History of miscarriage as a risk factor for hepatitis C virus infection in pregnant Iraqi women

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سوابق الإسقاط كعامل اخطار للإصابة بالتهاب الكبد «سي» لدى الحوامل العراقيات

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الخلاصة: تم تقييم دور سوابق الإسقاط كعامل اخطار للإصابة بالتهاب الكبد «سي». وذلك بدراسة المصول المأخوذة من 3491 حاملاً، بحثاً عن أضداد فيروس التهاب الكبد «سي»، ثم القيام بتحديد الأنماط الجينية على مصول 94 منهن. وقد وجد أن مجمل الانتشار المصلي لأضداد فيروس التهاب الكبد «سي» يبلغ 3.21٪، وأن هذا الانتشار يتناسب طرداً مع عدد الإسقاطات، وأن الإسقاط هو من عوامل الاختطار الهامة لاكتساب العدوى بفيروس التهاب الكبد «سي» من الإسقاط الأول حتى الإسقاط الخامس، وأن الخطر يزداد بتزايد عدد الإسقاطات. وأن نسبة مرتفعة من النساء اللواتي لديهن سوابق إسقاط لديهن النمط 1b من فيروس التهاب الكبد «سي» مقارنة باللواتي ليس لديهن سوابق إسقاط.

ABSTRACT Sera from 3491 pregnant women were screened for the presence of HCV antibodies (anti-HCV). HCV genotyping was also performed on the sera of 94 women. The overall anti-HCV seroprevalence was 3.21%. Anti-HCV seroprevalence was significantly positively correlated with the number of miscarriages. Miscarriage was a significant risk factor for the acquisition of HCV infection from the first miscarriage up to the fifth, the risk increasing with increasing number of miscarriages. A higher proportion of women with a history of miscarriage harboured HCV-1b compared to those with no miscarriage.

Les antécédents de fausses couches en tant que facteur de risque d'infection par le virus de l'hépatite C chez des femmes enceintes iraquiennes

RESUME La présence d'anticorps anti-VHC a été recherchée dans le sérum de 3491 femmes enceintes. Le génotypage du VHC a également été réalisé sur le sérum de 94 femmes. La séroprévalence globale des anticorps anti-VHC s'élevait à 3,21 %. Il y avait une corrélation positive significative entre la séroprévalence anti-VHC et le nombre de fausses couches. La fausse couche était un important facteur de risque d'infection par le VHC de la première fausse couche jusqu'à la cinquième, le risque augmentant avec le nombre de fausses couches. Une proportion plus élevée de femmes ayant des antécédents de fausses couches étaient porteuses du VHC-1b par rapport à celles qui n'avaient pas eu de fausses couches.

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Introduction

Since hepatitis C virus (HCV) immunoassay and molecular analysis has become commercially available [*1*], several studies have been published describing the seroprevalence of HCV antibodies (anti-HCV) in a variety of populations from both low risk (blood donor volunteers) and high risk groups (intravenous drug users, sex workers, homosexuals)[2]. Pregnant women are considered a good representative group of a healthy population [*3*]. Although HCV was first identified as responsible for almost all cases of post transfusion non A, non-B hepatitis [*1,4,5*], it is now recognized to occur more often from causes other than transfusion [*6*]. Unfortunately there are few studies of HCV seroprevalence among pregnant women, and only limited knowledge of maternal HCV infection, even though pregnant women are at risk of acquiring HCV infection via different sources (including obstetric or gynaecological management) [2]. Miscarriage may be one of these risk factors for HCV infection.

The aim of the current study was therefore to investigate the possible association between HCV infection and a history of previous miscarriage in pregnant women. A further aim was to ascertain whether miscarriage is a risk factor for the acquisition of HCV infection.

Methods

We randomly selected of 3491 apparently healthy women in their third trimester of pregnancy from 19 hospitals and primary health care units in the Baghdad area. Each pregnant woman was asked about the history and number of previous miscarriages. Women with a history of miscarriage occurring less than 9 months before the interview were excluded from the study.

A blood sample was drawn from each participant and serum samples were dispensed into two small screw-capped vials, stored at –20 °C and –70 °C. The former sample was screened for HCV-specific antibodies, while the latter was used to detect HCV-specific RNA and subsequently for genotyping and subtyping.

The initial screening for HCV-specific antibodies was performed on sera from all 3491 pregnant women, using the available commercial kits of a third generation enzyme immunoassay (UBI EIA 4.0, Lake Success, New York, United States of America). According to the manufacturer's instructions, a sample was considered positive if the optical density value was equal to or greater than that of a strong positive reaction control multiplied by 0.2 (cut-off). The positive samples were retested in duplicate. A sample was considered firmly positive when at least two positive results were obtained. Positive sera were then subjected to a confirmatory test using a third generation immunoblotting assay (Lia-Tek III, Organon, Belgium). According to the manufacturer's instructions, a sample was considered positive when the reaction to one antigen was rated at 2+ or higher, or the reaction to two or more antigens rated at 1+ or higher. For data analysis only cases that tested positive by Lia-Tek III were considered HCV-positive.

In addition, 94 sera samples were used for molecular analysis in the laboratories of Sorin Biomedica in Saluggia, Italy. Viral RNA extraction, cDNA synthesis, reverse-transcriptase polymerase chain reaction (RT-PCR) amplification and detection of HCV-specific RNA were performed using a more accurate and recently developed molecular method of DNA enzyme immu-

noassay (DEIA) (GEN-ETi-K-DEIA, Sorin Biomedica, Saluggia, Italy). The DEIA system relies on a monoclonal antibody that selectively hybridizes to double-stranded but not to single-stranded DNA. Hybridization was determined using a microtitre plate reader (vertical reader photometer ETI-system).

A sample was considered positive when the absorbency was equal to or greater than the cut-off value. Serum samples shown to contain HCV-specific RNA by DEIA were subjected to HCV genotype/subtype analysis, also using the DEIA method but using different probes specific for a different HCV genotype/subtype.

Statistical analysis was performed using the chi-squared (χ^2) test with Yates correction, to determine the association between variables. The odds ratios (OR) and 95% confidence intervals (CI) for the magnitude of this association were also calculated. Stepwise logistic regression (r) analysis was applied. $P < 0.05$ was considered significant.

Results

Lia-Tek III detected 112 positive serum samples, giving a true prevalence of anti-HCV of 3.21% in all pregnant women. Women with no history of miscarriage had a significantly lower anti-HCV seroprevalence (60/2519, 2.38%) than those with a history of miscarriage (52/972, 5.35%) (χ^2 = 18.95, P = 0.00001) (Table 1).

Among the 3491 women, there were 1506 miscarriage episodes involving 972 women, with a range of one to seven miscarriages per mother and a mean of 0.44 ± 0.84 for all women. Of the women with miscarriage, 623 (64.1%) had had only one miscarriage and showed a lower prevalence of seropositivity than those with more than one miscarriage (Table 2). Table 2 also shows that anti-HCV seroprevalence increased steadily with the number of miscarriages, with a range of 4.65%–18.75% for 1–5 miscarriages. A statistically significant association (χ^2 = 31.32, P = 0.0001) and a direct positive correlation (r = 0.842, P = 0.035) were detected between the number of previous miscarriages and the rate of anti-HCV seropositivity.

Table 1 **Anti-HCV seropositivity in relation to history of miscarriage among 3491 pregnant women in Iraq**

Anti-HCV serostatus	History of miscarriage					
	Present		Absent		Total	
	No.	%	No.	%	No.	%
Positive	52	5.35	60	2.38	112	3.21
Negative	920	94.65	2459	97.62	3379	96.79
Total	972	27.84	2519	72.16	3491	100

χ^2 = 18.95, P = 0.0001.
HCV = hepatitis C virus.

In order to measure the strength of association, ORs were estimated for various numbers of miscarriages. There was a significant positive trend in the estimated magnitudes of ORs with increasing number of miscarriages, with the demonstration of a significant direct association (r = 0.985, P = 0.0021). This significant association began with the occurrence of the first miscarriage (OR = 2.001, 95% CI: 1.283–3.119) and increased as the number if miscarriages increased (Table 3).

Molecular analysis showed that the prevalence of HCV-specific RNA was significantly higher among pregnant women with a history of previous miscarriage than in those with no miscarriage history (83.3% and 50.0% respectively) (χ^2 = 10.55, P = 0.001) (Table 4). We also found that pregnant women with history of miscarriage were at a significantly higher risk

Table 2 **Anti HCV seroprevalence in relation to the number of miscarriages**

Anti-HCV serostatus	Number of miscarriages (%) 0	1	2	3	4		Total
Positive	60 (2.4%)	29 (4.7%)	13 (5.5%)	5 (7.5%)	2 (6.9%)	3 (18.8%)	112 (3.21%)
Negative	2459	594	224	62	27	13	3379
Total	2519	623	237	67	29	16	3491

χ_5^2 = 31.32, P = 0.00001, r = 0.842, P = 0.035.
Of the 972 women with one or more miscarriage, 64.1% had had one miscarriage, 24.4% 2 miscarriages, 6.9% 3 miscarriages, 3.0% 4 miscarriages and 1.6% = 5 miscarriages.
HCV = hepatitis C virus.

of acquiring HCV infection (OR = 2.2, 95% CI: 1.06–6.73).

Both groups demonstrated similar patterns of HCV genotypes, in single and in a mixed pattern of infection. HCV-1b as a single infection was detected in both groups, but a pattern of single HCV-1b infection was found at a higher rate in mothers with a history of miscarriage (Table 5).

Discussion

HCV infection is a major public health problem worldwide [7]. Many risk factors for the acquisition of HCV infection are specific to women and not men [8]. Any medical or gynaecological intervention during pregnancy, labour, the puerperium or thereafter may be a risk factor for HCV infection [3,8]. The molecular studies carried out provide more evidence that miscarriage may be a risk factor for exposure to HCV (OR = 2.2, 95% CI: 1.06–6.73 to OR = 2.31, 95% CI: 1.60–3.35). A significant association between a history of miscarriage and a high level of HCV-specific antibodies and RNA is in agreement with the findings of an earlier study conducted in France [9],

Table 3 **Significant direct correlation between crude odds ratio (OR) and number of miscarriages**

Anti-HCV positive	χ^2	P*	OR	s	95% CI
1 versus no miscarriage	8.57	0.0040	2.001	0.231	1.263–3.119
2 versus no miscarriage	12.87	0.0001	2.104	0.205	1.419–3.120
3 versus no miscarriage	15.39	0.0001	2.189	0.199	1.496–3.203
4 versus no miscarriage	16.19	0.00005	2.214	0.196	1.52–3.225
5 versus no miscarriage	18.95	0.00001	2.316	0.193	1.601–3.351

**All significant at P < 0.05.*
r = 0.985, standard error = 0.023, P = 0.0021.
HCV = hepatitis C virus.
s = standard deviation.
CI = confidence interval.

Table 4 **History of miscarriage and its association with HCV-RNA seropositivity**

HCV-RNA	History of miscarriage				
	Present		Absent		Total
	No.	%	No.	%	
Positive	30	83.3	29	50.0	59
Negative	6	16.7	29	50.0	35
Total	36		58		94

χ^2 = 10.55, P = 0.001, odds ratio = 2.2, 95% confidence interval = 1.06–6.73.
HCV = hepatitis C virus.

although the rate detected there was higher (47.4%). This variation in the rates can probably be attributed to additional behavioural risk factors (intravenous drug use, unsafe sexual practices or use of alcohol) among the cohort of pregnant French women in that study.

The effect of repeated miscarriage on the acquisition of HCV infection, demonstrated through both antibody and RNA levels in the present study, has not been not reported in any other study. Only the in from France already mentioned showed an increased risk of HCV acquisition with a higher number of induced abortions in pregnant women with more than one episode of pregnancy loss (OR = 8.3, 95% CI: 2.2 39.8) [9]. Our study clearly shows that a woman with a history of a single miscarriage has a significant risk of HCV infection (χ^2 – 8.57, P – 0.0004) and that a single miscarriage could act as a possible risk factor for HCV infection. A possible explanation for the failure of the French study to demonstrate a similar finding [9] is that abortion in France is more usually an elective procedure, while miscarriages among Iraqi women are usually emergencies managed in inadequate conditions of care and sterility, particularly since the economic sanctions imposed by the United Nations.

The mechanism by which HCV infection may be acquired following miscarriage is likely to be the result of many factors. One is nosocomial transmission either as patient-to-patient, health care personnel-to-patient, or through diagnostic or therapeutic material. The direct correlation we observed between the increased prevalence of anti-HCV and a higher number of miscarriages strongly suggests nosocomial transmission. Blood transfusions that may occasionally [7] accompany miscarriage could play a role in the acquisition of HCV infection.

Our study demonstrated an association between HCV genotype and history of miscarriage. Unfortunately there are no comparable data in other studies. The HCV genotype 1b, which was found in a higher

Table 5 **Hepatitis C virus genotype and subtype distribution (single or mixed) by history of miscarriage among 57 pregnant women**

History of miscarriage	Hepatitis C virus genotype and subtype							Total
	1	1a	1b	4	1 and 4	1b and 4	3a and 4	
Present	2	7	7	5	–	5	3	29
Absent	3	6	3	8	3	5	–	28
Total	5	13	10	13	3	10	3	57

proportion of women with a history of miscarriage, is the most pathogenic genotype [*10,11*], and has a greater cumulative effect as it is eliminated from the body more slowly than other HCV genotypes [*12*].

In conclusion, our study provides strong evidence that pregnant Iraqi women are at risk of acquiring HCV infection following each miscarriage and are at risk of acquiring HCV infection following even a single miscarriage. It is still unclear, however, whether HCV infection is a causative factor of miscarriage, especially multiple miscarriage. The high prevalence of HCV infection among pregnant women with a history of miscarriage may provide a clue to an as yet unrecognized mode of viral transmission.

References

1. Busch MP. HIV, HBV and HCV: new developments related to transfusion safety. *Vox sanguinis*, 2000, 78(suppl. 2):253–6.
2. Roudot-Thoraval F et al. Anti-HCV seroprevalence in pregnant women in France. *Gut*, 1993, 34(suppl. 2):S55–6.
3. Caspari G et al. Non-specific and specific anti-HCV results correlated to age, sex, transaminase, rhesus blood group and follow-up in blood donors. *Archives of virology*, 1997, 142:473–89.
4. Alter MJ et al. Risk factor for acute non-A, non-B hepatitis in the United States and association with hepatitis C infection. *Journal of the American Medical Association*, 1990, 264:2231–5.
5. Goldman M, Long A. Hepatitis C lookback in Canada. *Vox sanguinis*, 2000, 78(suppl. 2):249–52.
6. Allain JP. Emerging viruses in blood transfusion. *Vox sanguinis*, 2000, 78 (suppl. 2):243–8.
7. Alter MJ et al. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. *New England journal of medicine*, 1992, 327: 1899–1905.
8. Love A et al. Characteristics of hepatitis C virus among intravenous drug users in Iceland. *American journal of epidemiology*, 1996, 143:631–6.
9. Dubois F et al. Hepatitis C in a French population-based survey, 1994: seroprevalence, frequency of viremia, genotype distribution, and risk factors. The Collaborative Study Group. *Hepatology*, 1997, 25:1490–6.
10. Mihm S et al. Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. *Hepatology*, 1997, 25:735–9.
11. Nousbaum JB et al. Hepatitis C virus type 1b (II) infection in France and Italy. Collaborative Study Group. *Annals of internal medicine*, 1995, 122(3):161–8.
12. Pozzetto B et al. Structure, genomic organization, replication, and variability of hepatitis C virus. *Nephrology, dialysis, transplantation*, 1996, 11(suppl. 4):2–5.