THE ASSOCIATION OF ACUTE HUMAN PARVOVIRUS B19 INFECTION AND SPONTANEOUS MISCARRIAGE IN BASRAH, IRAQ

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ABSTRACT

A case control study was carried out during October 2008 till September 2009 in Basrah. The study was designed to evaluate the occurrence of acute human parvovirus B19 infection among pregnant women and its association with spontaneous abortion.

A total of 182 blood samples were collected from the outpatient clinic, wards and emergency unit at Basrah Maternity and Children Hospital, 91 from women with spontaneous abortion and 91 from women with normal pregnancy as control matching cases in age and gravidity. Maternal serum was kept frozen at –20$^{\circ C}$ until serological investigation by Enzyme-linked immunosorbent assay to detect parvovirus B19 IgM (ELISA-B19-IgM), then the positive cases were investigated to determine the level of interferon-gamma by ELISA kit and acute phase protein C-reactive protein (CRP) by latex agglutination test.

The overall prevalence of parvovirus B19 in study population was 53.8% for cases and 41.8% for control. All B19 positive cases were positive for interferon-gamma in different titers and 77.6% of B19-IgM positive cases were positive to CRP as well. The data indicate that the diagnosis of B19 infection during pregnancy should be considered more often, particularly in complete and missed types of abortion, as missed abortion represents more than 50% of B19 seropositive of spontaneously aborted ladies. Significantly ($P<0.05$) high percentage of B19 positive cases as well as fetal losses were observed in the second trimester. Also the results showed significant difference in B19 antibodies in relation to gravidity ($P<0.05$).

The main conclusion that human parvovirus B19 is common with high prevalence rates among pregnant in our region, and cases of complete and missed abortion should be investigated to exclude parvovirus B19 infections.

INTRODUCTION

Exposure to and infection by human parvovirus B19 can lead to serious complications during pregnancy may result in fetal anemia, spontaneous abortion and hydrops fetalis.[1] About 30-40% of women are non-immune and do not possess neutralizing antibodies to B19, therefore are susceptible to infection by this virus. Pregnant women are most susceptible to B19 infection during epidemics and also when exposed to infected children at home.[2] Fetal death usually occurs 4-6 weeks post B19 symptomatic infection.[3] Fetal loss as a consequence of intrauterine B19 infection is highest in the first 20 weeks of gestation.[4] Parvovirus B19 has tropism for erythroid progenitor cells.[5] Parvovirus B19 replication within erythroid progenitor cells leads to apoptosis, fetal anemia is an underlying factor in the development of hydrops.[6] TH1 cells are the key regulators of cellular immunity. The cytokine primarily responsible for their proinflammatory action is interferon-gamma. Interferon-gamma induces various cells to secrete tumor necrosis factor-alpha (TNF-^{alpha}) and chemokines.[7] To date INF-gamma and TNF-alpha are the best investigated antiviral cytokines in patients with B19 infections. CD4+ T-cells, CD8+T-cells and NK-cells provide the main sources of INF-gamma. These cytokines are known to be harmful to concept.[8] This study aimed at determination of acute human parvovirus B19 occurrence among pregnant women and the association between acute parvovirus B19 infection and spontaneous abortion, and to study the proinflammatory mediators of cellular response (interferon-gamma) in association with recent B19 infection and to estimate the parameter of acute inflammatory (CRP) as acute phase protein.

MATERIALS AND METHODS

This case control study was carried out during October 2008 till Sept 2009. A total of 182 blood samples were collected from patients

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attended the outpatient clinics, wards and emergency unit at Basrah Maternity and Child Hospital. The study group comprised 91 pregnant women present with first and second trimester spontaneous abortion. The control groups comprised 91 normal pregnant women matching to cases in age and gravidity and they had no previous history of miscarriage, still birth, gestational hypertension or low birth weight, their age ranged from 15-45 years, they are collected from general population of Basrah. All relevant information were obtained from both cases and control groups using special questionnaire form. Each individual had one questionnaire form and was informed about the study and an agreement was taken. Blood was drawn from the veins of both patients and control using a sterile disposable syringes, 5ml of venous blood was taken and serum was kept frozen at -20ºC until the serological detection of human parvovirus B19-IgM antibody and human interferon-gamma by ELISA and CRP by latex agglutination test were performed. Recombinant human parvovirus B19 ELISA kit (DRG instrument GmbH: DRG-NOVUM branch Lab/Germany/catalog No. 3504) was used for the determination of acute parvovirus B19 infection according to the manufacturer's instructions. Human interferon-gamma was quantified using Bioassay ELISA kit produced by USA biological, South Texas/USA/catalog No. 17661-65B which is based on the international reference standard human interferon-gamma. Rapid latex agglutination Avitex CRP kit provided by Omega diagnostics, Scotland/UK. All the procedures were done according to the manufacturer's instructions including the interpretation of the cut-off values. Statistical analysis was performed using SPSS version -15. A P-value of <0.05 was considered to indicate statistical significance.

RESULTS

The study population divided into two groups; women with spontaneous abortion (study group) and women with no miscariage (control group). The overall prevalence of parvovirus B19 among study population is shown in (Table-1). The highest seropositivity was observed among study group (53.8%) compared with control group (41.8%). There was no statistical difference between study group and control group (P>0.05).

Table 1. Distribution of cases and control according to seropositivity to parvovirus B19 IgM antibody

B19 IGM antibody	Study group		Control group		Total	
	No.	*%*	*No.*	*%*	*No.*	*%*
Seropositive	49	53.8	38	41.8	87	47.8
Seronegative	42	46.2	53	58.2	95	52.2
Total	91	100	91	100	182	100
χ^2 = 2.664			df =1		P > 0.05	

The prevalence of parvovirus B19 antibodies in relation to age groups is presented in Table-2. The seropositivity was variable in different age groups, but generally the highest prevalence was observed in age group more than 40 years (60%). The association between age and seropositivity to B19 IgM was statistically not significant (P>0.05).

Table 2. The seroprevalance of B19 IgM antibodies in relation to age groups.

Age range in years	B19 IGM antibodies				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
Less than 20	21	47.7	23	52.3	44	100
20- 40	63	47.4	70	52.6	133	100
More than 40	3	60	2	40	5	100
Total	87	47.8	95	52.2	182	100
$\chi^2 = 0.308$		df =2		P > 0.05		

The seropositivity of parvovirus B19-IgM antibody in relation to pregnants gestational age is summarized in Table-3. The percentage of seropositivity was observed in the second trimester (56.9%) compared to the percentage of women with B19-IgM in first trimester (36.2%). The difference in this seroprevalence between the first and second trimester of pregnancy was statistically significant (P<0.05).

Table 3. Distribution of B19 IgM antibody in relation to gestational age

Gestational age	B19 IGM antibodies				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
1st trimester	29	36.2	51	63.8	80	100
2nd trimester	58	56.9	44	43.1	102	100
Total	87	100	95	100	182	100
Fisher's Exact Test = 0.007				P < 0.05		

Table-4, shows the association between gestational age at the time of miscarriage and parvovirus B19–IgM seropositivity. The highest fetal losses occurred in the second trimester (60.4%) with 67.3 of B19 positive. The association between fetal losses at the first and second trimester of pregnancy and B19 seropositivity was statistically significant (P<0.05).

Table 4. Distribution of B19 IgM antibody in relation to gestational age and fetal losses.

G.A of fetal losses	B19 IGM antibodies				Total	
	Positive		Negative			
	No.	*%*	*No.*	*%*	*No.*	*%*
1st trimester (39.6%)	12	33.3	24	66.7	36	100
2nd trimester (60.4)	37	67.3	18	32.7	55	100
Total	49	100	42	100	91	100
Fisher's Exact Test = 0.002				P < 0.05		

GA: gestational age

The relation between Parvovirus B19 serological results and the gravidity is presented in Table-5 which showed that 53.6% of multigravida women were seropositive compared to 35.1% of primigravida. This difference was statistically significant (P<0.05).

Table 5. Parvovirus B 19 IgM antibody in relation to gravidity.

Gravidity	B19 IGM antibody				Total	
	Positive		*Negative*			
	No.	%	No.	%	No.	%
Primigravida	20	35.1	37	64.9	57	100
Multigravida	67	53.6	58	46.4	125	100
Total	87	100	95	100	182	100
$\chi^2 = 5.377$		df=1		P < 0.05		

The overall seropositivity to parvovirus 19 among women with abortion was 53.8%. Complete abortion carried the highest seropositivity (100%), followed by missed abortion and threatened types (66.7% for both), Table-6. Inevitable abortion (55%) and incomplete abortion (26.9%) represented in a lower rates of seropositivty to parvovirus B19. These differences were statistically significant (P<0.05).

Table 6 . Distribution of B 19 IgM antibodies in relation to types of the abortion.

Type of abortion	B19 IGM antibody				Total	
	Positive		*Negative*			
	No.	%	No.	%	No.	%
Missed abortion	26	66.7	13	33.3	39	100
Incomplete abortion	7	26.9	19	73.1	26	100
Complete abortion	3	100	0	0.0	3	100
Inevitable abortion	11	55	9	45	20	100
Threatened abortion	2	66.7	1	33.3	3	100
Total	49	53.8	42	46.2	91	100
$\chi^2 = 12.943$		df =4		P < 0.05		

The levels of interferon-gamma among the study population were shown in Table-7. There were 63.3% of B19-IgM positive cases were positive to interferon-gamma with titers above 100pg/ml while 36.8% of B19-IgM positive control were positive to interferon-gamma with titers below 100 pg/ml. The difference in the level of interferon-gamma between cases and control was statistically significant (P<0.05).

Table 7. ***The level of interferon γ among the study population.***

Level of interferon γ in PG/ML	B19 IGM positive antibody				Total	
	Study group		*Control group*			
	No.	%	No.	%	No.	%
Below 100 pg/ml (Low level)	18	36.8	24	63.2	42	100
Above 100 pg/ml (High level)	31	63.2	14	36.8	44	100
Total	49	100	38	100	87	100
χ^2 = 5.984			df = 1		P < 0.05	

Among the study group 49(53.8%) were positive to parvovirus B19-IgM and 38/49(77.6%) were positive for CRP (Table-8). However as the levels of anti-B19-IgM increased, the frequency of CRP increased which showed the higher percentage (50%) among those with very high level of anti-B19 IgM a trend which was not observed among the control group. These differences were statistically significant (P<0.05)

Table 8. Correlation of B 19 IgM antibodies level to positivity of CRP .

B19-igm Antibody level	C - reactive protein Positive				Total	
	Study group		*Control group*			
	No.	*%*	*No.*	*%*	*No.*	*%*
Low	1	2.6	5	22.7	6	10.0
Moderate	9	23.7	5	22.7	14	23.3
High	9	23.7	8	36.4	17	28.3
Very high	19	50	4	18.2	23	38.3
Total (49)	38/49 (77.6%)	100	22/38 (22.4%)	100	60	100
χ^2 =10.103			df =3		P < 0.05	

DISCUSSION

The current opinion is that parvovirus B19 infection during pregnancy is influenced relatively by social level, but epidemic incidence rates in pregnant women have not been established. Furthermore, there are no data on the relative importance of parvovirus B19 infections on a population level of different risk factors.[4] Many serological and molecular studies blame human parvovirus B19 infection during pregnancy as causative agent of spontaneous abortion and non-immune hydrops fetalis.[1,2] The present study showed that the seropositivity of parvovirus B19 among pregnant women generally high in Basrah population; in spontaneously aborted and normal pregnant ladies. This is due to the high contagious nature of the virus B19 with no awareness to this viral infection in the community since some of B19 infections occur in mild form and mostly asymptomatic.[9] As many as 50% of women at childbearing age in our community may not immune to parvovirus B19 and are susceptible to infection as shown in this study, there is a state of viral persistency which modulate immune responses a finding which is supported by the presence of B19-IgM in both cases and control. The infection in a long run in asymptomatic form among our population that indicate a stressing pressure imposed by the presence of viral infection on immune responses especially on their low rate of spontaneous replication making availability of various parvovirus B19 proteins in contact with immune system possibly the role of NS-1,

VP1 and VP2 proteins of B19 which have variety of functions.[9] The high titers of parvovirus B19 antibody found in this study is consistent with a study carried out in Basrah 2007 that showed 65.2–72.2% of general population were positive to parvovirus B19-IgG.[10] Another study carried out in Brazil of an outbreak of exanthematous disease was reported that 58.3% of cases were positive to B19-IgM[11] which is almost in agreement with our figure. The current study showed that all parvovirus B19-IgM positive cases associated with an elevated levels of interferon-gamma which is proinflammatory antiviral cytokine, responses early in patients with recent B19 infections. This finding is consistent with a study obtained from Ireland showed higher levels of the inflammatory cytokines (interferon-gamma) were secreted by PBMCs obtained from B19 seropositive individuals than those obtained from seronegative donors.[12] Our data showed that more than 60% of positive B19-IgM cases were positive to interferon-gamma with a titer above 100 pg/ml. The variation in the interferon-gamma levels are probably attributed to the timing of the first sample taken in relation to severity of acute infection and stage of cellular immune responses as B19-IgM positivity persist up to 10 months.[13] It has been reported that CRP is fairly sensitive marker to the onset of inflammatory process,[14] including viral infections.[15] Our study showed a positive correlation between B19-IgM antibody titers and positive cases to CRP (77.6% compared to 22.4%) indicating a good overall agreement between the two tests and may be used as parameter in the prediction of acute B19 cases. This is supported by the finding of that early acute phase of B19 infection were always associated with positive CRP results. Knowledge on the prevalence rate of an infectious agent in relation to age is important in order to identify the target age of population for an effective control measures. The current study showed that the seropositivity to B19 is highest among age group more than 40 years that is because females at child bearing age have high risk factors for maternal infection, a situation which have been described in a Danish population.[3,16] Furthermore easy and rapid mode of spread through respiratory route may play an important role in the increased prevalence of this virus in our community.[17] Our data showed significant difference in parvovirus B19 antibodies among women with spontaneous abortion in relation to gestational age, where the highest percentage of B19-IgM antibody in the second trimester (67.3%) and this is associated with 60.4% of fetal losses occurred in the second trimester, this finding is consistent with many studies.[3,18,19] Parvovirus B19 may be considered as a significant cause of fetal loss throughout pregnancy, but has a higher impact in the second half of pregnancy when spontaneous fetal loss from other causes is relatively rare.[20] Parvovirus B19 infection during pregnancy has been associated with variety of fetal damage and a significant cause of fetal loss.[20] In the current study, types of abortion was determined by ultrasonic and pelvic examination and the serological results demonstrated high rate of B19-IgM seropositivity among ladies with complete type of abortion which may reflect the possible role of B19 infection in the causation of such of abortion. This result is consistent to the suggestion that maternal blood should be tested for parvovirus B19-IgM or on tissue biopsy for parvovirus PCR if patient presented with abortion.[21] In this study a significant difference in parvovirus B19-IgM antibody in relation to gravidity was found. This finding is consistent with the results reported from a study carried out in three regions in Denmark, which showed that the risk of acute parvovirus B19 infection during pregnancy increased significantly with the number of children already born into the family compared to the nulliparous women where the women with 1 child had a more than 3-fold greater risk increased to 7.54-fold in women with 3 or more children.[3]

In conclusion, parvovirus B19 is common and highly distributed among pregnant ladies in our locality and there is a significant association between B19 antibody and interferon-gamma, CRP in different titers. There were a significant relation between parvovirus B19 infection and fetal losses, gravidity and type of abortion, mostly during the second trimester of pregnancy.

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