Schistosomiasis Japonica During Pregnancy Is Associated With Elevated Endotoxin Levels in Maternal and Placental Compartments

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Schistosomiasis affects approximately 40 million women of reproductive age and has been linked to elevated levels of circulating endotoxin in nonpregnant individuals. We have evaluated endotoxin levels in maternal, placental, and newborn blood collected from women residing in Leyte, Philippines. Endotoxin levels in both maternal and placental compartments in pregnant women with schistosomiasis were 1.3- and 2.4-fold higher, respectively, than in uninfected women. In addition, higher concentrations of endotoxin in placental blood were associated with premature birth, acute chorioamnionitis, and elevated proinflammatory cytokines. By promoting endotoxemia, schistosomiasis may exert additional, maladaptive influences on pregnancy outcomes.

Keywords. endotoxin; pregnancy; S. japonicum.

Schistosomiasis is a parasitic disease affecting millions of people. It is estimated to be responsible for as many as 13–15 million disability-adjusted life years (DALYs) lost annually in endemic regions [1]. Despite a 2002 World Health Organization policy statement recommending treatment of pregnant and lactating women with praziquantel, many pregnancies remain complicated by schistosomiasis due to regional differences in adoption of this policy and barriers to drug availability and delivery [2].

Studies from our group have established schistosomiasis as a chronic infection eliciting proinflammatory immune responses that are thought to mediate much of the morbidity associated with schistosomiasis, including during pregnancy [3]. Recent studies have also demonstrated an association between helminthiasis and microbial translocation (MT), the leakage of gut contents, including microbes and their associated products, into the normally sterile bloodstream [4–6]. MT can lead to persistent stimulation of the immune system and has been associated with adverse outcomes in the context of many diseases that disrupt gut integrity [6, 7]. Depending on the intensity of schistosome infection, a significant number of eggs leave the systemic circulation through capillaries of the gut lamina propria, eventually penetrating the gut epithelium. Importantly, we have demonstrated evidence for this disruption in a cohort of adult Kenyan males “hyperexposed” to schistosomiasis who had significantly higher plasma levels of lipopolysaccharide (LPS; endotoxin) compared with uninfected Kenyan controls [4].

Schistosomiasis-associated MT likely exacerbates the negative consequences of infection during pregnancy; however, to our knowledge, no studies have addressed the role of MT in the context of human pregnancy. We evaluated the levels of endotoxin in our study population of pregnant women in Leyte, Philippines, a region endemic for Schistosoma japonicum. We quantified endotoxin levels in maternal, fetal, and placental serum samples and evaluated differences based on schistosome infection status. In addition, we assessed the relationship between endotoxin levels and pregnancy outcomes.

METHODS

Ethical Considerations and Informed Consent
This study was approved by the institutional review boards of Rhode Island Hospital and the Research Institute for Tropical Medicine in The Philippines. All subjects provided informed consent prior to enrollment in the study. Per the guidelines of the Philippine Department of Health, treatment for infections was withheld until after the women had given birth and stopped breastfeeding. Cord blood collection from North American pregnancies was approved by the institutional review boards of Rhode Island and Women and Infants Hospitals. Samples were obtained in a de-identified manner from women...
undergoing routine cesarean sections for nonpathological reasons.

**Study Site and Population**

Characteristics and enrollment strategies of this study population have been described elsewhere [3]. The study was conducted in Leyte, Philippines, where *S. japonicum* is endemic, HIV prevalence is estimated at <0.1%, and malaria is not endemic. All subjects presented at a municipal health center for routine prenatal care. Eligibility criteria included the presence of a singleton pregnancy in the second or third trimester, age 18 years or older, and provision of informed consent.

**Data Collection**

At the time of enrollment, we collected health-related epidemiologic data, including gravidity, parity, age, weight, height, smoking status, and socioeconomic status (SES), described in our original publication with this cohort [3]. Geohelminth (*Ascaris lumbricoides*, hookworm, *Trichuris trichiura*) and schistosome infections were determined at enrollment from 3 consecutive stool specimens using the Kato-Katz method. Each sample was read in duplicate and an average number of eggs per gram calculated. Maternal blood at 32 weeks’ gestation and cord blood samples at delivery were collected into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing serum separator gel. Placental blood was collected from the pool of blood resulting after placental wedge biopsy. Serum samples were aliquoted and stored at −80°C.

**North American Cord Blood Collection**

Cord blood from uninfected, healthy pregnancies was collected from North American women after routine cesarean section. The umbilical vein was cannulated immediately after delivery and blood collected into sterile Vacutainer tubes. Serum was stored at −80°C until use.

**Endotoxin Assays**

Endotoxin levels were quantified in serum samples from maternal, placental, and fetal compartments using a chromogenic LAL assay (Q1000, Lonza, Basel, Switzerland) according to manufacturer’s instructions.

**Cytokine Assays**

Multiplexed cytokine analyses were performed on maternal, placental, and fetal serum as previously reported [3].

**Histopathological Analysis**

Placental tissue samples were collected at delivery and contained basal plate, villous core, and chorionic plate with amnion. Tissue was fixed in 10% buffered formalin for 24 hours and stored in 70% ethanol until paraffin embedding, sectioning to 5 µm thickness, and hematoxylin and eosin staining.

Sections were evaluated for acute chorioamnionitis (ACA), fetal inflammatory response, chronic villitis, and chronic deciduitis as previously reported, according to established criteria [3, 8].

**Statistical Analysis**

All analyses were performed using JMP Pro 10 (SAS Institute, Cary, NC). Statistical significance was defined as *P* < .05. Endotoxin levels were natural log (ln)–transformed to better approach normality and evaluated as continuous variables. Schistosome infection status was evaluated as a nominal variable (yes/no). The relationship between schistosome infection status and endotoxin levels in each compartment (maternal, placental, fetal) was examined using multivariate linear regression. Models were built by first evaluating the role of potential confounders in bivariate analyses, with covariates associated with endotoxin level (*P* < .10) then evaluated for inclusion in multivariate models. Potential confounding and explanatory covariates included coinfection with *A. lumbricoides*, *T. trichiura*, or hookworm, body mass index, maternal age, gravida, parity, history of miscarriage, history of stillbirth, smoking status, and SES. A unique model for endotoxin levels within each compartment was defined.

The relationship(s) between endotoxin and cytokine levels within a specific compartment were assessed using bivariate analysis following ln transformation. Placental histopathology and birth outcomes were examined in relation to placental or maternal endotoxin levels using the same model-building strategy as outlined above.

**RESULTS**

Characteristics of the study population are provided in Table 1. The original cohort included 150 subjects; however, maternal blood at 32 weeks’ gestation was captured for 132, placental blood for 112, and cord blood for 110 pregnancies. Analyses of the relationships between schistosome infection and endotoxin levels in these compartments reflect these numbers. Endotoxin levels with each compartment were fit with individual regression models, in order to best control for confounders within each data set.

**Maternal Schistosomiasis is Associated With Elevated Levels of Endotoxin**

For maternal serum, the final model included schistosomiasis status, maternal age, and maternal BMI. The final model for placental endotoxin levels included schistosomiasis, *T. trichiura* status, and history of miscarriage. After adjusting for confounders, endotoxin levels in maternal and placental blood were 1.3- and 2.4-fold higher (*P* = .04 and < .001), respectively, in those pregnancies complicated by schistosomiasis, compared with their uninfected counterparts (Figure 1A).
The final model for endotoxin levels in cord blood included *S. japonicum*, *A. lumbricoides*, and SES. Endotoxin levels in cord blood were not different between infected pregnancies and uninfected pregnancies from the Philippines. All compartments from Filipino pregnancies displayed higher levels of endotoxin than cord blood from North American control pregnancies (Figure 1A).

### Elevated Endotoxin Levels Are Associated With Proinflammatory Cytokines in Maternal Circulation

We have previously found both tumor necrosis factor (TNF)-α and interleukin (IL)-10 to be elevated in maternal circulation in schistosome-infected women in this same study population [3]. Similarly, TNF-α and IL-10 levels in maternal circulation in this study population were 7.0- and 2.6-fold higher (*P < .05*), respectively, in women with high endotoxin levels, compared to those with relatively low endotoxin (Figure 1B). In addition, maternal endotoxin levels were correlated with cytokines in maternal circulation other than those classically associated with a proinflammatory response (TNF receptor type II [TNF-RII] and IL-5; Supplementary Table 1).

### Elevated Endotoxin Levels Are Associated With Proinflammatory Cytokines in Placental Blood

We have previously identified a number of cytokines that are elevated in the placental compartment among women with schistosomiasis [3]. All of these cytokines (IL-6, IL-1, TNF-α, and TNF-RII) were elevated in those women with the highest placental endotoxin (Figure 1C and D). In addition, C-reactive protein (CRP) and a number of other cytokines were correlated with endotoxin levels in the placental compartment, including, TNF-RI, IL-5, IL-10, and IL-13 (Supplementary Table 1).

### Adverse Pregnancy Outcomes Are Associated With Elevated Endotoxin in Placental Blood

We assessed endotoxin levels in relationship to histopathologic markers of inflammation of the placenta (ACA, fetal inflammatory response, chronic villitis, chronic deciduitis) and adverse pregnancy outcomes such as intrauterine growth restriction, low birthweight, and prematurity. After adjustment for smoking status, women with ACA and prematurity had 1.5- and 2.6-fold increased endotoxin levels, respectively, in placental serum samples (Figure 1E).

### DISCUSSION

We have previously demonstrated that chronic schistosomiasis during pregnancy results in a pronounced proinflammatory response, measurable in maternal, placental, and cord blood [3]. In addition, trophoblast cells of the placenta exhibit a robust proinflammatory response to schistosome antigens [9]. In this report, we have identified elevated endotoxin levels as another mechanism by which schistosomiasis may adversely affect the health of human pregnancy.

Extremely high levels of endotoxin have previously been associated with schistosome infection, and are likely tolerated due to immunosuppressive responses promoted by both the host and the schistosome, though potential mechanisms remain unclear [4]. Other helminthic infections have been associated with MT [5], and given the high percentage of coinfections,

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**Table 1. Characteristics of Study Population Stratified by Schistosome Infection Status**

<table>
<thead>
<tr>
<th>Schistosome Infection Status</th>
<th>S. japonicum infected (n = 66)</th>
<th>S. japonicum uninfected (n = 66)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hookworm infected; % (N)</td>
<td>48.5 (32)</td>
<td>27.3 (18)</td>
</tr>
<tr>
<td>Body mass index; median (IQR)</td>
<td>21.6 (19.6, 24.8)</td>
<td>22.5 (20.3, 25.4)</td>
</tr>
<tr>
<td>Maternal age; mean (95% CI)</td>
<td>29.4 (27.8, 31.0)</td>
<td>33.1 (31.3, 34.9)</td>
</tr>
<tr>
<td>Gravida; median (IQR)</td>
<td>3 (2.5)</td>
<td>4 (2.6)</td>
</tr>
<tr>
<td>History of miscarriage; % (N)</td>
<td>12.3 (8)</td>
<td>12.1 (8)</td>
</tr>
<tr>
<td>History of stillbirth; % (N)</td>
<td>4.8 (3)</td>
<td>4.5 (3)</td>
</tr>
<tr>
<td>Smoker; % (N)</td>
<td>0 (0)</td>
<td>3.0 (2)</td>
</tr>
<tr>
<td>Socioeconomic status; mean (95% CI)</td>
<td>14.4 (13.3, 15.6)</td>
<td>16.5 (15.3, 17.7)</td>
</tr>
<tr>
<td>Birthweight (kg); mean (95% CI)</td>
<td>2.9 (2.8, 3.0)</td>
<td>2.9 (2.8, 3.0)</td>
</tr>
<tr>
<td>Gestational age (weeks); median (IQR)</td>
<td>40.0 (38.0, 40.0)</td>
<td>38.5 (38.0, 40.0)</td>
</tr>
<tr>
<td>Low birthweight (&lt;2.5 kg); % (N)</td>
<td>6.8 (4)</td>
<td>13.7 (7)</td>
</tr>
<tr>
<td>Intrauterine growth restriction; % (N)</td>
<td>16.9 (10)</td>
<td>25.5 (13)</td>
</tr>
<tr>
<td>Premature birth; % (N)</td>
<td>8.5 (5)</td>
<td>5.7 (3)</td>
</tr>
<tr>
<td>Fetal inflammatory response; % (N)</td>
<td>14.0 (8)</td>
<td>5.9 (3)</td>
</tr>
<tr>
<td>Acute chorioamnionitis; % (N)</td>
<td>59.6 (34)</td>
<td>45.1 (23)</td>
</tr>
<tr>
<td>Chronic villitis; % (N)</td>
<td>8.8 (5)</td>
<td>3.9 (2)</td>
</tr>
<tr>
<td>Chronic deciduitis; % (N)</td>
<td>25.0 (11)</td>
<td>15.9 (7)</td>
</tr>
<tr>
<td>Maternal endotoxin (EU/mL); median (IQR)</td>
<td>0.17 (0.13, 0.29)</td>
<td>0.14 (0.12, 0.17)</td>
</tr>
<tr>
<td>Placental endotoxin (EU/mL); median (IQR)</td>
<td>1.02 (0.48, 2.76)</td>
<td>0.30 (0.22, 0.87)</td>
</tr>
<tr>
<td>Cord endotoxin (EU/mL); median (IQR)</td>
<td>0.29 (0.20, 1.14)</td>
<td>0.24 (0.18, 0.58)</td>
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</tbody>
</table>

Abbreviations: CI, confidence interval; epg, eggs per gram; EU, endotoxin units; IQR, interquartile range.
may help explain the elevated levels of endotoxin in all study subjects, compared with North American newborns. Despite the apparent host accommodation to schistosome-induced endotoxemia, we demonstrate that elevated endotoxin levels at the maternal-fetal interface are associated with adverse pregnancy outcomes, such as ACA and prematurity.

Inflammation of the placenta is a known risk factor for premature birth, and ACA is present in up to 85% of all preterm births [10]. Although the precise mechanisms by which placental inflammation results in early delivery are not clear, LPS has been shown to cause proinflammatory cytokine production by trophoblast cells and placental extracts in culture [11, 12], and...
is commonly used as a model for preterm delivery in animal models [13]. Our finding that elevated endotoxin in the placenta was also associated with increased levels of proinflammatory cytokines (TNF-α, IL-1, IL-6, CRP, and interferon-γ) at the maternal-fetal interface supports a role for LPS-induced placental inflammation. In rodent models, intraperitoneal LPS administration results in elevated proinflammatory cytokine production within the placenta. [13] This pattern is recapitulated in our studies involving treatment of trophoblast cells with schistosome egg antigens [9]. These data suggest that in the context of schistosomiasis, the trophoblast may receive a dual insult in the form of schistosome antigens and elevated endotoxin.

We have identified an association between endotoxin levels and a number of cytokines, including those associated with Thelper 2 and immune-regulatory responses. Given that our study population experiences chronic schistosome exposure, this may represent a host mechanism to downregulate the proinflammatory response initiated by schistosomiasis and/or endotoxin. Indeed, in LPS-induced rodent models of intrauterine growth restriction and premature birth, IL-10 protects against LPS-induced preterm birth [14]. Therefore, the impact of schistosomiasis on pregnancy outcomes may reflect a balanced host response to LPS and schistosome antigens.

Our data suggests that levels of endotoxin are highest in the placental compartment of schistosoma-infected women compared to either the maternal or fetal compartment. This is perhaps to be expected, as placental blood represents an admixture of blood from maternal and fetal sources. It is also possible that the placenta acts as a biological sink, protecting the fetus from additional insult by endotoxin from the maternal compartment. In support of this hypothesis, a mouse model of LPS administered intraperitoneally demonstrated a robust cytokine response in maternal circulation and within the placenta, with an absence of such a response in the fetus [15].

In conclusion, we have shown elevated endotoxin levels in maternal and placental blood from pregnant women infected with schistosomiasis, compared with pregnant uninfected controls. Despite relatively low subject numbers for some outcomes, we found that pregnancies complicated by prematurity or ACA had increased levels of placental endotoxin. Elevated endotoxin in maternal or placental compartments was also associated with a proinflammatory signature within that compartment. To our knowledge, this is the first report of microbial translocation in the context of S. japonicum infection as a contributor to placental inflammation and subsequent adverse birth outcomes.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References