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Maternal thyroid function during pregnancy as sero-diagnostic marker of pregnancy, delivery and birth outcome

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ABSTRACT

The burden of infection during pregnancy is of public health concern across the globe, in view of the pathogenesis, hormonal alterations, clinical sequelae, prevention and treatment of infections; all have unique features during pregnancy. The combinatorial effect of infection/s, physiological, metabolic alterations and various other obstetric complications during human pregnancy further complicates the prompt and accurate diagnosis of hormonal alterations and infections. This prompted us to investigate the prevalence of infections, haematological perturbations and the role of maternal thyroid function as reliable biochemical marker for diagnostic potential in pregnancy and delivery. Between September 2021 and December 2022, 1715 and 870 women at ANC and DU were enrolled, and screened for malaria by microscopy and RDT. Anaemia was defined as haemoglobin concentration. Thyroid function tests were measured in six stratified groups from ANC and two from DU using competitive ELISA kit. The prevalence of malaria during pregnancy was 5.4% and 4.3% at ANC and DU, respectively and 13.2% in non-pregnant women with malaria, majority of which were infected with *P. vivax*. Anaemia was significantly associated with malaria; however, severe anaemia was more common among women with parasitaemia. Further, observed gestational age specific increasing trend of TSH concentration and differences were significant for all the trimesters as compared to healthy women. TSH were significantly higher in infection as compared to healthy women and those with malaria without pregnancy. The TSH concentrations were highest in caesarean and still birth & higher in normal mode of delivery as compared to healthy women, whereas marginally elevated in caesarean and still birth as compared to normal delivery. Pre-term delivery had highest TSH followed by post-term and term delivery; whereas compared to term delivery, TSH was higher in pre-term and post-term delivery. High prevalence of infection in women and in pregnant women with associated anaemia suggests prompt diagnosis regardless of symptoms and comprehensive drug regime to be offered. Further, thyroid function evaluation could be considered to be reliable prognostic markers and possess promising rationale for diagnostic potential in association with existing measures in clinical spectrum of infection, pregnancy, delivery and its outcome.

Key Words - TSH, Delivery, Birth-Outcome, Diagnostic Marker, Pregnancy

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INTRODUCTION

A number of factors make diagnosing malaria during pregnancy more difficult, such as multiple pregnancy stages with compromised immunity, heightened vulnerability to severe illnesses, different obstetric complications, parasite sequestration in the spleen and placenta, different types of anemia, and variations in patient presentation. Therefore, one of the main objectives of MIP research is the creation of an accurate and timely diagnosis. Central to this effort we evaluated the thyroid function test to identify and step towards the development of a fast, specific, safe and cost-effective biochemical marker to serve as suitable and probable serodiagnostic marker. Reproductive hormones have been shown to impact thyroid physiology during pregnancy (Alexander *et al.,* 2004 and Soldin *et al.,* 2004) and likely lead to influence the maternal thyroid function and measures of thyroid functions (Brent, 1997). Normal maternal thyroid function during the periconception window defined as the transition from pre-pregnancy through the early first trimester is important as implantation disorders may predispose to adverse obstetrical outcomes (Lala *et al.,* 2003) most miscarriages occur during this interval (Wang *et al.,* 2003) and normal early fetal neurological development requires maternal thyroxin (Calyo *et al.,* 2008). Further, maternal thyroid dysfunction during pregnancy has been shown to be associated with an increased risk of pre-term birth, placental abruption, fetal death, low birth weight infants and impaired neurological development in the child (Casey *et al.,* 2005).

Thus, in view of the prevalence, association of maternal thyroid with adverse outcomes, hormonal changes and metabolic demands occur not only in pregnancy and various trimesters, but also in labour and puerperium; prompted us to investigate with an objective to evaluate the levels of thyroid hormones in various trimesters of pregnancy and/or with malaria and non-pregnant women with malaria as compared with healthy women to elucidate the role of thyroid regulation during malaria in pregnancy and its effect on delivery and birth outcome. Further, to explore the role of maternal thyroid function as reliable biochemical marker for diagnostic potential in malaria during pregnancy, in various modes of delivery and birth-outcome. As far as we are aware, no previous study has explored the profile, epidemiological links, and clinical correlations of malaria in pregnancy from Hazaribag, Jharkhand—one of India's malaria-endemic areas. Notably, our work represents the first attempt to examine the relationship between thyroid hormone levels and malaria during pregnancy, categorized by clinical groups in an adult population living in a region of perennial transmission where both *Plasmodium vivax* and *Plasmodium falciparum* are co-dominant (Jharkhand, Eastern India).

METHODS

Screening and Enrolment

The study comprised three components, with recruitment focused on women attending antenatal clinics (ANC), delivery units (DU), or admitted to the antepartum ward. In the ANC group, pregnant women aged 17 years and above visiting the facility for routine care were screened, and those who provided consent were enrolled. In the DU group, women aged 18 years or older who presented for childbirth and agreed to participate through written informed consent were included. For the inpatient group, pregnant women admitted with suspected malaria, anemia, or unexplained fever were screened, and those with confirmed malaria diagnoses were enrolled after consent. At each visit, participants underwent clinical assessment, microscopic examination of peripheral blood smears, and measurement of axillary temperature prior to enrolment. The enrolment strategy, sampling procedures, and classification into broad study groups are depicted in the schematic flow chart (Figure 1).

ANC procedures

Trained research staff interviewed enrolled participants to obtain data on socio-demographic details (such as age, educational status, and socio-economic background), reproductive history including gravidity, prior fever episodes, use of anti-

malarial medications, and preventive practices against malaria. A thorough clinical examination was carried out, with gestational age determined using fundal height palpation along with last menstrual period records. Axillary temperature was measured using a digital thermometer, and other vital signs were also recorded. From each participant, 3–5 ml of peripheral venous blood was collected for malaria microscopy, rapid diagnostic testing (RDT), hemoglobin estimation, and additional biochemical and molecular analyses. Women who tested positive on RDT or were found to be anemic were promptly referred to the hospital physician for treatment. Furthermore, hospital staff were notified of any parasitemia detected through microscopy to ensure timely clinical management

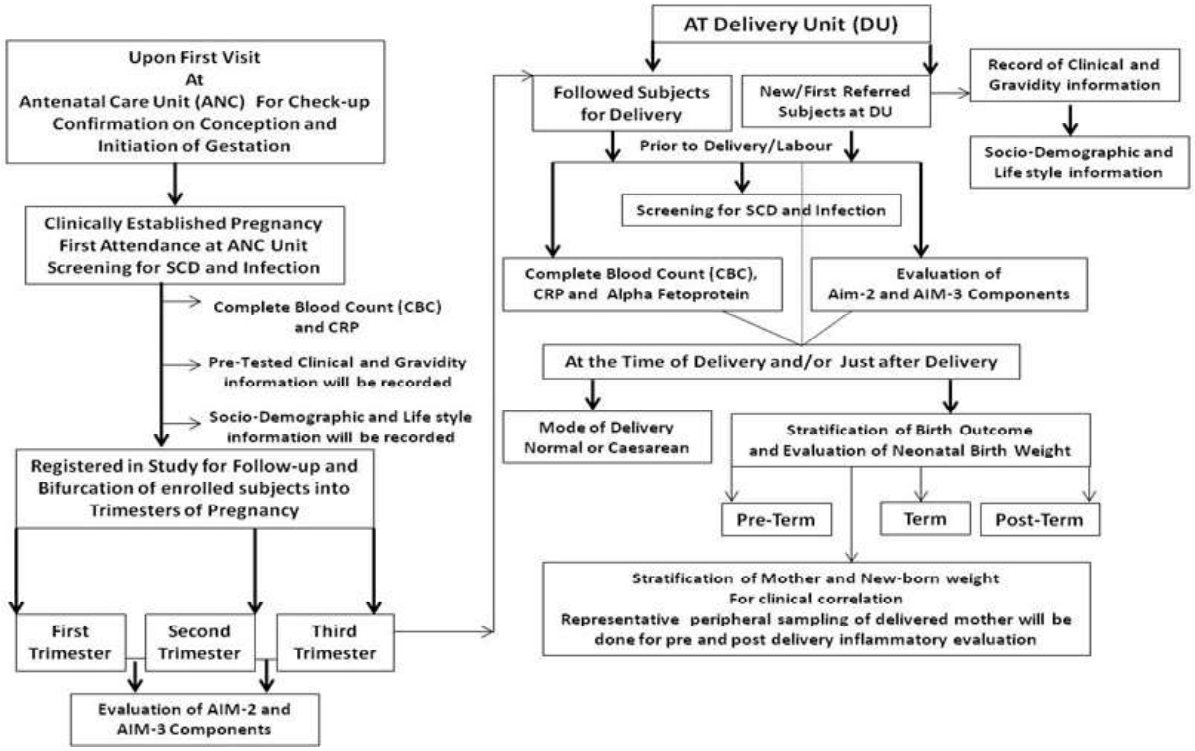

Figure-1 Schematic Flow Chart Summarizing the Sampling Strategy and Groups

DU procedures

At the delivery units (DUs), enrolled pregnant women were interviewed to gather information on socio-demographic and anthropometric characteristics, obstetric complications, fever history, use of anti-malarial drugs during pregnancy, preventive practices, delivery outcomes, and mode of childbirth. Following delivery, 3–5 ml of peripheral venous blood was collected for malaria microscopy, rapid diagnostic testing (RDT), hemoglobin estimation, as well as additional biochemical and molecular analyses. Women testing positive by RDT or blood smear were referred for appropriate treatment. In addition to assessing malaria prevalence in the DU cohort, clinical and demographic information and biological samples were obtained according to mode of delivery (normal, cesarean, or stillbirth) and classified further by delivery outcomes, including pre-term, term, and post-term births. The summarized details are presented in Table 1.

Table 1- Baseline characteristics of pregnant women attending antenatal and delivery units

Characteristics	Antenatal clinics n=1271	Delivery units n=870
	N, (%)	N, (%)
AGE (YEARS)		
<20	166(13.1)	109(12.5)
20-34	983(77.4)	708(81.4)
≥35	122(9.5)	53(6.1)

PRIOR PREGNANCIES		
Primigravid	423(33.3)	338(38.38)
Secundigravid	578(45.5)	209(24.1)
Multigravid*	270(21.2)	323(37.1)
GESTATIONAL AGE AT ENROLMENT (WEEKS)**		
<20 weeks	567(44.6)	n/a
20-36 weeks	641(50.4)	57(6.5)
≥37 weeks	63(5)	813(93.5)
CASTE		
Schedule caste	169(13.3)	93(10.7)
General caste	428(33.7)	307(35.3)
Other backward caste	311(24.5)	219(25.2)
Scheduled tribe	363(28.5)	251(28.8)
EDUCATION		
No formal schooling	357(28.1)	321(36.9)
Attended school any length of time	914(71.9)	549(28.8)
SOCIOECONOMIC CHARACTERISTICS		
Owns TV	567(44.6)	387(44.5)
Owns bicycle	1173(92.2)	687(78.9)
Owns house	958(75.4)	643(73.9)
Owns refrigerator	123(9.6)	83(905)
ROOF MATERIAL		
Mud	622(48.9)	513(58.9)
Corrugated iron/asbestos sheet	242(19)	182(20.9)
cement/concrete	329(25.8)	107(12.3)
Other	78(6.1)	68(7.8)
WALL MATERIAL		
Mud/sand/dung	673(52.9)	478(54.9)
Mud bricks	127(9.9)	93(10.7)
Cement bricks	419(32.9)	267(30.7)
Other	52(4.1)	32(3.7)
PRIMARY COOKING FUEL		
wood	619(48.7)	387(44.5)
charcoal	437(34.4)	279(32.1)
Gas	153(12.1)	136(15.6)
Other	62(4.9)	68(7.8)
MODE OF DELIVERY AMONG PREGNANT WOMEN		
Normal	n/a	586(67.3)
Caesarean	n/a	179(20.6)
Still Birth	n/a	105(12.1)
BIRTH OUTCOME		
Pre-Term Delivery(≤36 weeks)	n/a	129(14.8)
Term Delivery (31-41 weeks)	n/a	623(71.6)
Post-Term Delivery (after 41 weeks)	n/a	118(13.5)

Numbers may not add to sample size secondary to missing data.

* Defined as 3 or more pregnancies

** For ANC enrollees, gestational age assessed by fundal height. For DU enrollees, gestational age was assessed by Ballard score.

Laboratory procedures

Peripheral blood samples obtained from ANC and DU participants were used to prepare thick and thin smears, which were Giemsa-stained and examined microscopically under high power. Parasite density was estimated by counting asexual parasite stages per 200 leukocytes, assuming a standard leukocyte count of 8,000/µl of blood. Thin smears were utilized for species identification. All slides were independently verified by trained staff following strict diagnostic criteria to confirm *Plasmodium* infection. Additionally, the First Response Malaria pLDH/HRP2 combo rapid diagnostic test (RDT) kits (Premier Medical Corporation, Mumbai, India) were employed according to the manufacturer's instructions as a screening tool for malaria detection in pregnant women. Hemoglobin (Hb) concentration in peripheral blood was measured using a portable HemoCue hemoglobinometer (HemoCue AB, Ängelholm, Sweden)."

Sample processing and assay for thyroid function test

Three to Five ml venous blood was drawn as per the sampling strategy depicted in Figure-1; before administration of antimalarial therapy, aseptically by dripping from the syringe without anticoagulant into a sterile pro-clot activator coated tubes. Blood was allowed to coagulate in a refrigerator for 4 to 6 h at 4^{o}C before being processed by centrifugation. Sera were preserved in three to five aliquots immediately stored at -20^{o}C, and maintained at -80^{o}C until measurements were performed. The TSH, T3 and T4 serum concentration were measured by competitive ELISA micro well plate-based assay using commercially available kit form Bene SpheraTM (Avantor Performance Materials, PA, USA). The assays were performed according to manufacturer's instructions and optical densities measured using a microplate reader set to 450 nm wavelength. The sensitivity of the assay for TSH, T3 and T4 were 0.078µlU/ml, 0.04ng/ml and 0.4µg/dl, respectively. The intra-assay TSH, T3 and T4 coefficient of variation were 5, 5.5 and 6.7%, respectively; the TSH, T3 and T4 inter-assay coefficient of variation were 6, 7 and 8.3%,

respectively. The reference interval for TSH, T3 and T4 were 0.39-6.16 µlU/ml, 0.52-1.85ng/ml and 4.4-11.6µg/dl, respectively.

Ethics Statement and Subject Consent

All blood samples in this study were obtained only after informed consent was provided by the participants, following protocols approved by the Institutional Ethics Committee (IEC) of Vinoba Bhave University, Hazaribag, Jharkhand. The procedures adhered to the ethical standards outlined by the Medical Ethics Committee, Ministry of Health, Government of India. Approval for the study protocol was granted by the IEC, VBU under memo no. VBU/R/888/2012 dated 05-06-2012.

Data management and analysis

All clinical, demographic, and anthropometric records were thoroughly reviewed for accuracy, and discrepancies were resolved prior to analysis. Data were entered into MS Excel and analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, CA, USA). For comparisons between two groups, Student's *t*-test was applied when data followed a normal distribution, whereas the Mann–Whitney U test was used for non-normally distributed data. Categorical variables were expressed as frequencies (percentages) and compared using chi-square or Fisher's exact test, as appropriate. Continuous variables were presented as mean ± standard error and compared using *t*-test or analysis of variance. Since many participants were unable to recall their exact date of birth, age was categorized into ranges based on self-reported estimates. Risk factors associated with *Plasmodium falciparum* or *Plasmodium vivax* parasitemia were first assessed by univariate analysis, followed by multivariate analysis to adjust for significant predictors. A p-value <0.05 was considered statistically significant.

RESULTS

Recruitment and enrolment were carried out from September 2021 to December 2022. During this period, 1,890 pregnant women attending ANC visits at Sadar Hospital, Hazaribagh, Jharkhand, were screened (Figure 2). Of these, 1,746 expressed willingness to participate, and 1,715 consented to peripheral blood sampling, while 31 declined. Thus, a total of 1,715 women were enrolled. Based on pregnancy screening, structured interviews by trained staff, and clinical assessments, participants were grouped into pregnant and non-pregnant categories. The pregnant group was further stratified by trimester, comprising 135 women in the first, 492 in the second, and 644 in the third trimester. The non-pregnant group was classified as malaria-free (227 women) and malaria-infected (217 women). At the delivery unit, 870 pregnant women were screened, all of whom were eligible and subsequently enrolled.

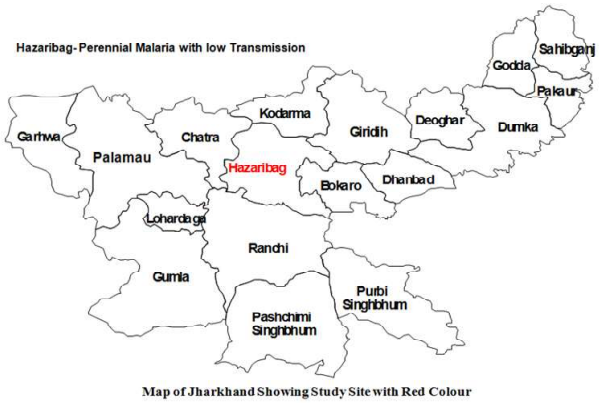

Figure 2- Map of Jharkhand Showing Study Site

Antenatal clinics

Most of the pregnant women attending ANC were between 18 and 38 years of age and had received some level of formal education (Table 1). A large majority were Hindi-speaking (97.6%) and non-smokers (98.7%). About three-fourths owned their homes (75.4%) and the majority were engaged in household activities (76.7%), while a smaller proportion worked in farming (12.3%). Participants had attended a median of one ANC visit (range: 0–9) during the current pregnancy, with nearly one-third being primigravidae (33.3%). Slightly more than half presented during the later half of pregnancy, while 44.6% reported before 20 weeks of gestation. Less than half of the women reported taking iron/folate supplements (46.3%), and about one-third were on multivitamins (33.2%). Regarding malaria prevention, most households owned untreated bed nets and reported recent use, though very few had insecticide-treated nets (ITNs). Only nine women

reported taking malaria prophylaxis, of whom seven (77.8%) were unable to name the medication; the remaining identified chloroquine as the drug used.

Among the total cohort, malaria was confirmed in 5.4% (68/1271) of participants. Microscopic examination of blood smears identified infection in 4.3% of pregnant women, while an additional 1.1% (14 cases) were detected through rapid diagnostic tests (RDTs).

Delivery units

Like the ANC cohort, most pregnant women attending DUs were aged 20-36 years and had some level of formal education (Table-1). All were non-smokers (100%) and nearly all spoke Hindi (97.2%). Most owned their own home (73.9%) and were involved in household work (84.3%); a minority engaged in farming (14.6%). Study participants had attended a median of three ANC visits (range 0-9) and about slightly less than two-thirds were primigravidae and secundigravidae (Table-1). The majority of pregnant women reported having untreated bed nets in their homes and using them recently but ITN ownership was uncommon. Only three women were taking chemoprophylaxis for malaria and none knew the name of the medication that they were taking.

Only 4.3% of the women enrolled at the DUs had peripheral parasitaemia (either a positive blood smear and/or RDT). *P. falciparum* was identified in 5.4% (2/37), *P. vivax* in 86.5% (32/37), and mixed infection in 8.1% (3/37).

As observed in the ANC participants, most episodes of parasitaemia occurred in July to September during the monsoon season. Nearly two third of the DU participants were anaemic whereas 16.3% had severe anaemia. For DU participants with peripheral parasitaemia, 83.7% had anaemia as compared to 47.6% of those who did not have parasitaemia (p=0.004). More women with peripheral parasitaemia had severe anaemia (5.7%) than those without parasitaemia (2.6%) and the difference was significant (p=0.02).

As we observed 5.4% and 4.3% malaria during pregnancy at ANC and DU unit, respectively as compared to 1.8% and 1.7% at ANC and DU unit, respectively reported by Hamer *et al.* (2009) from the series of cross-sectional study in Jharkhand. However, our study design is slightly broader than the earlier investigation from Hamer *et al.* (2009) in terms for subject stratification, as we also taken into the account of women with malaria without pregnancy and the prevalence of malaria were found to be 13.2%, which itself reflects the importance of the investigated region and population under malaria sensitive zone.

Thus, in view of the sizable prevalence, both in women with malaria without pregnancy and malaria during pregnancy, we thought to explore the serodiagnostic marker using thyroid function test. In order to investigate further, we selected 100 subjects (representative samples) each in all the groups as described previously except in women with malaria in pregnancy group (N=68) for the analysis of thyroid function tests. The experiments for this marker-based analysis were restricted with sample size due to financial constraint of the project and baseline subject characteristics are shown in Table-2.

Table 2- Baseline Characteristics of the Study Population

Parameters	Healthy Women N=100 Mean±SE	Women with Malaria N=100 Mean±SE	Pregnant Women N=300 Mean±SE	First Trimester N=100 Mean±SE	Second Trimester N=100 Mean±SE	Third Trimester N=100 Mean±SE	Delivery Unit N=200 Mean±SE	Pregnant Women with Malaria N=68 Mean±SE
Age (years)	24.1±0.7	33.4±1.3	22.1±0.5	22.3±1.0	21.6±0.2	22.6±0.4	27.3±0.2	22.8±0.7
Weight (Kg)	49.4±1.1	47.4±0.9	47.9±0.8	48.0±1.4	46.8±0.6	49.0±0.6	42.0±0.4	47.4±0.9
Height (Meters)	1.54±0.1	1.56±0.6	1.47±0.5	1.48±0.2	1.48±0.6	1.45±0.6	1.35±0.6	1.41±0.1
BMI (kg/m2)	20.6±0.4	19.3±0.4	22.4±0.5	22.1±0.7	22.1±0.7	23.0±0.3	22.0±0.2	24.0±0.6
Systolic (mm/hg)	118±2.8	119±1.8	106±3.3	105±4.0	105±4.0	109±2.1	119±2.3	103±2.5
Diastolic (mm/hg)	71±2.1	78±1.1	67±2.3	65±2.4	65±2.4	71±2.1	78±2.4	66±2.2
Pulse	96±2.0	96±1.0	107±3.1	110±3.9	110±3.9	101±1.2	121±1.7	105±2.3

Thyroid Functions during Pregnancy in serum obtained from Malaria Negative Pregnant Women

Little is known about thyroid function levels during normal pregnancies and to our knowledge of baseline levels at different gestational ages of Jharkhand populations have not been reported. We, therefore first determined the levels of thyroid panel (TSH, T_3 and T_4) in the women groups who remained infection-free and all the trimesters (from first trimester to third trimester) were observed to be significantly higher thyroid panel as compared to healthy women without pregnancy, as shown in Table-3 and Figure-3A-C. Interestingly, we observed the increasing trends of levels of the TSH are gestational age specific as compared to healthy women without pregnancy and their differences were statistically significantly for all the trimesters; *P=0.005, P=0.001 and P=0.0001*, respectively as shown in the Figure-3A. Further, T_3 was observed to highest in second trimester and higher in first trimester as compared to third trimester, whereas T_4 was highest in third trimester and higher in first trimester as compared to second trimester; although the differences were significant in all the cases as compared to healthy women as shown in Figure-3B-C.

Table 3- Serum triiodothyronine, thyroxin and thyroid stimulating hormone levels in the healthy women and other investigated clinical groups

Parameters Group of Subject	TSH (µIU/ml.) Mean ± SE	T3 (ng/ml.) Mean ± SE	T4 (µgm/ml.) Mean ± SE
Healthy Women (N=100)	1.1±0.11	0.52±0.06	0.8±0.1
First- Trimester (N=100)	1.7±0.13	0.91±0.10	1.7±0.2
Second-Trimester (N=100)	2.2±0.26	0.98±0.12	1.6±0.2
Third-Trimester (N=100)	2.4±0.24	0.81±0.07	2.1±0.3
Pregnancy with Malaria (N=68)	2.05±0.18	0.96±0.08	1.5±0.2
Women With Malaria (N=100)	1.01±0.14	0.88±0.12	1.3±0.1

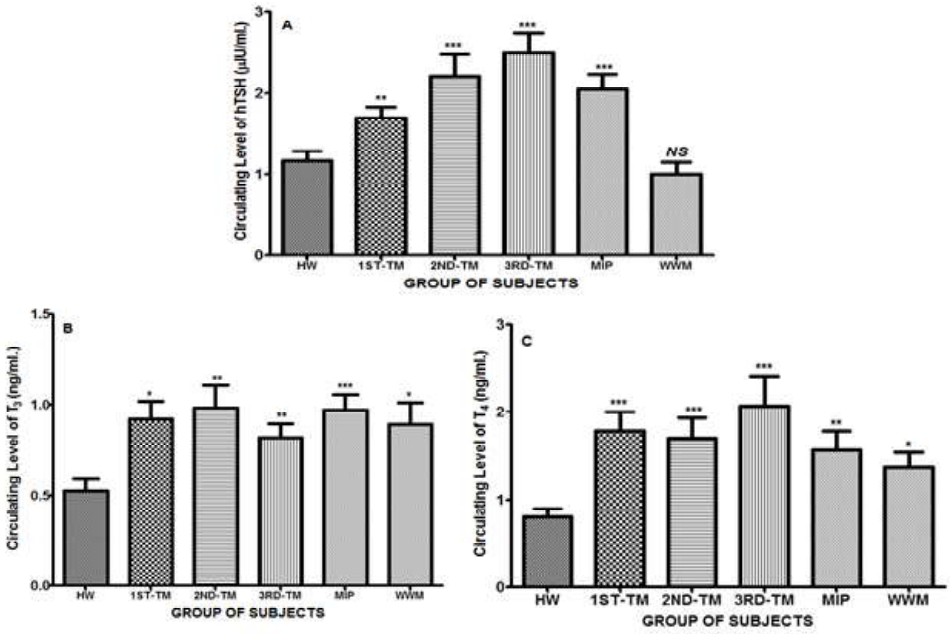

Figure 3 (A-C)- Circulating level of (A) TSH, (B) T_3 and (C) T_4 hormones among healthy women subject (HW), pregnant women in first trimester (First-TM), pregnant women in second trimester (Second-TM) and in pregnant women in third trimester (Third-TM); malaria infected pregnant women (MIP) and women with malaria (WWM). Data is presented as mean and error bar represent the plus or minus SE *p≤0.01; **p≤0.001; ***p≤0.0001 compared with healthy subjects by two-way analysis using paired 't' test through GraphPad Prism version 5.0

Thyroid Panel and Malaria Infection

To evaluate the effect of plasmodium infection we compared the concentration of the thyroid panel (TSH, T_3 and T_4) in the serum of women with malaria during pregnancy and women with malaria without pregnancy as compared to healthy women as shown in Table-3 and there statistical correlation in Figure-3A-C. Most interestingly, TSH level was found to be almost double in case of malaria in pregnancy as compared to malaria-free women and malarious women with without pregnancy and differences were significant (*P=0.0001, and P=0.002*) (Figure-3A). Additionally, concentration of TSH was slightly lower in malaria without pregnancy as compared to healthy women and difference was not significant.

Further, T_3 and T_4 during malaria in pregnancy were nearly double in the concentration and the differences for both were found to be significant (P=0.0001, and P=0.001, respectively) as compared to healthy women, whereas slightly higher as compared to women with malaria without pregnancy (Figure-3B-C) but in neither case (T_3 and $T_{4)}$ did the difference reach statistical significance.

Precisely, we observed that all the thyroid panel (TSH, T_3 and T_4) levels in malaria during pregnancy were markedly higher and their differences were statistically significant (P=0.0001, P=0.0001, and P=0.001, respectively) as compared to healthy women; whereas T_3 and T_4 were higher and TSH was marginally lower in case of malaria without pregnancy and their differences were statistically significant except TSH as compared to healthy women (Figure-3A-C).

Assessment of Thyroid panel in Birth-Outcome and mode of Delivery

To investigated the effect of thyroid function on mode and outcome of delivery, we compared the concentration of the thyroid panel (TSH, T_3 and T_4) in the serum of women from delivery unit; those who had delivered normal, caesarean, and still birth mode of delivery groups respectively and those who had pre-term, post-term, and term delivery groups, respectively as compared to healthy women shown in Figure-4A-C and Figure-5A-C, respectively. The TSH concentration were highest in caesarean and still birth and higher in normal mode of delivery and their differences were significant (P=0.001, P=0.01, and P=0.04, respectively) as compared to healthy women. As compared to normal delivery, we observed marginally elevated TSH in case of caesarean and still birth mode of delivery but the differences were not significant shown in Table-4. We observed highest T_3 elevation in caesarean followed by normal and still birth mode of delivery and the differences were significant (P=0.001, and P=0.006, respectively) except still birth as compared to healthy women, whereas compared to normal, T_3 level in caesarean and still birth mode of delivery was higher and lower, respectively and the differences were not significant. Further, T_4 concentration was observed to be highest in caesarean followed by still birth and normal mode of delivery and the differences were significant (P=0.0001, P=0.006, and P=0.001, respectively), whereas compared to normal, higher levels of T_4 were observed in caesarean and still birth mode of delivery although the differences were not significant. Precisely, we observed that the entire thyroid panel (TSH, T_3 and T_4) were reasonably elevated in caesarean and still birth mode of delivery except T_3 in case of still birth as compared to normal delivery; however, the differences were not significant. We also observed that the entire thyroid panel (TSH, T_3 and T_4) were significantly elevated in all the mode of delivery except lower and non-significant T_3 content in still birth as compared to healthy women.

In addition to this, we evaluated the effect of thyroid panel (TSH, T_3 and T_4) test in outcome of delivery, we observed highest TSH level in pre-term delivery followed by post-term and term delivery and the differences were significant (P=0.0003, P=0.003, and P=0.04, respectively) when compared to healthy women, whereas compared to term delivery, TSH were higher in pre-term and post-term delivery and differences were significant (P=0.03, and P=0.01, respectively). Though we observed significantly elevated level of T_3 in all the three

outcome of delivery (term, pre-term and post-term delivery) compared to healthy women but the content were almost equal in all the outcome of delivery; however, the differences were significant (P=0.0001, P=0.01, and P=0.01, respectively). The T_4 content was highest in pre-term delivery followed by term and post-term delivery and differences were significant (P=0.0004, P=0.0001, and P=0.0001, respectively) as compared to healthy women. Further, T_4 were found to be higher in pre-term and lower in post-term delivery and the differences were not significant as compared to term delivery. Precisely, we observed that the entire thyroid panel (TSH, T_3 and T_4) were reasonably elevated in all the three outcomes of delivery (term, pre-term and post-term delivery) and the differences were significant as compared to healthy women; whereas only TSH was found to be elevated in case of pre and post-term deliver and differences were significant as compared to term delivery.

Table 4-Serum triiodothyronine, thyroxin and thyroid stimulating hormone levels in the delivery based stratification and based on the birth-outcome

Parameters Groups of Subject	TSH (µIU/ml.) Mean ± SE	T3 (ng/ml.) Mean ± SE	T4 (µgm/ml.) Mean ± SE
Based on Delivery			
Normal Delivery	1.5±0.12	0.86±0.12	2.4±0.23
Caesarean Delivery	1.7±0.12	1.02±0.08	2.9±0.15
Still Birth	1.7±0.26	0.70±0.17	2.5±0.31
Based on Birth Outcome			
Pre-Term Delivery	2.1±0.24	0.93±0.17	2.8±0.30
Post-Term Delivery	1.6±0.07	0.89±0.11	2.6±0.20
Term Delivery	1.5±0.13	0.98±0.09	2.7±0.17

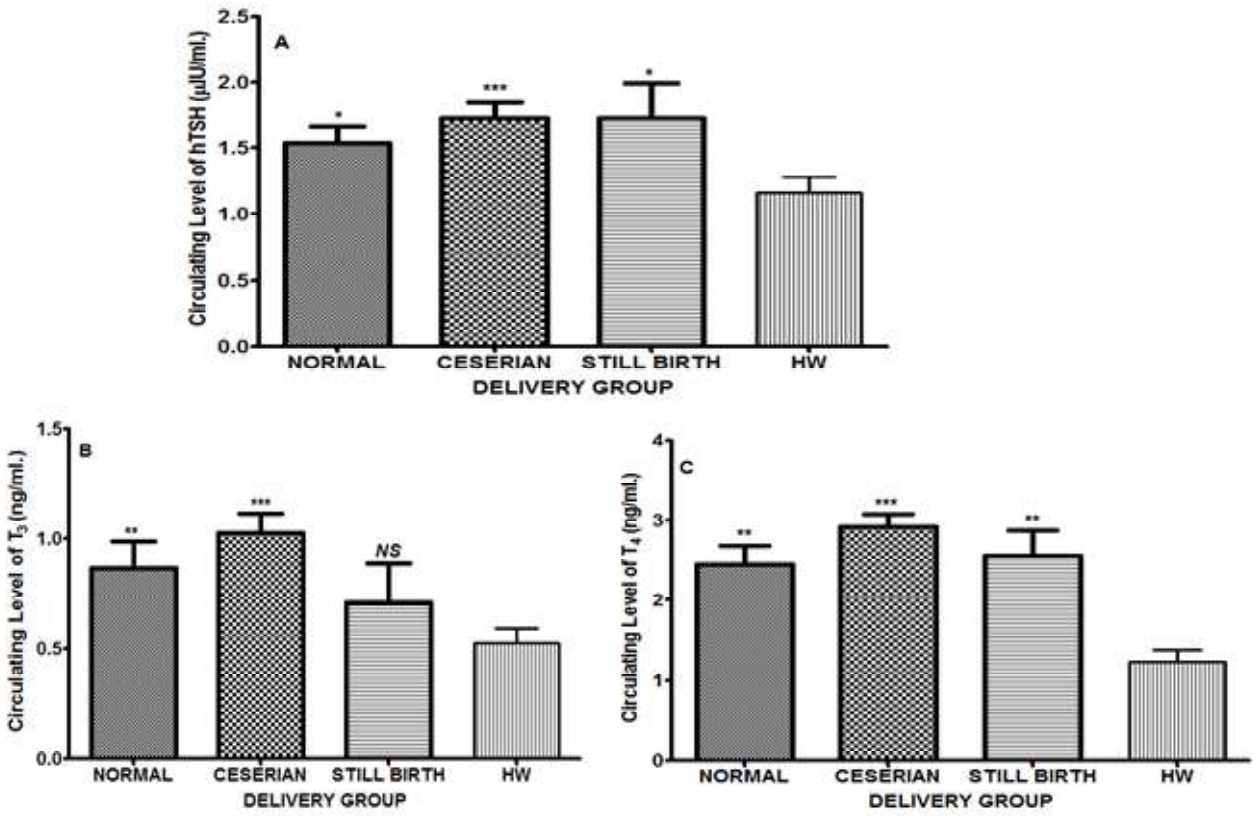

Figure 4 (A-C)- Circulating level of (A) TSH, (B) T3 and (C) T4 hormones based on the delivery group i.e. in normal delivery, ceserian delivery and still birth groups as compared to healthy women subject (HW). Data is presented as mean and error bar represent the plus or minus SE *p≤0.01; **p≤0.001; ***p≤0.0001 compared with healthy subjects by two-way analysis using paired 't' test through GraphPad Prism version 5.0

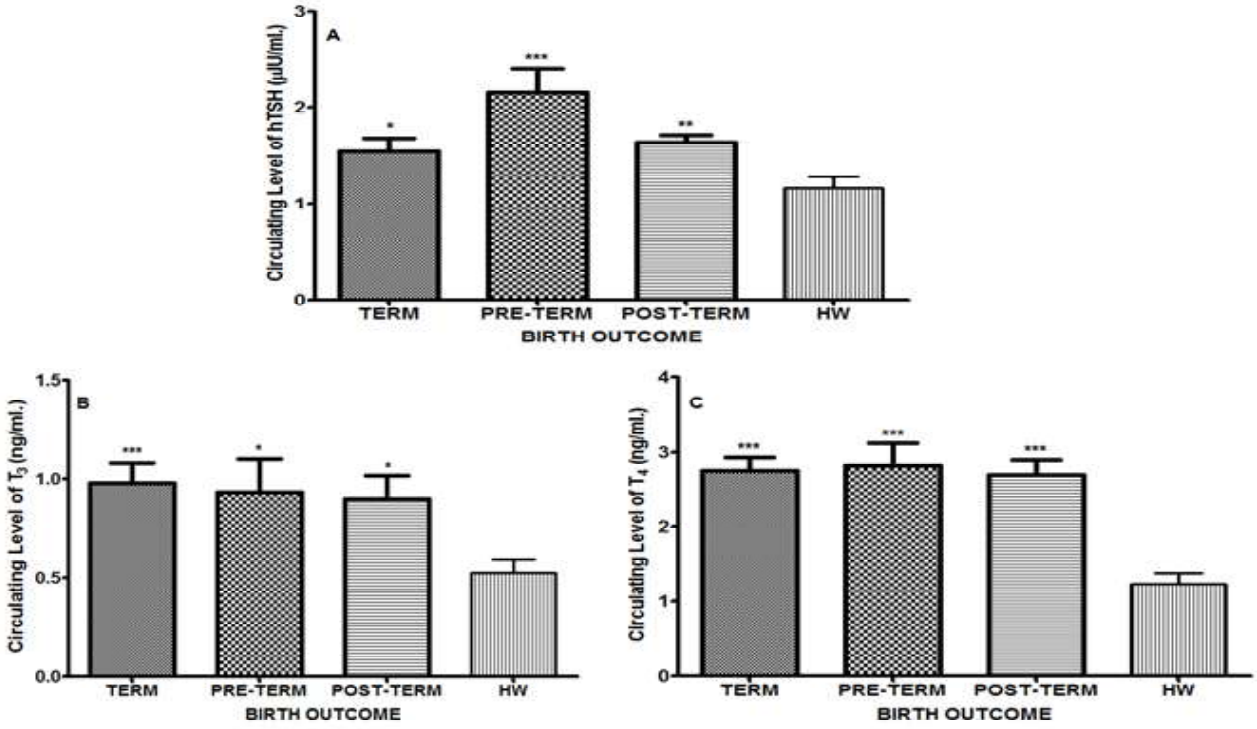

Figure 5 (A-C)- Circulating level of (A) TSH, (B) T3 and (C) T4 hormones based on the birth outcome i.e. in term delivery, pre-term delivery and post-term groups as compared to healthy women subject (HW). Data is presented as mean and error bar represent the plus or minus SE *p≤0.01; **p≤0.001; ***p≤0.0001 compared with healthy subjects by two-way analysis using paired 't' test through Graphpad Prism version 5.0

DISCUSSION

The burden of malaria in pregnancy remains a major challenge for maternal and child health worldwide, including India, and directly impacts the goals of the National Family Welfare Programme, which emphasizes 'healthy mothers and healthy babies.' The problem is even more severe in developing nations such as India, where poverty, limited literacy, socio-economic disparities, geographical variability, and multiple pregnancies collectively compromise maternal well-being.

In the present investigation, malaria was detected in 5.4% of ANC attendees and 4.3% of women at delivery units (DU). These values are higher than those reported by Hamer *et al.* (2009), who observed only 1.8% and 1.7% prevalence in ANC and DU, respectively, in a multicentric study across Jharkhand. A key distinction is that our design not only considered pregnant women but also included non-pregnant women with malaria, where prevalence reached 13.2%. This highlights the high endemicity of the study region, although unlike Hamer *et al.* (2009), our investigation did not assess placental malaria.

The difference in prevalence between our study and that of Hamer *et al.* (2009) may largely be attributed to site selection. Our study was limited to Hazaribag district, whereas Hamer *et al.* (2009) focused on Ranchi (an urban capital with better infrastructure, healthcare access, education, and socio-economic conditions) and Gumla. Ranchi has consistently reported lower malaria transmission compared to nearly 20 other districts in Jharkhand (State Malaria Control Program Report, 2009). Therefore, while their findings accurately reflect the chosen sites, they may underestimate the true burden of malaria in pregnancy in Jharkhand. In contrast, our prevalence values align with earlier Indian studies, which reported ranges from 1.7% to 20% (Hamer *et al.* 2009). Many of those investigations primarily screened febrile or recently febrile pregnant

women, introducing selection bias and likely inflating prevalence estimates. This case-detection approach is consistent with India's National Vector Borne Disease Control Programme guidelines (2008). By comparison, our study assessed all pregnant women regardless of symptoms, revealing that over 70% of ANC and DU attendees with malaria were asymptomatic. This suggests the need for region-specific screening interventions.

The elevated prevalence among both pregnant and non-pregnant women, irrespective of rural, urban, or semi-urban residence, points to perennial malaria transmission in Hazaribag and surrounding areas. Hence, all population groups remain vulnerable, with transmission peaks following the monsoon. Contributing factors include limited health literacy, inadequate awareness, favorable environmental conditions for mosquito proliferation, poverty-linked malnutrition and anemia, and poor access to preventive tools or antimalarial therapy. Notably, few participants reported antimalarial use during the week preceding enrolment or at any point during pregnancy. Indoor residual spraying (IRS), typically implemented by government programs, was reported more frequently in rural than urban areas; however, its seasonal application in a region with continuous transmission may suggest vector resistance, potentially explaining sustained malaria prevalence. These findings highlight the urgent need to strengthen IRS coverage and expand the distribution of insecticide-treated nets (ITNs).

A high burden of anemia was also observed among pregnant women in Jharkhand. This finding is consistent with earlier reports from the region (Hamer *et al.* 2009, Sohail, *et al.* 2015), nationwide data (Hamer *et al.* 2009), and international studies, notably Nosten *et al.* (1991), who demonstrated that malaria during pregnancy significantly increases the risk of anemia. Indeed, maternal anemia is widely recognized as the most frequent adverse outcome of malaria in pregnancy (Menendez *et al.* 1995). The clinical manifestations and complications vary with transmission intensity and acquired immunity. In low-transmission settings, women of reproductive age often lack immunity, making them more susceptible to severe disease, anemia, spontaneous abortion, stillbirth, prematurity, and low birth weight (Kumari *et al.* 2019). Malaria pathogenesis involves sequestration of infected erythrocytes in the spleen, contributing to folic acid deficiency and microcytic anemia. In pregnancy, additional sequestration occurs in the placenta, leading to disproportionate rates of severe anemia among expectant mothers (Desai *et al.* 2007).

Despite the varied frequency of MIP within the state, the large population of Jharkhand, nearly 22 million people (Hamer *et al.* 2009), means that there are nearly 100,000 women at risk for malaria-associated complications based on the 5.4% prevalence we observed in the ANC population and assuming that 25% of the population are women of child-bearing age. Consistent with this hypothesis, a recent re-evaluation of the worldwide burden of malaria in pregnant women suggested a much higher burden of disease in the Asia-Pacific region than previous estimates (Dellicour *et al.* 2008).

In view of the sizable prevalence of malaria and malaria in pregnancy, we explored the thyroid function test (TFT) as serodiagnostic marker and serum TFT levels undergo dynamic changes throughout pregnancy. We observed higher level of thyroid panel (TSH, T_3 and T_4) in all the trimesters and more particularly we found increasing trend of TSH level are gestational age specific as compared to healthy women and this has been in accordance with the observations in various other studies comparing thyroid hormones during pregnancy (Zarghami *et al.* 2005). The reason of elevated thyroid hormone concentrations in pregnancy may be attributed, partly due to the high levels of estrogen and due to the weak thyroid stimulating effects of human chorionic gonadotropin (hCG) that acts like TSH (Smyth *et al.* 1997). Thyroxine (T4) levels begin to increase between 6 and 12 weeks of pregnancy and typically reach their peak by mid-gestation, accompanied by reciprocal changes in thyroid-stimulating hormone (TSH).

During early pregnancy, the fetus relies entirely on maternal thyroxine, as the fetal thyroid gland becomes functional only in the second trimester. Since thyroxine plays a vital role in fetal brain development, ensuring adequate maternal supply to the fetus during early gestation is of critical importance. Further we observed distinctly elevated thyroid panel test in case of malaria during pregnancy and marginally elevated thyroid functions in case of malaria infection without pregnancy and our observations regarding thyroid functions are quite novel and pioneering in malaria during pregnancy as it potently lacks previously published studies, however very few earlier studies has been carried out systematically investigating thyroid function during malaria infection (Davis *et al.* 1990 and Talwar *et al.* 1977). The role of the orchestration of the pituitary-thyroid axis during infection was studied and probably during infection, TSH responses to TRH stimulation were preserved, while prolactin secretion showed a slight increase. During infection, serum T3 levels rose abruptly, whereas serum T4 remained stable or showed a mild increase, indicating possible alterations in peripheral thyroid hormone metabolism and leading to the assessment of reverse T3. Fluctuations in serum T3 concentrations were consistently associated with reciprocal changes in reverse T3

As these changes may be an adaptation to accelerated catabolism, though, the role of thyroid replacement in such patients is uncertain (Davis *et al.* 1990). Additionally, hormone secretion by the thyroid and its responsiveness to endogenous TSH are maintained during fever. The alteration in T_3 levels are not suggestive of a hypothyroid state, but perhaps could be due to decreased peripheral conversion of T_4 to T_3 and to decreased binding of T_3 to serum proteins. The exact mechanism or significance of these alterations in thyroid function during febrile illness remains to be elucidated (Talwar *et al.* 1977). Notably, these observations provide some clarification of previously unknown aspects of thyroidal economy during infection. Our study also indicates the important influence of labour process on thyroid axis at birth, as we found higher TSH level in caesarean and still mode of delivery and our observations are in agreement as were reported by Casey *et al.* (2005). However, some contrasting observations have also been previously reported that there was no relation between TSH, T3 and T4 values and the delivery route, duration of labor or uterotonic agents (Eltom *et al.* 2001). The probability of increase in serum thyrotropin level during the birth may be attributed to exposure of neonates to the cold in extra uterine environment (Christensson *et al.* 1993). Infants delivered by caesarean section have lower axillary, and skin temperature than those delivered per vaginum (Christensson *et al.* 1993). Therefore, lower body temperature of the neonate in cesarean delivery may be a stimulus for the higher TSH levels, which evokes increased secretion of T4 and T3 by the thyroid.

The evaluation of thyroid alterations, especially in preterm birth was high in this study and this is in accordance with the finding by Negro *et al*. (2006) also reported that pregnant women with higher TSH level has increased risk of pre-term delivery and miscarriage also. Thyroid hormone is associated with the neurodevelopment of preterm birth (Gressens 2002). Despite many studies on thyroid function in preterm birth, it's significant to investigate on region and diseases specific. Moreover, there has been much need for exploring the thyroid function tests for preterm birth (Rapaport *et al.* 2002) as sensitive biomarker in malaria during pregnancy.

CONCLUSION

Given the substantial burden of pregnancy-associated infections, often accompanied by significant anemia and parasitemia, prompt diagnosis irrespective of the presence of symptoms and the provision of a comprehensive antimalarial treatment regimen are essential for pregnant women. In addition, evaluation of thyroid function may serve as a reliable prognostic marker and holds potential as a complementary diagnostic tool within the broader clinical spectrum of infection, pregnancy, delivery, and associated outcomes.

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