Soil-transmitted Helminth Infection and Perinatal Outcomes in Pregnant Women in Primary Care Settings in South India: A Cohort Study

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Abstract

Background: Soil-transmitted helminth infections (STHs) in pregnancy have been linked to maternal anemia, low birth weight, and adverse perinatal outcomes. STH includes *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*. However, conflicting evidence exists regarding these effects. Hence, we aimed to assess the association of STH infections with perinatal outcomes. **Methods and Materials:** A community-based cohort study was conducted among pregnant women in primary care settings in Puducherry, South India, during December 2019 to April 2021. Stool samples were collected for STH screening and confirmed using polymerase chain reaction. Information on perinatal outcomes and hemoglobin levels were extracted from case records. The association of pre-term birth, low birth weight, anemia, and miscarriages with STH infection was analyzed using the Chi-square test. **Results:** Among the 650 women enrolled, 58 (8.9%) had one of the STH infections. At the time of recruitment, 336 (52.0%) women had anemia. Twenty-four women (3.7%) had miscarriages, 14 (2.2%) had pre-term birth, and 65 (10.4%) had low-birth-weight (LBW) babies. All 14 pre-term babies had LBW, and out of 65 LBW, 40 were in mothers with anemia. Incidence of LBW, pre-term deliveries, and anemia were higher in women with STH than in those without STH (LBW: 12.3% vs 10.2%, P = 0.62; pre-term: 3.5% vs 2.1%, P = 0.5; and anemia: 54.4% vs 51.85, P = 0.7). **Conclusion:** Though the incidence of anemia, LBW, and pre-term delivery was higher in women with STH infections compared to those without STH, we did not find any significant association between the presence of STH infections and perinatal outcomes.

Keywords: Anemia, low birth weight, pre-term birth, primary care setting, STH, and perinatal outcomes

INTRODUCTION

Soil-transmitted helminth (STH) infections, caused by nematodes such as *Ascaris, Trichiuris*, and hookworm, are among the most common parasitic infections globally.^[1] According to the Global Burden of Disease 2013 report, continuous biannual school and community-based de-worming programs and improvements in living conditions have led to a significant reduction in the prevalence of *Ascaris*, a modest reduction in *Trichuris*, and a minimal reduction in hookworm (-5.1%).^[2] However, pregnant and reproductive-age women remain vulnerable to STH infections, and an estimated 44 million pregnancies were affected worldwide.^[3]

Pregnancy leads to a reduction in cell-mediated immunity, thereby increasing the susceptibility of women to infections caused by STHs.^[4] In India, the burden of STH varies between

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12.5% and 66%,^[5-9] with a varying prevalence for different parasites. These infections are predominantly seen in regions with poor sanitation and hygiene.

India faces several challenges in maternal and child health, including an infant mortality rate of 28 per 1000 live births.^[10] Moreover, 17.3% of infants are born with a low birth weight (LBW)^[11] and 45.7% of the pregnant women aged 15–49 years suffer from anemia.^[12] Hookworm infection leads to intestinal blood loss,

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Methods

Study design and general setting: We conducted a prospective cohort study among pregnant women registered for care at specific primary health centers (PHCs) in Puducherry, South India. Of the 27 PHCs in Puducherry, five rural and four urban PHCs were chosen by simple random sampling for the study. The population of the PHCs included in the study varies from 30,000 to 80,000. Hence, the average number of new registrations of ANC ranged from 25 to 50 registrations per month. Puducherry is a union territory with good health indicators, which includes an infant mortality rate (IMR) of 3 per 1000 live births and an institutional delivery of 99.7%, and women with at least four antenatal check-ups were 87% for the year 2019–2021.^[21]

Specific setting and study population

All women attending ANC at PHCs and who agreed to provide one stool sample were included. In routine, pregnant women register at PHCs at 10–12 weeks of gestation. They visit PHCs once in a month till 28 weeks of gestation, twice a month during 29–32 weeks, and once a week till delivery. All women receive iron-folic acid supplements, tetanus toxoid (TT) vaccination, and calcium supplements free of cost from PHC. Details of the ANC mothers up to 42 days of post-partum were recorded in the Mother and Child Tracking System (MCTS) card. The MCTS card contains details regarding the type of delivery, gender of the baby, birth weight, and complications faced during pregnancy and hemoglobin values. It is maintained by the auxiliary nurse and mid-wife.

Mothers are not screened for parasitic infection routinely at the PHCs and all the pregnant women receive a single dose of albendazole 400 mg as a preventive therapy after the first trimester.^[22] In children, the biannual de-worming program has been started since 2015.^[23]

Sample size estimation

This study was a part of a project which assessed the prevalence of STH infections in pregnancy. Considering the prevalence of intestinal parasitic infection among pregnant women as 12.4%,^[15] an absolute precision of 3, and a design effect of 1.4, the calculated sample size was 649 for 95% confidence level.

Study procedure

Information on age, parity, gestational age, monthly family income, and educational status was collected using a structured questionnaire. After obtaining an informed consent, the women were provided with a plastic, wide-mouthed labeled leak-proof container for stool sample collection. They were instructed to collect one stool sample without urine contamination the following morning. They were asked to submit the samples to the laboratory located in the PHCs. One stool sample was collected from each woman at the time of recruitment. The collected samples were transported to the laboratory within 4 hours and stored at -800C for the purpose of screening. Detailed methods of collection and transportation are described elsewhere.^[24] A cohort of pregnant women was established at the time of registration and was followed up until their delivery to gather the information regarding mode of delivery, infant gender, birth weight, gestational age at delivery, and any complications faced during the pregnancy. MCTS cards were utilized for this purpose. Information regarding the same parameters was entered in the MCTS cards by PHC staff after cross-checking with the discharge summary from the hospitals. The women were contacted through mobile phone to cross-check, confirm the information, and collect the data which were missing on MCTS registers. Hemoglobin levels during the first and last trimesters were extracted from lab registers and MCTS cards. The detailed study procedure is described in Figure 1.

Screening of STH infection

Screening took place at the parasitology laboratory within the Microbiology Department of a tertiary care hospital situated in Puducherry. Samples were screened for the presence of STH by an individual trained in molecular techniques under the supervision of a microbiologist responsible for overseeing the parasitology laboratory. A Qiagen stool extraction kit (Qiagen, Hilden, Germany, Cat.no. 160022126) was used to extract the deoxyribonucleic acid (DNA) from stool samples preserved at -80°C. Polymerase chain reaction (PCR) was performed to detect the presence of STHs, which includes Ascaris lumbricoides, Necator americanus, and Ancylostoma duodenale. Since the prevalence of Trichuris trichiura was negligible in our setting, we excluded the same. The PCR yielded the products of 420, 662, and 510 base pairs, respectively, to the above-mentioned parasites. Approximately 5% of representative samples were sent for sequencing for confirmation. Details on the molecular identification methods are documented elsewhere.[25]

Statistical analysis

The data were entered in EpiData Manager, and analysis was done using STATA17.0. Birth weight and gestational age at delivery were categorized and expressed as proportions. Categorical variables like delivery type, abortion, and pregnancy complications were expressed as percentages. Ulaganeethi, et al.: Soil-transmitted helminth infection and perinatal outcomes



Figure 1: Flow chart showing the study methods

Anemia (hemoglobin <11 gm/dl), miscarriage (pregnancy loss before 20 weeks), pre-term delivery (delivery before <37 weeks), and LBW (<2.5 kgs) of the baby were considered for perinatal outcomes.

The proportion of women with STH infection, delivering LBW babies, pre-term delivery, and anemia were reported with proportions with 95% confidence interval (CI). The association between STH infection and perinatal outcomes was assessed using Chi-squared test, while the independent association of STH on perinatal outcomes was determined through log-binomial regression. After controlling for age, occupation, education, and gravida, the independent effect of STH on perinatal outcomes was reported using adjusted risk ratios with 95% CI. A P value less than 0.05 was considered statistically significant.

RESULTS

We enrolled 650 pregnant women for the study. Among these women, half of them aged 25 years or younger, 59.2% were primigravida, 58% had completed their bachelor/ master's degree, 31% were employed, and 52% were anemic. Socio-demographic and obstetric characteristics are described in Table 1.

Among the 650 women, 58 (8.9%, 95% CI: 6.8–11.4) had one of the parasitic infections. Of these 58 women with STH infection, 41 (70%) were infected with *Necator americanus* and 17 (30%) were infected with *Ascaris lumbricoides*. No species of Ancylostoma was found. Of the 650 women included, 24 (3.7%, 95% CI: 2.5–5.7) had miscarriages. Excluding 24 women who had miscarriage, the data on perinatal outcomes (birth weight, gestational age at birth) were available for 626 women. Out of 626 women, 246 (39.5%) delivered through cesarean section, 76 (12.3%) had gestational diabetes, 39 (6.3%) had pregnancy-induced hypertension, and 6 (1%) had both. About 14 women (2.2%, 95% CI: 1-3-3.8) had pre-term delivery and 65 (10.4%, 95% CI: 9.1-14.6) had a birth weight of less than 2.5 kg. A total of 336 women (52.0%, 95% CI: 48.0-55.9) had anemia during enrolment, and 282 (45.9%) had anemia in the third trimester. Of the 58 women with STH, 31 (54.4%) had anemia at recruitment and the following de-worming 26 out of these 31 (83.9%) remained anemic.

Of the women with STH infections, 2 (3.5%) had pre-term delivery compared to 12 (2.1%) in women without STH infection. The incidence of LBW babies was higher in women with STH (12.3% vs 10.2%; P = 0.62). Even though the incidence of LBW was slightly higher in one group, we could not find a statistically significant difference in mean birth weight between the groups [Table 1]. Anemia was slightly higher in women with STH during enrolment and during the third trimester than in women without STH (enrolment: 54.4 vs 51.8%, third trimester: 55.4 vs 54%). One woman (1.7%) had abortion in the STH-infected group compared to 23 (3.9%) in women without STH [Table 2]. The proportion of women with anemia was higher in hookworm infection than in *Ascaris* (60% vs 41%; P = 0.2).

Variable	Total	STH	(Yes)	STH	Р	
		п	%	п	%	
Total	650	58	8.9	592	91.1	
Age categories (years)						
≤25	317	28	8.8	289	91.2	0.99
26-30	232	21	9.1	211	90.9	
>30	101	9	9.9	92	90.1	
Monthly family income (INR)*						
Up to 6000	139	16	11.5	123	88.5	0.01
6001-10000	222	24	10.8	198	89.2	
10001-25000	206	7	3.4	199	96.6	
>25000	70	8	11.4	62	88.6	
Occupation						
Home maker	445	44	9.9	401	90.1	0.20
Working	205	14	6.8	191	93.2	
Education [#]						
No/Up to primary	17	0	-	17	100.0	0.39
Secondary to higher secondary	252	21	8.3	231	91.7	
Bachelor/Master's	372	35	9.4	337	90.6	
Gravida						
Primi gravida	385	39	10.1	346	89.9	0.19
Multi gravida	265	19	7.2	246	92.8	
Residence						
Rural	323	39	12.1	284	87.9	0.005
Urban	327	19	5.8	308	94.2	
Period of gestation						
First	282	21	7.4	261	92.6	0.51
Second	347	35	10.1	312	89.9	
Third	21	2	9.5	19	90.5	

Table	1: So	cio-der	nographic	; and	obstetric	characte	eristics	of the	antenatal	women	attending	primary	care	centers,
Puduc	herrv.	India.	durina D	ecem	ber 2019	to April	2021 (n = 65	0)					

*Information on income was missing for 13 women, "education was missing for 9 women, and $^{\text{s}}$ hemoglobin values were missing data for 4 women; a P<0.05 was statistically significant

The proportion of pre-term deliveries and LBW were higher among the women who were anemic at enrolment than among those who were non-anemic (pre-term delivery: 3.1% vs 1.3%; LBW: 12.4% vs 8.4%). Similar association was seen in third-trimester hemoglobin. Among 65 mothers who delivered LBW babies, 40 (61.5%) were identified as anemic [Table 3]. However, no significant difference was found in the mean (SD) hemoglobin levels in women with STH before and after deworming, which were 10.7 (1.4) and 10.74 (0.8), respectively.

Multi-variable analysis showed that the incidence of pre-term birth, LBW babies, and anemia at the first trimester were higher in women with STH compared to women who did not have STH. However, the associations were not statistically significant [Table 4].

DISCUSSION

Our study found that nearly one in 11 pregnant women had STH infection. The proportion of mothers delivering pre-term and LBW babies was comparatively higher in women with STH than those without STH. The proportion of mothers with anemia was slightly higher in women with STH. In our study, the prevalence of STH infections was 8.9%. A previous study conducted in Bangalore, India, about a decade ago reported a higher prevalence of 12.4%^[15] using traditional microscopic diagnosis. The present study used a highly sensitive molecular technique for identification, yet the prevalence was lower. This lower prevalence of STH could be due to improvement in sanitation, socio-economic and environmental conditions, and increased awareness. Also, continuous biannual mass de-worming campaigns among the high-risk groups could have played an important role in the reduction.^[26]

In the present study, 54% of women diagnosed with STH infections had anemia. Previous studies conducted in South India and Ethiopia reported higher and lower rates of anemia at 88%^[15] and 40%,^[3] respectively. Anemia is a multi-factorial condition, and STH infection is one of the frequently observed causes. The differences in the prevalence of anemia among these studies might be due to variations in dietary habits, underlying physiological, biochemical parameters, or certain genetic conditions.^[27] A study from North Ethiopia reported that women with helminth infection were 1.8 times higher odds of being anemic than women without STH infection. Ethiopia

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Variable	STH infection*					
	Yes		No			
Gestational age at delivery ^a (<i>n</i> =626)						
Preterm (<37 weeks)	2	3.5	12	2.1	0.5	
Term (≥37 weeks)	55	96.5	557	97.9		
Abortion (n=650)						
No	57	98.3	569	96.1	0.4	
Yes	1	1.7	23	3.9		
Birth weight ^b ($n=624$)						
Mean (SD) in kg	2.86 (0.42)		2.92 (0.42)		0.34	
Normal birth weight (≥2.5kg)	50	87.7	509	89.8	0.62	
LBW (<2.5 kg)	7	12.3	58	10.2		
Hemoglobin at enrolment ^c (n=646)						
Mean (SD)	10.7 (1.4)		10.8 (1.3)		0.65	
Non anemic (≥11)	26	45.6	284	48.2	0.7	
Anaemic (<11)	31	54.4	305	51.8		
Hemoglobin at third trimester ^d ($n=614$)						
Mean (SD)	10.7 (0.7)		10.8 (0.8)		0.94	
Non anemic (≥11)	25	44.7	257	46.0	0.85	
Anemic (<11)	31	55.4	302	54.0		

Table 2: Association of STH infections during pregnancy and perinatal outcomes among women attending primary care centers. Puducherry, India, during December 2019 to April 2021

STH, Soil-transmitted helminth infection; STH includes *Ascaris lumbricoides* and hookworm. For analysis related to a, b, and d, women who had abortion were excluded. ^bbirthweight missing for two women; ^chemoglobin values were missing for 4 women at recruitment; ^dhemoglobin values were missing for 12 women at third trimester. A *P*<0.05 was statistically significant

Variable		Total	Р			
	Preter	m <i>n</i> (%)	Term <i>n</i> (%)			
Total	14 (2.3%)		608 (97.7%)		622	
Anemia (Enrolment)						
Non-anemic	4	1.3	295	98.7	299	0.14
Anemic	10	3.1	313	96.9	323	
Anemia (third trimester)						
Non-anemic	4	1.3	295	98.7	299	0.14
Anemic	10	3.1	313	96.9	323	
Total	65 (10.5%)		555 (89.5%)			
Anemia (Enrolment)						
Non-anemic	25	8.4	272	91.6	297	0.1
Anemic	40	12.4	283	87.6	323	
Anemia (third trimester)						
Non-anemic	26	9.3	255	90.8	281	0.37
Anemic	38	11.5	293	88.5	331	

Table 3: Association of anemia with pre-term delivery and LBW among the pregnant women recruited from selected primary health centers of Puducherry, India, during December 2019 to April 2021 (n=626)

LBW, low birth weight; NBW, normal birth weight; hemoglobin values of <11g/dl are considered as anemic and ≥11 is normal

is a country where the prevalence and intensity of the parasite were higher and severe intensity of infection was associated with anemia, which could be the possible reasons. They have assessed malarial parasite along with the STH.^[28] The current study shows that the mean hemoglobin was almost similar in women with and without STH. The prevalence and intensity of STH infection are decreasing in India, and this would reduce the impact of STH on anemia. Hence, continuing the de-worming programs in school children will help in reducing the STH

infection in adolescents and later in expectant mothers. Along with this, anemia-focused programs in India like Anaemia Mukt Bharat covering the complete life course (infants to adults) will reduce the levels of anemia. A review conducted among pregnant women and children less than 5 years reported that there was no improvement in the status of anemia after de-worming.^[29]

About 12.3% of mothers with STH delivered LBW babies compared to 10.2% in women without STH, and all the

Table 4: Log binomial regression showing the association of STH infection in pregnancy with pre-term delivery, LBW, and anemia during the first trimester among the women recruited from selected primary health centers of Puducherry, India, during December 2019 to April 2021

	Model 1	Model 2	Model 3	Model 4	
	Preterm birth, RR (95% CI)	LBW, RR (95% CI)	Anemia, RR (95% CI)	Miscarriage, RR (95% CI)	
STH infection ^a	1.2 (0.3-5.6)	1.3 (0.5-3.0)	0.9 (0.7-1.2)	0.5 (0.07-4.1)	
STH infection ^b	1.3 (0.3-5.6)	1.1 (0.5-2.7)	1.0 (0.8-1.3)	0.4 (0.06-3.2)	

Model 1: adjusted for age categories, gravida, income, residence, and hemoglobin at enrolment. Model 2: adjusted for age categories, gravida, income, the residence anemia during the first trimester and preterm delivery. Model 3: adjusted for age categories, gravida, occupation, income, residence, and education. Model 4: adjusted for gravida, occupation, income, residence, and hemoglobin at enrolment. (a) Results from unadjusted analysis; (b) Results from adjusted analysis

LBW babies were pre-term. A review published in 2021 also supported that there was no or little difference in birth weight in pregnancy after anti-helminthic treatment.^[30] Studies conducted in Nigeria and Central America reported that women with higher intensity of STH infection were significantly associated with anemia, LBW, and intra-uttering growth retardation.^[18,19] In our study, almost all the women received albendazole regardless of their STH infection and the mean birth weight in the two groups was comparable (STH vs no STH: 2.86 vs 2.92 kgs). Hence, we could conclude that in our study, STH infection does not have a substantial impact on birth weight, probably due to mild intensity of infections. Future studies are recommended to assess the impact of intensity of STH infections on birth weight.

In India, pregnant women are administered a single dose of 400 mg albendazole for STH infection. The literature supports the safety of de-worming medications in pregnancy after the first trimester as it does not have any harmful effects. In accordance with the WHO targets for 2030, India has successfully achieved a coverage of \geq 75% for preventive chemotherapy in children under the age of 5. In order to meet the 2030 targets, it is crucial to prioritize continuous de-worming efforts in children and women of reproductive age. To achieve these targets, it is recommended to conduct periodic STH prevalence surveys among high-risk groups de-wormed for a duration of 5 to 6 years. The findings of these surveys will help in determining the reduction in the frequency of de-worming. If the prevalence for any of the STH is ≥ 2 to <10% prevalence, preventive chemotherapy of once in 2 years could be considered.^[31] This would allow for a targeted reduction in the frequency of de-worming.

Strengths and limitations

We adopted a community-based design and the use of molecular techniques for diagnosing STH infections, which were the strengths. However, information on pregnancy weight gain, maternal calorie intake, and compliance with iron supplements is lacking, which are known to influence birth weight. We did not assess the genital tract microflora, which is known to be associated with the risk of miscarriage and pre-term birth. This information is crucial and could have provided valuable insights into the potential link between STH infections and these adverse outcomes. The study may not have had sufficient statistical power to demonstrate a significant association between STH infection and perinatal outcomes due to limitations in sample size.

CONCLUSION

A higher incidence of anemia, LBW, and pre-term delivery was observed in women with STH infection than in women without STH. However, we did not find any significant association between the presence of STH infections and the perinatal outcomes.

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Ethics

The study protocol was reviewed and approved by scientific advisory committee and Institute Ethics committee (for human studies) of a tertiary care hospital, South India (JIP/IEC/2019/137, dated 02/07/2019). A written informed consent has been obtained from each participant before enrolment.

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Conflicts of interest

There are no conflicts of interest.

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