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Original Article

Seroprevalence of dengue infection in pregnant women and placental antibody transfer

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ABSTRACT

Background: Pregnant women and infants are vulnerable for developing severe dengue. This study was conducted to determine the seroprevalence of dengue infections among pregnant women, their offsprings and its association with outcomes.

Methods: A cross-sectional study was conducted among pregnant women, admitted for delivery in a tertiary mother and child hospital in Thiruvananthapuram. Blood specimens (2 ml) were collected from the women during hospitalization in the first stage along with blood samples being drawn for other investigations. Umbilical cord blood was collected from the neonates. The samples were tested using IgG enzyme-linked immunosorbent assay (ELISA). Quantitative titres were also obtained, and index ratios were calculated using optical density values.

Results: Seroprevalence of dengue in antenatal women was 6.9% (95% confidence interval [CI]: 3.4–12.48). Among cord blood samples, the seropositivity was 10.8% (95% CI: 6.3–16.6). A significant correlation (Spearman rho: 0.653 and p value <0.001) was obtained between maternal and cord sample IgG index ratios. Agreement between maternal and cord blood IgG values was obtained using kappa as 0.742. The mean weight of newborns born to IgG-positive mothers was significantly lower than babies of IgG-negative mothers (2.3 vs 2.8; t = 2.64; p = 0.01). The mean gestational age at delivery was also significantly lower in IgG-positive mothers (36.29 vs 38.04 weeks; t = 2.48; p = 0.01).

Conclusion: Seroprevalence of dengue in antenatal women and in their offsprings is lower than other areas endemic for dengue. Dengue infection (any time before pregnancy) may result in preterm delivery and low birth weights.

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Introduction

Dengue is an emerging tropical disease. It is one of the most serious disease among mosquito-borne diseases. Its burden globally is 465000 disability-adjusted life years.¹ About 2.5–3 billion people (40% of the global population) live at risk of infection. The estimate of dengue infection annually was 80–100 million in 1988. This was subsequently revised by the World Health Organization as 50–100 million. The recent estimate of dengue infection is 390 million (95% confidence interval [CI]: 254–528). India regularly reports dengue outbreaks. India is becoming hyperendemic for dengue. Newer areas being struck by the disease.^{2,3}

Dengue cases and deaths in Kerala were first reported in 1997. During the epidemic in 2003, 3546 cases and 68 deaths were reported. Kerala also reported the highest number of deaths due to dengue in the country during the year. The burden of the disease is increasing over the years. Kerala has become hyperendemic and has multiple challenges to face namely the prevalence of multiple serotypes, high coinfection rates, and evolution of the virus. Thiruvananthapuram district reports the highest number of cases in the state.^{4,5}

Severe clinical forms of dengue occur in pregnant women and children. Therefore, they are considered to be a vulnerable group globally. Various reports on the effects of dengue on pregnancy and neonates are available.^{6,7} Dengue infection in a mother results in transfer of antibodies to the newborn. These antibodies may protect the newborn and infant against an infection of dengue with the particular serotype. However, it also carries the risk of a dengue hemorrhagic infection following infection with a different serotype^{8–10} The evidence of severe primary infection in children from South East Asia supports this hypothesis.^{11,12}

Transplacental transmission and vertical transmission were first reported in Malaysia.¹³ Perinatal transmission to the newborn has also been reported from Malaysia in 2007. Clinical presentation of antenatal and postpartum dengue is similar to dengue infections in adult. However, management of perinatal dengue deserves special attention.^{14,15} Mothers and newborn are prone to dengue shock syndrome and other bleeding manifestations. It may be intensified by the wounds and trauma during childbirth. The mortality and morbidity due to perinatal dengue can be significantly reduced by improving awareness for early recognition. This requires the strengthening of laboratories for early case detection.¹⁶ Evidence regarding symptomatic dengue and its effects on pregnancy is abundant, but few seroprevalence studies have been undertaken in pregnant women. Maternal and foetal outcomes in women with symptomatic dengue infection during pregnancy have been studied, but effects of dengue seropositivity have not been looked into.

Objectives

Primary objectives

1. To determine the seroprevalence of dengue infections among pregnant women during late pregnancy.

2. To find the proportion of IgG dengue positivity in cord blood.
3. To find the correlation between the IgG positivity of mother and offspring, as measured by the index ratios.

Secondary objective

1. To find the association of antenatal, natal and postnatal events with dengue seropositivity of parturients.

Materials and methods

Study design

This is a cross-sectional study.

Setting

The study was conducted at a tertiary mother and child hospital in Thiruvananthapuram, Kerala.

Study population

The study population included pregnant women in the active phase of labour (cervical dilatation more than 3 cm). Those women who were not willing to give informed written consent and pregnant women from outside Kerala were excluded.

Sample size and sampling technique

A minimum sample of 122 was sufficient to estimate the frequency (p) of ~45% of the dengue infection with 9% (relative precision “p” = 20% of 45) precision at a 95% CI using the formula $4pq/l^2$, where $q = 100 - p$.

Data collection

Pregnant women admitted for delivery were recruited. The necessary information was recorded in a questionnaire. Blood specimens (2 ml) were collected during hospitalization of women in the first stage, along with blood drawn for other purposes. Umbilical cord blood was collected as a routine procedure at the time of delivery. The serum samples were stored until analysis in aliquots at -20°C for serological tests. All samples of mothers and neonates were tested for IgG using IgG enzyme-linked immunosorbent assay (ELISA). The quantitative titres were obtained, and index ratios were calculated using optical density (OD) values. Only good-quality samples were tested and analysed. Therefore, 10% extra samples were taken.

Cut-off value was evaluated each time the test was performed. The mean of the three calibration values was found. Then, this mean was multiplied by the calibration factor to obtain the cut-off OD value. The sample OD value was divided by the cut-off OD value and then multiplied by 10 to find the index ratio. An index ratio of more than 22 was considered positive, 18–22 was considered equivocal and <18 was considered as negative.

Statistical analysis

The seropositivity for IgG in pregnant mothers and newborns is expressed in % with 95% CI. The agreement between the IgG positivity of the pairs of maternal and neonatal samples was calculated using the Kappa test. The correlation between the maternal and corresponding umbilical cord serum IgG antibodies indices were calculated and presented in a scatter plot. Case-control comparison of IgG-positive and IgG-negative mothers for significant differences in antenatal, natal and postnatal events was carried out using chi-squared test for qualitative variables and t-test for quantitative variables.

Results

Baseline characteristics of the study population

The mean age of the study group was 24.3 (standard deviation [SD] = 3.8) years, and median was 24 (interquartile range: 21–27); 35.8% of the study subjects were educated till high school, 37% studied till higher secondary or diploma and 27.1% had degree and higher education. Majority were housewives (94.1%) and the rest employed (5.9%).

Twenty-three of 189 (12.2%) pregnant women had gestational diabetes mellitus and nine of 189 (4.6%) had hypertension. Nineteen (10%) women had a previous history of one or more abortions. Nine (4.8%) mothers had fever during antenatal period. None of these mothers or their cord samples were positive for IgG. There was one mother with a previous history of dengue.

Dengue seroprevalence in maternal and cord blood

One hundred forty-three samples of mothers tested for IgG showed that 4.9% (95% CI: 2.1–9.4) were positive and 93.0% were negative. For further analysis, the three mothers (2.1%) with equivocal status were also considered as positive because it could be that their antibodies are waning off after the infection long back. Hence, seroprevalence of dengue in antenatal women can be taken as 6.9% (7/143; 95% CI: 3.4–12.48). None of these mothers had a previous history of dengue.

Among cord blood samples, 6.4% (10/158; 95% CI: 3.1 to 11.3) were positive and another 4.4% (7/158) was equivocal. Therefore, the positivity in cord samples was 10.8% (17/158;

95% CI: 6.3–16.6). It is higher than that of the mother positivity for dengue (6.9%).

Fifty initial maternal samples were tested for NS1, IgM and IgG using the card test. None of them were positive.

Correlation between maternal and cord samples IgG OD values and index ratios

One hundred twenty-six pairs of mother and cord samples were analysed. Before looking for correlations, test of normality was performed. The distribution of the values was not normal (Table 1). Therefore, we did Spearman rank correlation for nonparametric distribution. A significant correlation ($r = 0.653$ and p value < 0.001) was obtained between maternal and cord samples IgG index ratios (Fig. 1).

Agreement between maternal and cord IgG positivity

Out of the 126 pairs, 8 maternal and 13 cord samples were positive, considering a cut-off of 18 and above. There were 8 concordant pairs (6.3%) with both mother and cord blood samples positive. Five cord samples were positive for dengue without maternal samples being positive (discordant pairs). One hundred thirteen pairs were negative for IgG (Table 2). Agreement was measured using kappa, and it was obtained as 0.742. Taking cord IgG ELISA ratio as the dependent variable and mother IgG ELISA ratio as the independent variable, regression was performed, and it was found that the r^2 value was only 0.57. The cord blood IgG ELISA index ratio was higher than maternal values in 61.1% of the samples.

The mean OD value of cord samples (0.40) was higher than that of maternal samples (0.34). However, this difference was not statistically significant ($t = 1.31$, p value = 0.19).

Associations between dengue seropositivity among mothers and maternal and newborn problems

We looked for associations between IgG positivity status of mother and maternal antenatal problems. It was seen that hypertension is significantly higher among antenatals with IgG positivity compared with those who are negative (33% vs 4%). Similarly, a greater % of mothers with IgG positivity had preterm delivery compared with those who were negative (20% vs 6.01%). This difference was, however, not statistically significant (Table 3).

Table 1 – Distribution of maternal and cord samples IgG OD values and index ratios.

One-sample Kolmogorov–Smirnov test					
Test	Results	IgG mother OD values	IgG cord OD values	IgG cord index ratio	IgG mother index ratio
N		126	126	126	126
Normal parameters	Mean	0.33	0.38	7.46	6.44
	Standard deviation	0.34	0.39	6.83	6.09
Kolmogorov–Smirnov Z		2.41	2.36	2.03	2.13
p value		0.00	0.00	0.00	0.00
OD, optical density.

Fig. 1 – Correlation between mother and cord IgG ELISA ratio. ELISA, enzyme-linked immunosorbent assay.

Table 2 – Agreement between cord blood and mother blood samples for IgG.

Test	Results	Mothers blood IgG	
		N = 126	
		Positive	Negative
Cord blood IgG	Positive	8 (61.5%)	5 (38.5%)
	Negative	0	113 (100%)

Newborn weight and gestational age at delivery were significantly lower for newborns born to mothers with IgG positivity for dengue. The mean weight of newborns born to IgG-positive mothers was 2.3 (which falls in the range of low birth weight babies), compared with a mean birth weight of 2.8 among babies of IgG-negative mothers. The mean gestational age at delivery of IgG-positive mothers was 36.29 weeks (pre-term birth) compared with a mean of 38.04 weeks for IgG-negative mothers, which was also significantly lower (Table 4.).

Discussion

The mean maternal age in our study was 24.3 (SD = 3.8), which was lower than that of the study from Brazil, in which the

mean maternal age was 25.8 (SD = 6.4).¹⁷ Seroprevalence of dengue in antenatal women was 7%. It is much lower when compared with the study from Brazil which reports 53% of seroprevalence.¹⁷ The sample size was calculated in this study using a seroprevalence of 45%. Therefore, the absolute reworked precision for the study comes to four, and the power to conclude on the seroprevalence is only 40%, with this finding. The study from Singapore which showed a 45% seroprevalence was conducted on participants in the age group of 18–45 years. It states that with every 10-year increase in age, the odds ratio of seroprevalence increased by four. In the same study, the seroprevalence in the age group of 18–25 years (where the mean age of our study population falls) is only 17%. Seroprevalence among pregnant women in this part of the world is less than expected. Earlier studies in the general population in the same setting (Thiruvananthapuram) have yielded higher seroprevalence. It could be because we did the study in a hospital setting which draws patients not only from Thiruvananthapuram district but also from other parts of Kerala with lower prevalence. A serological survey was carried out using three methods for dengue virus antibody detection in the Democratic Republic of Sao Tome and Principe; 39.74% were found positive for dengue antibodies using immunofluorescence assay, 67.95% were positive for dengue IgG, using

Table 3 – Maternal IgG positivity and maternal problems.

Maternal problems	Mother IgG positive, N = 10	Mother IgG negative, N = 133	p value ^a	OR (95% CI of OR)
Diabetes	1 (10%)	16 (12.03%)	1.000	0.81 (0.02–6.60)
Hypertension	2 (33.3%)	3 (4.1%)	0.005	11.67 (1.49–90.93)
Blood group O	5 (50%)	49 (36.8%)	0.50	1.71 (0.40–7.28)
Blood group A	2 (20%)	19 (14.28%)	0.64	1.50 (0.20–8.61)
Induced labour	3 (30%)	39 (29.3%)	1.00	1.03 (0.20–4.77)
Preterm	2 (20%)	8 (6.01%)	0.15	3.91 (0.48–25.79)
PROM	1 (10%)	12 (9.02%)	1.00	1.12 (0.02–9.41)

OR, odds ratio; CI, confidence interval; PROM, premature rupture of membranes.

^a Fisher exact test.

Table 4 – Maternal IgG positivity and newborn outcomes.

Newborn outcomes	Mother IgG status	N	Mean	SD	SE	T value	p value
Newborn weight	Positive	7	2.35	0.61	0.23	2.64	0.01
	Negative	93	2.82	0.44	0.05		
APGAR	Positive	7	9.00	0.00	0.00	0.63	0.53
	Negative	91	8.95	0.23	0.02		
Gestational age	Positive	7	36.29	2.93	1.11	2.48	0.01
	Negative	79	38.04	1.67	0.19		
Haemoglobin	Positive	7	11.47	1.09	0.42	1.63	0.11
	Negative	92	10.86	0.95	0.09		

SD, standard deviation; SE, standard mean; APGAR, appearance, pulse, grimace, activity, respiration.

ELISA and 48.72% were positive for NS1 IgG; 35.90% were positive for all three tests. In our study, we used only a single test, IgG ELISA. Using a single test in our study would not have been an underestimation because, in the aforementioned study, IgG ELISA detected the maximum seroprevalence. This study also reported cross reactivity with Japanese encephalitis and West Nile.¹⁹ We did not check for cross reactivity.

The dengue IgG positivity in cord samples was 10.8%. Cord positivity in a Brazilian study was 55%.¹⁷ Cord blood seropositivity was higher than that of the seropositivity of maternal samples for dengue, and the mean OD value of cord samples was higher than that of maternal samples. The higher levels of the antibody in offsprings compared with their mothers have been reported in other studies as well.^{17,20} Dynamics of IgG placental transfer is seen clearly in this result. The antibody levels increase as the gestational age increases and rises to a maximum towards late pregnancy. The cord blood antibody levels were higher than maternal in 53% of the samples, detected by haemagglutination inhibition method also.²⁰ This kind of an immune response is also seen in other infectious diseases.¹⁸ Compared with the Brazilian study (99.3% copositive), 61.5% of pairs were copositive in our study. The agreement (kappa 0.742) was also lower than that in the study from central Brazil (kappa = 0.96).¹⁷

Newborn weight and gestational age at delivery were significantly lower for newborns born to mothers with IgG positivity for dengue. To study the effects of dengue on pregnancy, a systematic review was performed, which included 30 studies.⁷ It included 19 case reports, 9 case series, and 2 comparison studies. The case reports show high rates of caesarian (44%) and pre-eclampsia (12%). The rates of preterm deliveries (16.1%) and caesarians (20.4%) were high in case series also. The comparison studies showed that higher low birth weight babies were born to dengue-infected mothers compared with non-infected mothers. In our study also, we identified that the number of preterm deliveries and low birth weight babies were significantly higher among women with dengue seropositivity. Earlier publications refer specifically to dengue infections during pregnancy. Our study differs in the fact that we hypothesise that such effects are possible with dengue infection any time before pregnancy also. Several risk factors have been identified for preterm delivery. Along with these identified risk factors, we also need to think whether infections such as dengue in the prepregnancy or pregnancy period are additional risk factors because this study gives us an observation in this direction. It could also be because of other intermediate risk factors such

as poor socioeconomic status which is a common risk factor for both dengue and poor foetal outcomes (confounders). Because this was a cross-sectional study, we cannot prove causality. The analysis of antenatal problems and outcomes associated with IgG positivity has to be viewed in this context, and further research is required in this regard. An analytical study using a research question as “Is dengue infection a risk factor for low birth weight, preterm births?” needs to be taken up with appropriate sample size calculation to throw more light in this regard. Because the exposure would be a rare event, a cohort study will be more suitable than a case-control design. Fever during pregnancy was not tested for dengue. We may not have captured those who had abortions or pregnancy terminated and their dengue status. Cross-reactions with other flaviviruses such as Japanese encephalitis and West Nile is a possibility. We have not looked into this. Despite these limitations, this study gives insight into the effects of dengue seropositivity on pregnancy and its outcomes.

Conclusion

This study has thrown light on prevalence of transplacentally transferred maternal-infant antidengue antibodies. Seroprevalence of dengue in antenatal women and their offsprings is lower than that found in many other parts of the globe, endemic for dengue. However, we need to do studies on larger samples before we arrive at this conclusion. Cord blood seropositivity was higher than that of the seropositivity of maternal samples for dengue. A significant correlation was obtained between maternal and cord samples dengue IgG index ratios. Newborn weight and gestational age at delivery were significantly lower for newborns born to mothers with IgG positivity for dengue.

The study is the first of its kind to show that dengue infection any time before pregnancy can also be detrimental to the neonatal outcomes. Previous studies have shown that the gestational age and birth weight could be lower in women with dengue infection during pregnancy. This study shows that even an earlier infection (before pregnancy) with dengue can result in preterm delivery and low birth weights. It needs to be proved further by studies using cohort or case-control studies with sample size estimated according to this objective.

Research is required to generate evidence for a separate cut-off value to decide on the seropositivity of cord samples because we find that the titres are higher in cord than in maternal samples.

Conflicts of interest

All authors have none to declare.

Ethical approval

Institutional research and ethical committee clearance (IEC No. 02/43/2014/MCT dated 14/02/2014) was obtained. Informed written consent was taken from all study participants. Confidentiality and privacy was maintained at every stage of the study.

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