridae*, and there are 7 major genotypes of HCV[1].

During initial infection, patients often have mild or no symptoms, or occasionally fever, abdominal pain, and dark urine. The virus persists in the liver over many years and it often leads to liver disease and cirrhosis, and in some cases cirrhotic patients may develop complications such as liver failure, liver cancer, or varices in the esophagus and stomach. A woman with HCV have fewer life birth and face a higher risk of miscarriage. In the year 2000 a large cohort of rhesus negative patients in Ireland were infected with HCV as compared to an age and parity matched control group of rhesus positive women. The output was 4 premature births, 11 spontaneous miscarriages, and 2 neonatal deaths among HCV positive women. Also researchers in Italy found that women with HCV infection had a greater risk for miscarriage, infertility, gestational diabetes, and pre-eclampsia[4].

This study aimed to determine and compare the seroprevalence rate of HCV among women with miscarriage and women with no history of miscarriage to identify potential risk factors, clinical outcome, and symptoms that are associated with a positive serological antibody test.

Materials and methods

This was a case-control study was conducted at Wad Madani Teaching Hospital (Al Gezira

State, Sudan) during the period from July-October, 2018. The study was approved by the Scientific Research Committee of Al Neelain University, Khartoum (Sudan), and the Ethical Board of the Ministry of Health (Sudan). Data confidentially was maintained, and the information collected from all specimens had not been used for any purpose other than this study. Permission to investigate the specimens was granted from the director of Wad Madani Teaching Hospital (Al Gezira State, Sudan). Informed consent was obtained from patients before specimens collection.

45 women with recurrent miscarriage (test group) and 45 healthy, non-miscarriage, pregnant women (control group). The sample size had an over 80% power to detect a difference of 5% at $\alpha = 0.05$; assuming a10% of the women might have incomplete data or samples.

A volume of 5 ml blood specimen were collected from each patient through venipuncture technique, then displaced into plain containers, allowed to clot, centrifuged, and kept at -20°C until serological analysis.

Complete blood count was performed using the hematological analyzer (Sysmex–XP 300). The 3 main physical technology used were direct current impedance, advanced optical light scatter technology, and fluorescent flow cytometry and spectrophotometry.

These are used in combination with chemical reagents that lyses or alter blood cell to extend the measurable parameters. The specimens were analyzed for detection of HCV IgG and IgM antibodies by the commercially ELISA machine. The HCV IgG and IgM kits (Fortress English Company) were used.

The tests were performed as instructed by the manufacturer. The reagents which had positive and negative controls were used for specific detection of HCV antibodies.

Samples giving absorbance greater than or equal to the cut-off value (1.0 IU/ml) were considered positive. Samples giving absorbance less than the cut-off value (1.0 IU/ml) were considered negative. Samples with absorbance O.D $\leq$ cut-off $\times 2$ were considered borderline. (Cut-off = mean of 3 negative controls + 0.12).

Statistics analysis: The collected data were analyzed using IBM-SPSS, version 21, and double checked before analysis. Means and proportions of the socio-demographic and clinical characteristics were calculated for HCV seropositive groups. Univariate and multivariate logistic regression analysis were used for HCV 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.

Results

Sero detection of HCV IgG and IgM antibodies was performed using ELISA techniques. A total of 45 miscarriage women (test group) were investigated. 19 cases (21.1%) were found positive for HCV IgM antibody, 17 cases (18.9%) were found borderline for HCV IgM antibody,, and 9 cases (10%) were found negative for HCV IgM antibody.

A total of 45 non-miscarriage women (control group) were investigated. 6 cases (6.7%) were found positive for HCV IgM antibody, 31 cases (34.4%) were found borderline for HCV IgM antibody, and 8 cases (8.9%) were found negative for HCV IgM antibody. No positive or negative or borderline HCV IgG antibody were detected in neither test nor control groups.

Table (1): Socio-demographical and clinical data of test and control groups

No	Parameter	Control group Mean ± SEM	Test group Mean ± SEM	p - value
1	Age incidence	26.02 ± 0.8531	30.89 ± 0.9504	p = 0.0003 "-7.409 to -2.324"
2	Biomass index	25.66 ± 0.6089	27.85 ± 0.5751	p = 0.0104 "-3.860 to -0.5250"
3	RBCs	10.65 ± 6.849	3.843 ± 0.1349	p = 0.3235 "-6.836 to 20.44"
4	Hb	10.93 ± 0.2420	10.58 ± 0.3481	p = 0.4187 "-0.4995 to 1.188"
5	TWBCs	9.109 ± 0.4661	7.907 ± 1.214	p = 0.3577 "-1.386 to 3.790"
6	Platelets	251.7 ± 12.61	243.8 ± 14.61	p = 0.6803 "-30.43 to 46.39"
7	PCV	33.94 ± 0.6871	31.84 ± 1.053	p = 0.0984 "-0.4025 to 4.602"
8	MCV	90.72 ± 1.057	84.22 ± 1.010	**p = 0.0001 "3.590 to 9.410"**
9	MCH	29.00 ± 0.5027	28.11 ± 0.5391	p = 0.2311 "-0.5784 to 2.356"
10	MCHC	31.91 ± 0.3579	33.16 ± 0.3316	p = 0.0125 "-2.216 to -0.2733"
11	MPV	8.687 ± 0.1015	9.593 ± 0.2327	p = 0.0006 "-1.412 to -0.4012"
12	PCT	0.2115 ± 0.01113	0.2579 ± 0.03219	p = 0.1762 "-0.1143 to 0.02136"
13	RDWCV	15.88 ± 0.2821	14.59 ± 0.3397	p = 0.0044 "0.4121 to 2.170"
14	RWDSD	52.48 ± 0.8195	44.98 ± 0.8974	p = 0.0001 "5.078 to 9.917"
15	Neutrophils	65.34 ± 1.864	66.43 ± 1.908	p = 0.6829 "-6.403 to 4.216"
16	Lymphocytes	27.42 ± 1.617	32.19 ± 4.928	p = 0.3599 "-15.10 to 5.551"
17	Monocytes	4.627 ± 0.3153	5.324 ± 0.3098	p = 0.1180 "-1.578 to 0.1822"
18	Eosinophils	2.553 ± 0.1767	2.267 ± 0.14	p = 0.2108 "-0.1660 to 0.7394"
19	Basophils	00.00	00.00	Constant

(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, MCHC: Mean cell haemoglobin concentration, PCT: Platelets, RDWCV: Red blood cell distribution width, RWDSD: Red cell distribution width).

As shown in Table (1), the socio-demographic variables between test group and control group cases showed a significant difference in the age incidence (p = 0.0003), in biomax index (p = 0.0104), in MCV(p = 0.0001), in MCHC (p = 0.0125), in MPV (p = 0.0006), in RDWCV (p = 0.0044), RWDSD (p = 0.0001).

There was no significant difference between the test and control groups in RBCs, Hb, TWBCs, platelets, PCV, MCH, PCT, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. (p - value was considered as 95% confidence interval).

The predicators factors for miscarriage (preeclampsia, microcytic hypochromic anemia, vaginal bleeding, menstruation cycle, and biomass index) were found significantly associated with miscarriage in both univariate and multivariate cases. Diabetes mellitus, age incidence, and family history were found significantly associated with miscarriage in univariate analysis only.

Discussion

In this study a significant association (21.1%) between anti-HCV IgM sero-positivity and miscarriage; while there was no association (0.0%) between anti-HCV IgG sero-positivity and miscarriage. This finding reflects the recent HCV infection in the patients investigated.

There was 19 cases (21.1%) with seropositive anti-HCV IgM among the miscarriage cases; and there was 6 cases (6.7%) with seropositive anti-HCV IgM among non-miscarriage cases. This finding indicated the high prevalence rate of HCV infection among miscarriage women. This finding agreed with the findings of the study conducted in the year 2000 where a large cohort of rhesus negative were found infected with HCV as compared to an age and parity matched control group of rhesus positive women[5].

Jabeen and his colleagues[6] reported 4 premature births, 11 spontaneous miscarriages, and 2 neonatal deaths among anti-HCV IgM positive women, while there is a high prevalence rate of anti-HCV IgM among miscarriage women in the present study. However, the biological mechanisms that are involved in this process are incompletely understood.

Another case control study (2017) was conducted in America and showed that the HCV positive women had significantly lower levels of anti-müllerian hormone, and associated with a high rate of miscarriage[6]. This finding indicated that HCV can increase the rate of miscarriage as shown in the present study.

The effect of HCV infection on children may begin in utero. One study evaluated the effect of HCV on neonates and found that there are low levels of the regulatory T- cells, CD4+, and CD8+ T- cells activation. Also, there were low levels of pro-inflammatory markers suggesting the relative suppression of the immune activation in neonates infected with HCV[7].

Fateux and his co-workers[8] reported that HCV can be transmitted to the fetus as early as the first trimester especially in women co-infected with HIV which would have implications on early organ development. This may interfere with the normal fetal development and may lead to fetal death. In the United States there has been an increase in HCV infection in women infected

during pregnancy with 5-fold increase from 1998 to 2011[9].
Pregnancy represent a time of engagement of medical care and a unique opportunity to detect HCV infection[10].
All women enrolled in this case-control study had no symptoms of HCV infection. The current study elucidated different predictors for miscarriage, e.g. preeclampsia, microcytic hypochromic anemia, and the sero-positivity of anti-HCV IgM. These factors are considered univariate and multivariate risk factors that have significant effects. While women with thyroid disease, diabetes mellitus, vaginal bleeding, disturbance of menstruation cycle, and family history have univariate risk factors for miscarriage.
In this study the socio-demographical and clinical characteristics in test and control groups were found associated with miscarriage, e.g. age incidence ($p = 0.0003$), 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 ($p = 0.0001$). The limitations of this study is the small sample size investigated and the application of only one diagnostic serological test (ELISA technique). **T**he prevalence rate of anti-HCV IgM was 21.1% and 6.7% among the test and control groups studied. These findings should be retested by other researchers for more confirmation, and HCV screening need to be performed among pregnant women.
Conclusion: Sero-positive hepatitis C virus IgM antibody was associated with miscarriage in pregnant women.
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