

# Seroprevalence study of Parvovirus B19 among women with miscarriage in Mosul city

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## Abstract

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**Background:** Miscarriage and stillbirth are among the most common pregnancy complications, and could be caused by several factors, among these factors are viral infections some of them are obvious teratogenic and others are associated with fetal defects like severe anemia in the case of Parvovirus B19 which could be transmitted vertically causing fetal infection and miscarriage or stillbirth.

**Objective:** To investigate the seroprevalence of Parvovirus B19 among women with miscarriage and stillbirth and compare them with normal pregnant women in the city of Mosul-Iraq.

**Patients and Methods:** A case-control study conducted on pregnant aged 16-45 years with miscarriage and stillbirth admitted to Al-Khansa Teaching Hospital in Mousl city. The samples were collected during the period from November 2022 and January 2023 from 160 from pregnant women, 80 of them had miscarriage or stillbirth, and the other 80 had normal uneventfull pregnancy (control). Serum samples were subjected for ELISA Study of anti-B19V IgG and IgM antibodies.

**Results:** This study showed that the seroprevalence rate of anti-B19V IgG among pregnant women was about 23% (37 out of 160), subdivided into (40%) 32 out of 80 patients, and (6.2%) 5 out of 80 controls had positive anti-B19V IgG antibody which is significantly different. And IgM seroprevalence was about 22% (35 out of 160), in which (38.8%) 31 out of 80 patients, and (5%) 4 out of 80 controls had positive anti-B19V IgM antibody. Both anti-B19V IgG and IgM were higher among patients who had stillbirth than those who had miscarriage both qualitatively and quantitatively. On the other hand, there is a highly significant association of anti-B19V IgM positivity with maternal anemia.

**Conclusion:** The study found high association between B19V and miscarriage or stillbirth as compared to normal pregnancy, and this was more common among patients who had stillbirth than those who had miscarriage, with obvious association with maternal anemia, all of the above is among pregnant women from Mosul City in Iraq.

**Keywords:** miscarriage , stilbirth

## Introduction

A miscarriage is the loss of a pregnancy with a gestational age of under 20 weeks.

According to estimates from the American College of Obstetricians and Gynecologists

(ACOG), it is the most prevalent type of pregnancy loss [1]. An estimated 23 million miscarriages occur every year worldwide, translating to 44 pregnancy losses each minute [2].

Stillbirth is described as the death of a fetus after a predetermined gestational age and/or fetal weight, both of which previously lacked uniformity among various nations [3]. Despite advances in obstetric treatment, it is a frequent pregnancy complication, occurring in around one in 160–200 deliveries per year [4].

Recent studies have shown that miscarriage and stillbirth are associated with some causes including ((genetic factors, Thrombophilias, immune factors, endocrine factors, lifestyle factors, , bacterial, parasitic and viruses infections)), One of the common causes of miscarriage and stillbirth is viral infection, and among these viruses is Parvovirus [5].

Parvovirus B19 (B19V) is a tiny, non-enveloped DNA virus that belongs to the genus Erythrovirus of the family Parvoviridae, B19V is most recognized as the causative agent of erythema infectiosum, a generally mild febrile rash disease that mainly affects children [6]. However, the range of clinical symptoms associated with B19V infection might include asymptomatic conditions including arthritis and arthropathy, as well as chronic or recurring disorders [7]. B19V is typically spread through respiratory droplets, but it can also be transmitted through contaminated blood products. Because of the efficient replication of B19V in the erythroid progenitor cells, the infection can also result in chronic anemia in immunocompromised patients as well as aplastic crisis, which can be fatal. [8].

A fairly prevalent viral infection, particularly among school-aged children, is human parvovirus B19. Due to the mother's lack of immunity, an illness during pregnancy might have an impact on the fetus. Although there is yet no proof that parvovirus B19 causes teratogenic consequences in fetuses, vertical transmission of the virus during pregnancy may induce non-immune fetal hydrops and miscarriage [9]. It is significant to note that B19V can also be transferred from mother to fetus vertically. If this happens, it may result in severe fetal anemia, miscarriage, fetal death, or hydrops fetalis. One-third of pregnant women with acute B19V infection are at risk for vertical transmission [10]. This study aimed to investigate the seroprevalence rate of anti-B19V IgG and IgM antibodies among women with miscarriage and stillbirth in Mosul City.

### **Patients and Methods**

This case-control study was conducted on 160 pregnant women between the ages of 16 and 45 years, and these pregnant women were admitted to Al-Khansaa Teaching Hospital in Mosul city. Samples were collected during the period from November 2022 and January 2023. This study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine- Al-Nahrain University. Study groups included: Group A: 40 pregnant women who had miscarriage within the first 20 weeks of pregnancy. Group B: 40 pregnant women who had stillbirth after the 20<sup>th</sup> week of pregnancy. Group C: 40 pregnant women who had not experienced any complication during the first 20 weeks of pregnancy. Group D: 40 pregnant women who had not experienced any complication after 20 weeks

of pregnancy. Exclusion Criteria: women who had Diabetes mellitus, hypertension, antiphospholipid syndrome, positive TORCH, urinary tract infections, and patients had known chromosomal anomalies or had

family history of genetic diseases that cause abortions .

Serum samples were separated from the whole blood and used for detection of anti-parvovirus B19 IgG and IgM antibodies by ELISA study, Table (1).

**Table (1):** List of ELISA kits used in this study with their manufacturer company and origin

No.	Kits	Origin
1	H. Parvovirus B19 IgG, ELISA Kit (Quantitative)	SunLong . China
2	H.Parvovirus B19 IgM, ELISA Kit (Quantitative)	SunLong . China

These ELISA kits use Sandwich-ELISA as the technique. The Microelisa strip plate provided in this kit has been pre-coated with an antigen specific to B19-Ab. Standards or samples are added to the appropriate Microelisa strip plate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for B19-Ab is added to each Microelisa strip plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain B19-IgG and Horseradish Peroxidase conjugated B19 antigen will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The optical density value is proportional to the concentration of B19-Ab. you may determine the amount of B19 viral Ab. present in the samples by

comparing the optical density of the samples to the standard curve.

### Statistical Analysis

The Statistical Analysis IBM SPSS Statistics 26 program was used to detect the effect of different factors on study parameters. One-way ANOVA and T-test was used to significantly compare between means. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study. GraphPad Prism 9 program was used to draw the figures in this study (166,167).

### Results

The mean age of the patients and control was (28.96±6.38), (27.86±6.53) respectively Table (2), with no significant difference between them which means that they are comparable in age.

**Table (2):** Mean age values of the study groups

		Groups		Subgroups			
		Patients	Control	Group A	Group C	Group B	Group D
Age/ years	Mean	28.96	27.86	28.63	28.88	29.3	29.85
	Standard Deviation	6.38	6.53	6.73	6.91	6.06	6.05
	Minimum	16	18	16	18	18	18
	Maximum	44	42	44	42	42	38
P value		0.364 <sup>NS</sup>		0.759 <sup>NS</sup>		0.344 <sup>NS</sup>	

\* NS: not significant

Additionally, Table (3) demonstrated no significant deference in the mean gestational age (GA) which is demonstrated in details in Table (4) . In which the mean GA in group A

of patients was 9.55±304 weeks, and the mean GA in group B of patients was 26.75±4.5 week.

**Table (3):** Comparison of the gestational age between patients and control

		Group		P value
		Patients	Control	
Gestational Age/weeks	Mean	18.15	18.24	0.954 <sup>NS</sup>
	Standard Deviation	9.46	9.63	

\* NS: not significant

**Table (4):** Comparison of the gestational age among the four groups

		Study Goups			
		Group A	Group C	Group B	Group D
Gestational Age /weeks	Mean	9.55	9.6	26.75	26.88
	Standard Deviation	3.04	3.02	4.5	5.06
P value		0.891 <sup>NS</sup>		0.832 <sup>NS</sup>	

\* NS: not significant

The study subjects' hemoglobin (Hb) levels were taken from their reports and Hb value less than 11g/dl is regarded anemia in pregnancy, Table (5) showed significant

deferent between the mean Hb level in patient and control p=0.034, and 26.20% of patient had low Hb level as compared to 16.20% in the control.

**Table (5):** Study subjects' Hb values and comparison of the number of anemic patients and controls

		Group		P value
		Patients	Control	
Hb g/dl	Mean	11.56	11.91	0.034*
	Standard Deviation	1.09	0.97	
Anemia	Normal	59	67	0.088 <sup>NS</sup>
	%	73.80%	83.80%	
	Anemia	21	13	
	%	26.20%	16.20%	

\* P value is significant, NS: not significant

**Table (6):** Comparison of the subjects' Hb values among the four groups

		Study Goups			
		Group A	Group C	Group B	Group D
Hb g/dl	Mean	11.96	11.97	11.16	11.85
	Standard Deviation	0.92	0.93	1.1	1.02
P value		0.891 <sup>NS</sup>		0.005*	

\* P value is significant, NS: not significant

On comparing the presence and absence of anemia among the subgroups there was no

significant deference as demonstrated in Table (7).

**Table (7):** Comparison of the presence of anemia among the four groups

		study groups			
		Group A	Group C	Group B	Group D
Anemia	Normal	33 82.50%	34 85.00%	26 65.00%	33 82.50%
	Anemia	7 17.50%	6 15.00%	14 35.00%	7 17.50%
p value		0.998 <sup>NS</sup>		0.126 <sup>NS</sup>	

\*NS: not significant

**Results of ELISA study of anti-B19V IgG and IgM antibodies**

In the current study anti-B19V IgG seroprevalence rate among pregnant women was about 23% (37 out of 160), and IgM was about 22% (35 out of 160). Divided into (40%) 32 out of 80 patients, and (6.2%) 5 out

of 80 controls had positive anti-B19V IgG antibody, which is significantly higher in the patients than in the controls (p<0.001). In addition, the median IgG titer was also significantly higher in patients than in the controls (p=0.001), Table (8).

**Table (8):** comparison of the anti-B19V IgG values between the patients and controls

		Group		P value
		Patients	Control	
IgG Titer	Median	27.53	24.84	0.001**
	Percentile 05	17.48	10.75	
	Percentile 95	55.58	45.44	
IgG	Negative	48 60.00%	75 93.80%	
		Positive	32 40.00%	5 6.20%
	p value		<0.001	
odds ratio (95% CI)		4.512	1.97-10.32	

\*\* p value highly significant

Similarly, as shown in the Table (9); (61.3%) 31 of 80 patients (38.8%) had positive IgM for parvovirus B19 and compared to (5%) 4

at of 80 normal pregnant control, which significantly higher both quantitatively and qualitatively, P <0.001.

**Table (9):** comparison of the anti-B19V IgM values between the patients and controls

		Group		P value
		Patients	Control	
IgM Titer	Median	4.5	3.3	<0.001**
	Percentile 05	2.42	1.25	
	Percentile 95	8.35	6.51	
IgM	Negative	49 61.30%	76 95.00%	
		Positive	31 38.80%	4 5.00%
	p value		<0.001	
odds ratio (95% CI)		5.32	2.09-13.52	

\*\* p value highly significant

On the other hand, median serum IgM titer for anti-parvovirus B19 is significantly higher among both Patients who had miscarriage and who had stillbirth as compared to the normal groups, P values 0.007 and 0.001 respectively, and the median

IgM titer was 4.94 in women with stillbirth while it was 3.87 in women who had miscarriage. Also, IgG titer was 35.1 in in women with stillbirth while it was 22.1 in women who had miscarriage, Table (10).

**Table (10):** Comparison of the anti-B19V IgG and IgM values among the four groups

		Study Goups			
		Group A	Group C	Group B	Group D
IgG Titer	Median	22.1	25.32	35.1	22.41
	Percentile 05	15.88	14.69	24.31	9.52
	Percentile 95	48.77	31.51	59.78	69.15
P value		0.672 <sup>NS</sup>		<0.001**	
IgM Titer	Median	3.87	3.14	4.94	4
	Percentile 05	2.42	1.2	2.51	1.29
	Percentile 95	7.14	4.9	8.6	8.7
P value		0.007*		0.001**	

\* NS: not significant, \* P value is significant, \*\* p value highly significant

Additionally, in Table (11) the number of IgM positive cases in both groups who had miscarriage and who had stillbirth was significantly higher than in the control group, P= 0.001. The number of patients had

positive IgG was 25 in stillbirth group and 7 in miscarriage group, additionally, the number of patients had positive IgM was 19 in stillbirth group and 12 in miscarriage group.

**Table (11):** Comparison of the number of positive anti-B19V IgG and IgM among the four groups

		study groups			
		Group A	Group C	Group B	Group D
IgG	Negative	33	39	15	36
		82.50%	97.50%	37.50%	90.00%
	Positive	7	1	25	4
		17.50%	2.50%	62.50%	10.00%
p value		0.056 <sup>NS</sup>		<0.001**	
Odds ratio		8.27 (1.32 to 95.22)		15 (4.38 to 43.69)	
		study groups			
		Group A	Group C	Group B	Group D
IgM	Negative	28	39	21	37
		70.00%	97.50%	52.50%	92.50%
	Positive	12	1	19	3
		30.00%	2.50%	47.50%	7.50%
p value		0.001**		0.001**	
Odds ratio		16.71 (2.6 to 183.2)		11.16 (3.23 to 37.85)	

\* NS: not significant, \*\* p value highly significant

**Table (12):** The relation between IgG positivity and anemia among patients and controls

Group			anemia		Total	P value
			Normal	Anemia		
Patients	IgG	Negative	39	9	48	0.055 <sup>NS</sup>
			81.20%	18.80%	100.00%	
	Positive	20	12	32		
		62.50%	37.50%	100.00%		
	Total		59	21	80	
		73.80%	26.20%	100.00%		
Control	IgG	Negative	63	12	75	0.598 <sup>NS</sup>
			84.00%	16.00%	100.00%	
	Positive	4	1	5		
		80.00%	20.00%	100.00%		
	Total		67	13	80	
		83.80%	16.20%	100.00%		

\* NS: not significant

On studying the association of IgG positivity of parvovirus B19 with presence or absence of anemia there was no significant relation among both patient and control, as shown in Table (12). On the other hand, Table (13) showed that IgM positivity higher among patient who had anemia ( $P < 0.001$ ), with no significant association between IgM positivity and presence of anemia in the control group.

In addition, on the studying association between PCR positivity of parvovirus B19 and presence or absence of anemia it was significantly higher, in which all the 13 PCR positive patients had anemia as compared to none of the nonanemic patients had positive B19V DNA ( $P < 0.001$ ).

**Table (13):** The relation between IgM positivity and anemia among patients and controls

Group			anemia		Total	P value
			Normal	Anemia		
Patients	IgM	Negative	46	3	49	<0.001 <sup>**</sup>
			93.90%	6.10%	100.00%	
	Positive	13	18	31		
		41.90%	58.10%	100.00%		
	Total		59	21	80	
		73.80%	26.20%	100.00%		
Control	IgM	Negative	64	12	76	0.515 <sup>NS</sup>
			84.20%	15.80%	100.00%	
	Positive	3	1	4		
		75.00%	25.00%	100.00%		
	Total		67	13	80	
		83.80%	16.20%	100.00%		

\*NS: not significant, \* P value is significant, \*\* p value highly significant

## Discussion

Human Parvovirus B19 is a ubiquitous virus, Congenital parvovirus B19 infection during pregnancy has been linked to adverse outcomes, such as miscarriage, fetal anemia, and stillbirth [11].

The study groups were subdivided into four groups group A and C are pregnant women in the first 20 weeks (group A had miscarriage and C had normal uneventful pregnancy till time of taking sample), and groups B and D are pregnant women after the 20th weeks of pregnancy ((group B had stillbirth and D had normal uneventful pregnancy after 20th week of gestation till time of taking sample), in each two groups there was age and gestational age matching as demonstrated by none significant p values.

Congenital parvovirus B19 infection during pregnancy has been linked to adverse outcomes, such as miscarriage, fetal anemia, and stillbirth [11]. The present study showed that the frequency of anti-parvovirus antibodies IgG was 23% and the anti-parvovirus IgM antibody was 22% among the 160 women included in the study, the mean age of the patients and control was ( $28.96 \pm 6.38$ ), ( $27.86 \pm 6.53$ ) respectively.

This seroprevalence is comparable with previous estimates reported worldwide for instance, in a similar study conducted in Diyala - Iraq the results were seropositivity rate was 11.11% for an individual occurrence of parvovirus B19 IgM and 13.33% the rate of human parvovirus B19 IgG [12].

Some investigations' findings disagreed with this conclusion. For example, a research by Adam et al. in Sudan found that pregnant women (n=500) had a seroprevalence of B19 IgG of 61.4%, one person had IgM positivity,

and B19 DNA could not be identified using PCR [6].

In a different research conducted in Mwanza, Tanzania, by Mirambo et al., 258 pregnant women with a median age of 21 (19–25) years were tested using ELISA for parvovirus B19 IgM and IgG prevalence; B19 IgM prevalence was 32.8%, while B19 IgG prevalence was 55% [13].

Another study conducted in Nigeria for 122 pregnant women who suffered miscarriage and stillbirth, the prevalence of IgG antibodies reached 44% and IgM antibody prevalence reached 41%. [14] In a cohort study conducted in Italy, 1893 samples were examined from pregnant women. (69.5%) samples were IgG-positive, (1.1%) IgM-positive. Of the 21 IgM-positive women, (1%) were confirmed positive also by Polymerase chain reaction [15].

Also, a study was conducted in Tanguiéta, a rural district of Atacora, in northern Benin, among 227 women suffered from miscarriage and the stillbirth during pregnancy, (67.4%) were positive for anti-B19V IgG, and (3.1%) for IgM. After that, the virus DNA was detected by PCR and was positive in 1.3% [16].

In a study conducted in Zanjan, 110 pregnant women who sought care at the Mousavi Hospital in Zanjan over the course of a year were examined in terms of the findings of serologic and Real-Time PCR tests in an effort to identify Parvovirus B19 infection. Real-Time PCR findings revealed that none of the 110 pregnant women who were the subject of the investigation had Parvovirus B19 infection. However, serology research revealed that in 2 (1.8%) IgM and



41 (44.1%) IgG of the patients were both positive [17].

The reasons of this variation could be due to geographical regional variation, method of investigation and kits variability, variation in age of the study groups, and most importantly the presence of B19V epidemic at the time of sampling [18].

The most common risk factors mentioned in the literature for B19V infection is the presence of virus epidemic that increases the risk of transmission to the none-immune pregnant females especially from young children [18].

However, to the best of our knowledge there was no reported epidemic of B19V infection in Mosul City at the time of sampling (despite the fact that there was no screening for such virus, so that epidemic cannot be definitely excluded).

On the other hand, the current study demonstrated a high association between the presence of maternal anemia and B19V IgM positivity which might be a risk for infection or a consequence of infection which can be found in other studies on maternal B19 infections [19,20] In addition, several studies on B19V- associated anemia whether mild, chronic, or aplastic anemia due to the viral tropism to erythrocytes, the virus could be a risk factor for anemia in pregnant women and fetal anemia also which is the most important risk factor for fetal loss [21,22,23].

On the other hand, unlike other vertically transmitted viruses, B19 can cause fetal infection in the second and also the third trimesters of pregnancy and might cause fetal loss, which is mainly due to the main effect of the virus that is anemia and damage to the fetal liver and heart (due to expression of P

antigen in these organs), rather than teratogenic effect of the virus (which is not proved yet), this could explain the common effect of the virus in the second trimester rather than the first trimester like other teratogenic viruses [24].

This supports the results of the present study, in which anti-viral IgM and IgG antibodies are more frequent in group B (pregnancy loss after 20 weeks of gestation) than in group A (pregnancy loss before 20 weeks of gestation), even the titers of IgM and IgG are higher in group B than group A. In conclusion, anti-B19V antibodies are not as high as in some other countries, however the high incidence of these antibodies among patients with miscarriage and stillbirth as compared to normal control made it an important risk factor that needs screening during pregnancy to avoid such complications.

### **Conclusions**

1- Parvovirus B19 DNA, IgM and IgG antibodies are all more frequent in women who had stillbirth than in women who had miscarriage, even the titers of IgM and IgG are higher. And all these virus parameters are higher among patients with these bad pregnancy outcomes as compared with normal controls.

2-There is high association between Parvovirus B19 infection and maternal anemia.

3- The seroprevalence rate of anti-B19V IgG among pregnant women was about 23% (37 out of 160), and IgM was about 22%

### **Recommendations**

1-In view of the seroprevalence results in this study it is recommended to make screening test for Parvovirus B19 antibodies among

pregnant women at least every three months even in the second half of pregnancy, with increasing frequency of screening in women who had anemia during pregnancy

2-Study the seroprevalence of Parvovirus B19 among children and adults in Iraq to have an obvious idea about the frequency of this infection in Iraq, and follow up of virus epidemics that should be detected because they are dangerous on pregnant women.

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**Conflict of interest:** Nil

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## دراسة الانتشار المصلي لفيروس Parvovirus B19 بين النساء المصابات بالإجهاض في مدينة الموصل

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### الملخص

**خلفية الدراسة:** يعد الإجهاض وولادة الجنين ميتاً من أكثر مضاعفات الحمل شيوعاً، ويمكن أن يكون سببها عدة عوامل، من بين هذه العوامل الالتهابات الفيروسية، بعضها ماسخ بشكل واضح والبعض الآخر يرتبط بعيوب الجنين مثل فقر الدم الشديد في حالة فيروس بارفو B19 الذي يمكن أن ينتقل عمودياً مسبباً عدوى الجنين والإجهاض أو ولادة جنين ميت.  
**اهداف الدراسة:** لتقصي الانتشار المصلي لفيروس البارفو B19 بين النساء اللاتي يعانين من الإجهاض والإملاص ومقارنتهن مع النساء الحوامل الطبيعيات في مدينة الموصل-العراق.

**المرضى والطرائق:** دراسة الحالات والشواهد التي أجريت على الحوامل الذين تتراوح أعمارهم بين 16-45 سنة الذين يعانون من الإجهاض والإملاص الذين تم إدخالهم إلى مستشفى الخنساء التعليمي في مدينة الموصل. تم جمع العينات خلال الفترة من نوفمبر 2022 ويناير 2023 من 160 امرأة حامل، 80 منهن مصابات بالإجهاض أو ولادة جنين ميت، والـ 80 الأخريات حاملهن طبيعي غير مكتمل (السيطرة). تم إخضاع عينات المصل لدراسة ELISA للأجسام المضادة لـ B19V IgM و IgG. **النتائج:** أظهرت هذه الدراسة أن معدل الانتشار المصلي لمضاد B19V IgG بين النساء الحوامل بلغ حوالي 23% (37 من 160)، مقسمة إلى 32 من أصل 80 مريضاً، و(6.2%) 5 من 80 شاهد. كان لديه جسم مضاد إيجابي لـ B19V IgG والذي يختلف بشكل كبير. وكان معدل الانتشار المصلي لـ IgM حوالي 22% (35 من 160)، حيث كان (38.8%) 31 من 80 مريضاً، و(5%) 4 من 80 ضابطاً لديهم جسم مضاد إيجابي لـ B19V IgM. كان كلا من الأجسام المضادة لـ B19V IgM و IgG أعلى بين المرضى الذين ولدوا ميتين مقارنة بأولئك الذين تعرضوا للإجهاض من الناحيتين النوعية والكمية. من ناحية أخرى، هناك ارتباط كبير للغاية بين إيجابية الأجسام المضادة لـ B19V IgM وفقر الدم لدى الأمهات.

**الاستنتاجات:** وجدت الدراسة ارتباطاً عالياً بين B19V والإجهاض أو ولادة جنين ميت مقارنة بالحمل الطبيعي، وكان هذا أكثر شيوعاً بين المرضى الذين ولدوا ميتاً مقارنة بالنساء اللاتي أجهضن، مع ارتباط واضح بفقر الدم لدى الأمهات، كل ما سبق يكون بين النساء الحوامل. من مدينة الموصل في العراق.

**الكلمات المفتاحية:** الأجهاض , ولادة جنين ميت

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