International Journal of Infectious Diseases 39 (2015) 20–24

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Cholera in pregnancy: Clinical and immunological aspects

Ashraful I. Khan [a], Fahima Chowdhury [a], Daniel T. Leung [a,f], Regina C. Larocque [b,c], Jason B. Harris [b,c], Edward T. Ryan [b,c,e], Stephen B. Calderwood [b,c,d], Firdausi Qadri [a,*]

[a] Centre for Vaccine Sciences, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh
[b] Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA
[c] Department of Medicine, Harvard Medical School, Boston, MA, USA
[d] Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA
[e] Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA
[f] Division of Infectious Diseases, University of Utah, Salt Lake City, UT, USA

ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 13 July 2015
Accepted 9 August 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Cholera
Immune response
Pregnancy

SUMMARY

Background: The objective of this study was to examine the clinical and immunological features of cholera in pregnancy.
Methods: Women of reproductive age presenting to the icddr,b Dhaka hospital with cholera, and enrolled as part of a larger cohort study, were tested for pregnancy on admission. We compared initial clinical features and immune responses of pregnant patients with non-pregnant female patients at days 2, 7 and 21 after infection.
Results: Among reproductive age women enrolled between January 2001 and May 2006, 9.7% (14/144) were pregnant. The duration of diarrhoea prior to admission tended to be higher in pregnant compared to non-pregnant patients (p=0.08), but other clinical characteristics did not differ. Antibody responses to cholera toxin B subunit (CtxB), toxin-coregulated pilus A (TcpA), *Vibrio cholerae* lipopolysaccharide (LPS), and serum vibriocidal antibody responses, were comparable between pregnant and non-pregnant patients. There were no deaths among the pregnant cases or non-pregnant controls, and no adverse foetal outcomes, including stillbirths, during 21 days of follow up of pregnant cases.
Conclusions: To our knowledge, this is the first report of immune responses in pregnant women with cholera. We found that pregnant woman early in pregnancy has comparable clinical illness and subsequent immune responses compared to non-pregnant women. These findings suggest that the evaluation of safety and immunogenicity of oral cholera vaccines in pregnancy should be an area of future investigations.

1. Introduction

Cholera is a life threatening diarrheal disease caused predominantly by infection with *Vibrio cholerae* O1. Though cholera is rare in developed countries, it is prevalent in many areas of South and Southeast Asia and in Africa and may also cause major outbreaks worldwide.[1] Bangladesh is a country in South Asia where cholera is endemic and is consistently present throughout the year in high risk areas.[2] Cholera toxin (CT), the primary toxin produced by *V. cholerae* O1 and O139, causes the hypersecretion of electrolytes and water, sometimes with fatal results. The lipopolysaccharide of *V. cholerae* is an important determinant of protection, and is the primary antigen found in the most recent formulations of the oral cholera vaccine (OCV).

Pregnancy is an immuno-altered state where both humoral and cellular immunity are affected.[3,4] Several pregnancy outcomes, including preeclampsia, poor foetal growth, and preterm birth, have been linked to abnormalities in immune responses during pregnancy.[5–7] Pregnancy has also been associated with decreased inflammatory responses and increased anti-inflammatory responses to immune challenges in humans as well as in animal models.[8,9] In some cases, pregnant women are more susceptible to

* Corresponding author. Centre for Vaccine Sciences, icddr,b, 68 Shahid Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh. Tel.: +880 2 9827001 10; fax: +880 2 8823116/2 8826050x2431.
E-mail address: fqadri@icddrb.org (F. Qadri).

http://dx.doi.org/10.1016/j.ijid.2015.08.006

certain infections, and when infected, may experience a higher severity of illness. For example, pregnant women infected with influenza virus are at increased risk for serious complications when compared to other groups,[10] though a recent study on influenza virus vaccine during pregnancy showed that pregnancy did not significantly alter antibody responses.[11]

Women living in areas endemic for cholera are at risk of acquiring the disease during pregnancy, and studies from South Asia, Africa, and Haiti have demonstrated that cholera during pregnancy may increase the risk of poor outcomes.[12] However, there is a lack of data on the immunological responses to cholera during pregnancy to determine if vaccination might play a role in prevention. Thus, the objective of this study was to examine the clinical characteristics and immunological responses of pregnant women following severe cholera.

2. Materials and Methods

2.1. Study population and patient enrolment

The Cholera Immune Response Study (CIRS) was a prospective, observational study, undertaken as a collaboration between the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) and Massachusetts General Hospital in Boston. The icddr,b in Dhaka, Bangladesh cares for approximately 120,000 patients with diarrheal diseases each year. Patients presenting to the icddr,b Dhaka hospital with acute watery diarrhoea (study day 1) were eligible for inclusion in this study if their stool cultures were subsequently positive for *V. cholerae*, and if they were without significant co-morbid conditions; patients were enrolled prospectively in the study between 2001 and 2006.[13,14] Stool cultures for *V. cholerae* were done on taurocholate-tellurite-gelatin agar (TTGA). After overnight incubation of plates, serological confirmation of suspected *V. cholerae* colonies was carried out by slide agglutination.[15] Patients were enrolled on day 2 of admission (study day 2) if a stool culture was positive for *V. cholerae* O1 or O139. Information regarding clinical features, demographics, and history of diarrhoea were collected from patients at enrolment. Samples of venous blood were collected, for determining antibody titers, from patients on study day 2 and again at follow-up visits on study days 7 and 21. Informed written consent for participation in this research was obtained from participants or their guardians. This study was reviewed and approved by the Ethical and Research Review Committees of the icddr,b and the Institutional Review Board of the Massachusetts General Hospital.

2.2. Pregnant women and case control comparison

All women of reproductive age (15-49) enrolled in the CIRS study were screened for pregnancy by urine strip test (hCG One Step Pregnancy Test Strip, TUV product service, USA) on enrolment. A total of 14 women had a positive pregnancy test. We also selected all non-pregnant cases as controls from the same age cohort.

2.3. Treatment of patients

Patients enrolled for the study received the normal standard of care provided at the icddr,b for cholera. Dehydration was corrected either by infusing intravenous cholera saline or by oral rehydration solution depending on the severity of the dehydration and clinical condition of the patient. A short course of oral antibiotics was given. Non pregnant adult females with stool culture positive for *V. cholerae* received 300 mg of doxycycline in a single dose, whereas pregnant women with cholera received erythromycin (500 mg every six hours) for three days.

2.4. Immunological assessment

We measured vibriocidal antibodies in patient plasma using *V. cholerae* O1 El Tor Ogawa (strain X25049) or Inaba (strain 19479) or *V. cholerae* O139 (strain 4260B) as target bacteria, and adding guinea pig complement.[16] Antibody responses to recombinant cholera toxin B subunit (CtxB), toxin-coregulated pilus A subunit (TcpA), and lipopolysaccharide (LPS) were determined using a kinetic enzyme-linked immunosorbent assay (ELISA), as previously described.[17] In all cases, the vibriocidal and LPS responses were measured to the same serotype, Ogawa or Inaba of *V. cholerae* O1 and *V. cholerae* O139 as was present in the patient.

2.5. Data analyses

Data analyses were performed using SPSS for Windows (version 12; SPSS Inc., Chicago, IL, USA) and Epi Info (version 6.0; USD, Stone Mountain, GA, USA). For non-normally distributed data, comparisons were carried out as median (25th–75th percentile) using the Mann–Whitney U-test. The significance of differences in proportions was evaluated by the Chi-square test, and Fisher's exact test was applied when appropriate. A p-value of <0.05 was considered statistically significant.

3. Results

A total of 399 patients with cholera were enrolled in the study between January 2001 and May 2006 and the data obtained prospectively were analysed in a retrospective fashion. Overall, 144 cholera patients were reproductive age (15-49 years) women and of those, 9.7% (14/144) had a positive pregnancy test. Average duration of pregnancy was 14 weeks at enrolment (minimum 6 weeks, maximum 24 weeks, mean ±SD = 14±5.6 weeks) (data not shown). The clinical and microbiological features of the pregnant and non-pregnant cholera cases are presented in Table 1.

Table 1
Clinical and microbiological features of pregnant and all non-pregnant study participants

Characteristics	Pregnant (n=14)	Non-pregnant (n=130)	p-value
Demographics			
Median age (yr), (25^{th}, 75^{th} percentile)	24.5 (21 - 29)	27 (21 - 36)	0.14
Blood group, n (%)			
O	4 (28.6)	58 (44.6)	
A	2 (14.3)	36 (27.7)	
B	6 (42.9)	31 (23.9)	
AB	2 (14.3)	5 (3.9)	0.09
Clinical			
Dehydration			
Some	1 (7.1)	9 (6.9)	
Severe	13 (92.9)	121 (93.18)	1.00
No. of stools (> 20 in last 24hrs)	10 (71.4)	111 (85.48)	0.24
Fever in the last week	2 (14.3)	27 (20.8)	0.74
Vomiting in the last 24hrs			
No or <10 times	5 (35.7)	65 (50.0)	
≥ 10 times	9 (64.3)	65 (50.0)	0.40
Abdominal pain at presentation	11 (78.6)	93 (71.5)	0.76
Helminth present	2 (14.3)	16 (12.3)	0.69
Received IV fluid	13 (92.9)	126 (96.9)	0.41
Duration of diarrhoea, hr			
Median (IQR)	100 (9 - 105)	12 (7 - 23)	0.08
Duration of stay, hr			
Median (IQR)	102 (41 - 104)	103 (100 - 105)	0.25
Microbiological, n (%)			
Dark field positive stool			
V. cholerae O1, Ogawa	3 (21.4)	41 (31.5)	0.55
V. cholerae O1, Inaba	7 (50.0)	66 (50.8)	1.00
V. cholerae O139	4 (28.6)	23 (17.7)	0.30