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Serostatus of Coxsackie B in a sample of women with miscarriage in Baghdad, Iraq

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Abstract.
The antibody that crosses transplacentally from mother to fetus is very important origin of protective passive immunity against infection neonatal with enterovirus. Important varieties of coxsackievirus B3 (CVB3) are responsible for infections in newborns. The purpose from this study is to investigate in the prevalence of Coxsackie B virus in a sample of Iraqi women with miscarriage and potential role of miscarriage risk.
METHODS: Between November 2022 and June 2023, we included 91 parturient women (gestational age: 4–20 weeks) who were between the ages of 15 and 40. Every participant completed a questionnaire, and blood was drawn to assess maternal antibodies against CVB3.
RESULTS: The blood seropositive rates were 46 out 91(50.54%), 2 out 46 were IgM positive (4.34%), (8–12 weeks) 23 from **46** (**50%**) (p-value 0.0294) gestational age more frequent among aborted women that positive for anti-coxsackie B antibody, The 25–35 age group was significantly overrepresented (51/91, 56%) compared to other age groups.
CONCLUSION: This investigation posits Coxsackie B virus (CBV) as a possible etiology for miscarriage in the Iraqi female population. Further studies employing larger cohorts and robust methodologies, beyond the current detection technique, are warranted to corroborate these observations and elucidate the potential mechanisms by which CBV might induce miscarriage.

Keywords: Coxsackievirus B, non-polio enterovirus, maternal IgM, missed abortion

1. Introduction

Non-polio enterovirus (NPEV) infections are incredibly common in children around the world and can cause serious illnesses, particularly in young children. Coxsackievirus B1 to 5, Echo virus 6 to 11, and other NPEVs are medically urgent [1]. Neonatal are still at significant risk from enterovirus infection due to its high rate of case fatalities and challenging early detection. A miscarriage risk factor is enterovirus infection in pregnancy, particularly coxsackievirus B (CVB) [2, 3].

Coxsackie viruses type B (CVB) are frequently encountered pathogens, are mainly restricted to illnesses that are subclinical or asymptomatic, are have been known for their broad tropism and for their wide range of associated diseases. There are two major groups of coxsackie viruses: group A has 24 coxsackie viruses, whereas group B contains 6 [4,5].

Pregnancy-related enterovirus infections are slightly prevalent. Of the 198 unselected pregnant women investigated, the NIH Collaborative Perinatal Project discovered that around 9% had serologic evidence of group B coxsackie virus infection [6].

Furthermore, a study conducted in Korea on placental specimens and abortive tissues discovered a notably elevated incidence of coxsackie virus B3 (CVB3) in cases of abortion. The most prevalent type of neonatal myocarditis, CVB 1–5, with a 30–50% death rate [7].

The most of enterovirus infections that occur during pregnancy result in the delivery of healthy, unaffected infants. On the other hand, a number of reports have suggested that enterovirus infection could spread

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through the placenta. Following maternal infection, Basso et al. 5 isolated the coxsackie virus and echovirus from placental and fetal tissues [8].

Recent years in Iraq have witnessed a concerning rise in unexplained pregnancy losses. Identifying the causative factors behind these abortions is crucial. Viral infections during pregnancy represent a potential contributor, and Coxsackie B virus (CBV) is one such pathogen under investigation. This study aims to be a pioneering effort in diagnosing CBV presence in pregnant women and elucidating its potential role in miscarriage. We hypothesize that CBV infection during pregnancy might adversely impact the embryo and fetus, potentially leading to fetal and neonatal mortality, with cardiac anomalies being a possible manifestation. While perinatal maternal infections are recognized risk factors for serious neonatal complications, data on the prevalence of Coxsackie B virus (CVB) in women experiencing pregnancy loss remains elusive within the existing literature. This study's findings aimed to address two key questions: (1) whether the presence or level of Coxsackie B virus antibodies varied based on the gestational age at miscarriage, and (2) whether Iraqi women with miscarriages exhibited differences in Coxsackie B virus antibody prevalence or levels compared to women with miscarriages from another country with similar sociodemographic characteristics.

2. Materials and methods

2.1. Case enrolment

In this study about 91 aborted women were enrolled (under gestational age 20 weeks) aged 15–45 years old Study continued from November 2022 to June 2023, Exclusion criteria; Patients had positive TORCH, Patients had UTI, Patients had known chromosomal anomalies or had family history of genetic diseases that cause abortions, Patients with pre-existing physiological (hypertension, diabetes) or immunological disorders, or with a family history of pregnancy loss. The written informed consent had been obtained, a questionnaire investigation conducted to take demographic data, medical history, pharmacological history and understanding of infection control. The sample of blood was collected from all patients on the same day of abortion, to detect the IgM and IgG antibodies against CVB.

2.2. Detection antibodies against coxsackievirus B

The samples were collected as 3 ml of blood for each patient and then centrifuged to obtain serum. The serum stored at -4°C for the qualitative assessment of IgM and IgG using enzyme linked immunosorbent assay (ELISA) technique (DRG® Coxsackie B Virus EIA-5175, EIA-5493 Elisa kit). The sensitivity and specificity of test were over 80%, 95% respectively, also the negative control, positive control, and internal quality control were included in the test.

Samples were processed in accordance with the manufacturer's instructions after being brought to room temperature. At 450 nm, color detection was measured. At 630 nm, optical error correction was carried out. A standard curve was plotted and results were calculated.

2.3. Statistical analysis

The Statistical Analysis System-SAS (2018) program had been used to investigate the effect of difference groups. In this investigation, the chi-square test was utilized in order to compare percentages (0.05 and 0.01 likelihood) statistically significant.

2.4. The procedure

The procedure must be followed in accordance with what reported below, making sure that each tested sample is incubated for the same duration of time.

1. The samples were diluted 1:101 into a defined dilution tube.
2. The Microwells are numbered. In order to do the blanking process, A1 was left empty.
3. Each correctly labeled well contained 100 μL of diluted samples, 100 μL of duplicated low control, and 100 μL of single high control.
4. The microplate for 60 minutes of incubation at $+37^{\circ}$C.
5. As previously stated, an automatic washer was used to wash the microplate.
6. With the exception of the A1 well, 100 μL of enzyme conjugate was added to each well before being sealed. Every well has received the red component, except the A1.
7. For sixty minutes, the microplate was incubated at $+37^{\circ}$C.
8. The micro wells were washed.
9. Each well absorbed 100 μL of the Chromogen/Substrate mixture, and a blank well was added as well. After that, the microplate was incubated for 20 minutes at room temperature (18°C to 24°C).
10. Approximately 100 μL of sulfuric acid were pipetted into each well, following the same procedure as in step 9.

Table 1
The demographic data of aborted women

		Percentage out of total number	P value
Maternal age	15–25	20 from 91(21.9%)	0.0063
	25–35	51 from 91(56%)	
	35–45	20 from 91(21.9%)	
Type of abortion	Missed abortion	88 from 91(96.7%)	0.0001
	Incomplete abortion	3 from 91(3.29%)	
The gestational age	0–4 weeks	8 from 91(8.79%)	0.0294
	4–8 weeks	34 from 91(37.3%)	
	8–12 weeks	46 from 91(50.5%)	
	12–20 weeks	3 from 91(3.2%)	

Table 2
Symptoms among seropositive aborted women

	Fever		Headache		Sore throat		Abdominal pain		Cough	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Positive	31 from 46	15 from 46	32 from 46	14 from 46	31 from 46	15 from 46	36 from 46	10 from 46	27 from 46	19 from 46
	67.39%	32.6%	69.5%	30.4%	67.39%	32.6%	78.2%	21.7%	58.6%	41.3%
P-value	0.0369*		0.049*		0.042*		0.877 NS		0.031*	

*($P \leqslant 0.05$), NS: Non-significant.

Table 3
The gestational age among seropositive aborted women

Seropositive	1st trimester 0–4 weeks	1st trimester 4–8 weeks	1st trimester 8–12 weeks	Second trimester 12–20 weeks	P-value
	5 from 8	13 from 34	23 from 46	2 from 3	0.0294*
	62.5%	38.2%	50%	66.66%	
P-value	0.083 (NS)	0.0427*	1.00(NS)	0.804(NS)	–

*($P \leqslant 0.05$), **($P \leqslant 0.01$), NS: (Non-significant).

11. Following the instructions in Section 9.5, the color intensity of the solution was measured in each well, blanking the instrument on A1, both possible at 620–630 nm (background removal) and at 450 nm filter (reading).

2.5. Calculation

If the test is found to be legitimate, the results are computed using the Low Control (NC) mean OD450nm value as well as a cut-off value (Co) that is found using the following formula:

$$\text{Cut-Off} = \text{NC} + 0.250$$

3. Results

The results of Table 1 depict Of the 91 participants, 46 (50.54%) tested positive, among the positive cases ($n = 46$), only 2 individuals (4.35%) were positive for IgM antibodies, The 25–35 age group was significantly overrepresented (51/91, 56%) compared to other age groups (p-value $= 0.0063$), this suggests a potential association between age and the studied outcome, while missed abortion appeared to be the most frequent type compared to other types, although the specific frequencies are not provided.

Table 2 presents the symptom profile associated with CBV(E2) infection. The following symptoms: headache, sore throat, fever, and cough, exhibited higher prevalence in individuals who tested positive compared to the negative group. Notably, fever demonstrated the strongest significant association with positive cases (p-value $= 0.0369$). Conversely, abdominal pain did not reveal a statistically significant relationship with seropositive women who experienced an abortion.

Table 3 presents the distribution of gestational age among aborted women. Gestational age of 8–12 weeks is significantly (p-value $= 0.0294$) more frequent among sero-positive coxsackievirus B cases compared to other gestational ages in this group.

Table 4 demonstrates a predominant representation of the 25–35-year age group (51 out of 91, 56%) among aborted women (p-value $= 0.0063$). However, within

Table 4
The age group among aborted women

Seropositive	15–25 years	25–35 years	35–45 years	P-value
	15 from 20 75%	30 from 51 58.8%	11 from 20 55%	0.0219*
P-value	0.0042**	0.0318*	0.074 NS	–

*($P \leqslant 0.05$), **($P \leqslant 0.01$), NS: Non-significant.

Table 5
The type of abortion and positive case

Sero-positive	Missed abortion	Incomplete abortion	P-value
	45 from 88 51.13%	1 from 3 33.33%	0.0071**
P-value	0.792 NS	0.804 NS	–

**($P \leqslant 0.01$), NS: Non-significant.

seropositive individuals, the 15–25-year age group exhibits greater frequency (p-value $= 0.0042$).

Table 5 demonstrates the type of abortion and positive case and reveal that missed is more frequent and highly significant (45 from 88 51.13% p-value 0.0071) among seropositive aborted women.

4. Discussion

The outcome of a viral infection during pregnancy is influenced by several factors, including the specific viral pathogen, the maternal immune response, and the gestational timing of infection. Maternal infection with coxsackie B virus during pregnancy or delivery can lead to severe neonatal infection. Documented consequences of intrauterine hematogenous transmission of coxsackie B3 virus include fetal necrotic meningoencephalitis, cerebral calcifications, interstitial pneumonitis, and placental villitis [9,10,11,12,13,14]. Due to the frequent asymptomatic nature of coxsackie type B virus infections, data on the effects of exposure during early pregnancy remains limited. Most research on coxsackie B virus infection during pregnancy up until the 1980s relied on indirect maternal serological data, which is typically obtained after delivery and lacks virological or molecular confirmation. An investigation of 91 women with a history of miscarriage identified that 46 (50.54%) possessed IgG antibodies, whereas only 2 (4.34%) exhibited maternal IgM antibodies. These findings suggest that the presence of IgG antibodies confers significant protection against Coxsackievirus B3 (CVB3) infection in neonates. A number of studies show that among women who had abortions before 13 weeks, IgM antibody frequency against CVB1–5 was 42%, which was significantly greater than that of control women (18%). Additionally, CVB5's isolation rate was 26%. Ornoy and Tenenbaum suggest a potential causal link between CVB infection and an increased risk of spontaneous abortion [15].

Since the 1990s, a few reports of clinical case have provided virological proof linking spontaneous abortion and congenital cardiac disease to infection of the mother with CVB during the first trimester of pregnancy. While Ornoy and Tenenbaum suggest a link between Coxsackievirus B (CVB) and spontaneous abortion, our study predominantly observed missed abortions [13,15,16,17]. This discrepancy may be due to our limited sample size. Our findings tentatively support the work of Hwang et al. [17],who established a strong correlation between CVB infection and missed abortion in a Korean cohort [18].

In current study most of aborted women were in the first trimester 88 of 91(96.7%) were first trimester and 3 of 91(3.29%) were second trimester highly no of aborted women were had missed abortion while the remain number were had complete abortion and this agree with the study conducted in. Hwang JungHye, Kim JeongWook, Hwang JiYoung, Lee KyungMin, Shim HyeMin, Bae YoungKyung, Paik SeungSam and Park HoSun [18] and disagree with study conducted by according to. Axelsson, Bondestam, Frisk, Bergström and Diderholm [15], women who miscarried were more likely to have CVB infection than those who had a deliberate abortion before 13 weeks of gestation.

The idea that because CAR is expressed in both villous trophoblast cells and extra villous trophoblast cells of the human placenta at 11 weeks, adenovirus infection would be particularly vulnerable during the first trimester. Since CVB and adenovirus have the same receptor, CVB infection would also be vulnerable in the first trimester and could have a negative impact on the course of the pregnancy [17].

Thus, inaccurate CAR adhesion activities may prevent organ formation if CVB infection occurs early in pregnancy, which could result in miscarriage. In this context, Watson et al. have isolated CVB1 from a fetus with a congenital cardiac defect through amniotic fluid.

In this study, the gestational ages of CVB3-positive missed abortion cases were between 7 to 11 weeks, an active period of heart development [19].

Based on previous research suggesting a detrimental effect of the Coxsackie B virus (CV-B) on various aspects of fetal development, particularly heart cell growth, and considering the widespread nature of this virus evidenced by our recent seroprevalence study, further investigations are warranted to elucidate the pathogenic mechanisms of CV-B infection and its potential association with miscarriage.

5. Conclusion

In conclusion, this study investigated the seroprevalence of Coxsackie B virus among women experiencing miscarriage in Baghdad, Iraq. Our findings revealed that 46% of the participants tested positive for Coxsackie B antibodies IgM and IgG. While this study suggests a potential association between Coxsackie B infection and miscarriage risk, the relatively small sample size and restricted sampling location limit the generalizability of the findings. Further research with a larger and more geographically diverse sample is warranted to confirm these findings and elucidate the potential role of Coxsackie B virus in miscarriage etiology.

Conflict of interest

There is no conflict of interest.

Ethical statements

There are no ethical issues relevant to this work.

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