Prevalence of Asymptomatic *Plasmodium Vivax* Infection is Associated with Anaemia during Pregnancy in Malaria Endemic Population of Hazaribag, Jharkhand, India

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VINOBA BHAVE UNIVERSITY HAZARIBAG

INTRODUCTION

❖*Plasmodium vivax*, the most widespread parasite causing human malaria, is responsible for an estimated 130–435 million infections annually and is the major cause of malaria in most of Asia and Latin America [Baird JK. 2007].

❖Every year, in India, 28 million pregnancies take place with 67,000 maternal deaths (Registrar General of India. Sample Registration System. Special Bulletin on Maternal Mortality in India. 2004-06), 1 million women left with chronic ill health, and 1 million neonatal deaths [UNICEF. 2009]

❖Although *P. vivax* infection during pregnancy has been recognized for many years, the impact of such infection during pregnancy has been assessed only recently.

❖In studies from Thailand and India, women with *P. vivax* infection were more commonly anaemic and delivered lower birth weight neonates, compared with uninfected women, but the effects were less pronounced than those associated with *P. falciparum* infection [Nosten F. 1999].

❖Although earlier studies carried out primarily in central India suggest that both *P. falciparum* and *P. vivax* are associated with adverse pregnancy outcomes, these studies primarily focused on symptomatic pregnant women infected with *vivax* [Singh N. 1995, 1999].

❖Hazaribag, the region under investigation, was primarily dominated by *P.vivax* whereas some buffering, bordering and adjoining regions have lower prevalence of *P.falciparum* and mixed infection.

❖Relatively little information is available from India about *vivax* associated malaria during pregnancy, particularly from Jharkhand, an understudied and tribal dominant region with perennial malaria transmission zone where malaria is rampant and causing sizable annual malaria deaths, second to Orissa in India as per the latest observations published by Dhingra et al. 2010 and Hussain et al. 2011.

❖Interestingly, Hamer et al. in 2009 published a very significant piece of information regarding malaria during pregnancy, which reflects the importance of the area and its necessity of undertaking extensive investigation in terms of malarial pathology during pregnancy.

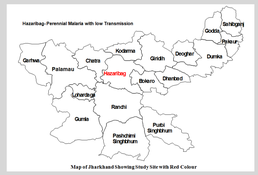
❖To the best of our knowledge, such profile, epidemiological association and clinical correlation has not been investigated before on isolates of malaria in pregnancy from Hazaribag, Jharkhand, among malaria endemic regions of India.

OBJECTIVES

The escalating burden of malaria during pregnancy (MIP) is of public health concern across the globe, in view of the pathogenesis, epidemiology, clinical sequel, prevention and treatment; all have unique features during pregnancy. The combinatorial adverse impact on maternal health during MIP further perplexed the situation of prompt diagnosis, treatment and preventive strategies.
Thus, we evaluated in view of the limited information on asymptomatic and vivax infection during pregnancy in India, with an objective to better define the estimate of MIP, the prevalence of asymptomatic malaria, and the relative contribution of plasmodium infection during pregnancy and at delivery in Hazaribag, Jharkhand, a malaria-endemic state in central-eastern India.

METHODS

Study Sites/design and Population
This study consisted of cross-sectional surveys conducted in two units, i.e. antenatal care units (ANC) and delivery units (DU) ward of Sadar hospital in Hazaribag districts of Jharkhand, India.



Map of Jharkhand Showing Study Site with Red Colour

Figure-1

Hazaribag (total population according to 2011 census is 1,734,005) is selected to represent a rural-cum-semi urban district with low but perennial transmission of malaria. Hazaribag had a yearly average SPR of 7.3% for symptomatic individuals over the last three years, with *P. falciparum, P.vivax* and Mix infection accounting for 14%,73% and 13% of the cases, respectively [30]. The majority of the indigenous population is mix of tribals, schedule caste, schedule tribes and other casts; exceptionally typical social stratification having gender disparity. Moreover, the district and state lies in the tropical zone with an annual rainfall of 1234.5 mm with favorable geo-climatic and ecological conditions conducive for perennial malaria transmission. Most interestingly, the monthly climatic temperature when compared with monthly malaria episode, we observed significant correlation between ambient temperature and subsequent rise and fall in malaria episode as shown in Figure-2. The recent (2010-2012) data on malaria epidemiology has been analyzed during investigation in this project and we observed the increasing trend of malaria episodes as shown in Figure-3; despite of consistent interventions and preventive measures implemented by various national and international bodies.
Thus, the selected study district is meant to provide a representation of typical conditions that would be found in malaria endemic districts of Jharkhand.

STUDY SITE-CONDUCIVENESS FOR MALARIA

Correlation between Malaria Prevalence and Temperature of Hazaribag

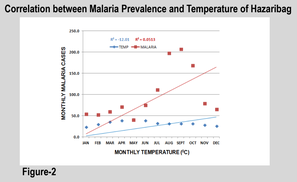


Figure-2

Transmission Pattern of Malaria (values in number) in Hazaribag (2010-2012).

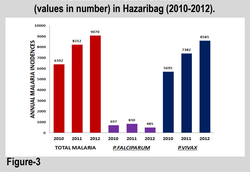


Figure-3

ENROLLMENT, SMAPLING AND SUB-GROUPS

Cross-sectional evaluation between September 2012 and December 2013 at antenatal clinics (ANC) and delivery units (DU) were performed at Sadar hospital, Hazaribag, Jharkhand, India.

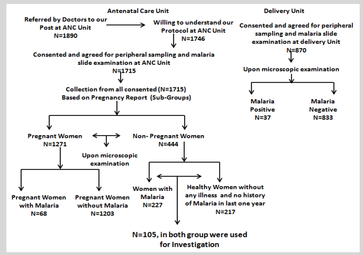


Figure-4. Schematic Flow Chart Summarizing the Sampling and Sub-Groups

LABORATORY PROCEDURES

❖Malaria was screened by Giemsa-stained blood smear and/or rapid diagnostic test. We have also used the PCR technique to diagnose malaria but in selective samples due to budgetary constraint. The selective samples were; all the MIP positive samples at ANC and DU were verified by PCR, those subjects who were disputed on microscopy and RDT were also verified by PCR and clinically most suspected cases with strong sign and symptoms but microscopically negative were verified by PCR.

❖Haemoglobin (Hb) levels were recorded at the first ANC and DU visit. Concentrations of haemoglobin (Hb) were performed in peripheral blood samples using a portable HemoCue haemoglobinometer (HemoCue AB, Ängelholm, Sweden) as stated by the manufacturer. Anaemia was defined as haemoglobin concentration following WHO guidelines.

❖Pre-tested questionnaires were used to gather socio-demographic, clinical and obstetrical data.

STUDY DEFINITIONS

Severe malaria was defined as a malaria attack associated with any of the following; cerebral malaria, severe anaemia, renal failure, pulmonary oedema, hypoglycaemia, shock, spontaneous bleeding, or repeated convulsions [35]. Maternal height and weight were taken at the first visit to ANC and DU, based on this information the body mass index (BMI) were calculated as weight (kg) divided by the squared height (meters); a low BMI was defined as a BMI < 22.0 kg/m^2. A documented fever was defined as an auxillary temperature ≥37.5 ^{0}C.

ETHICAL STATEMENT AND SUBJECT CONSENT

All human blood samples used in this study were collected after obtaining written consent from the study participants under protocols activities approved by the Institutional Ethics Committee (IEC) of the Vinoba Bhave University, Hazaribag, Jharkhand and human ethical guidelines as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Govt. of India. Present study does not involve any minor/children. Thus, signed and written approval was given by adult subject herself. All study participants were included only after informed consent. The study protocol and consent proposal is approved from IEC, VBU having memo no. VBU/R/888/2012 dated 05-06-2012.

DATA MANAGEMENT AND ANALYSIS

All clinical, demographic and anthropometric information were carefully checked for correctness and inconsistencies were resolve before analysis. Data were entered in MS-Excel and analyses were performed using SPSS v.16 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism version 5.0 (GraphPad Software, Inc., CA, USA). For comparisons of means between two groups of subjects, the student's t-test were used for normally distributed data and when data were not normally distributed; non-parametric tests (Mann-Whitney U) were used to analyze the data. Categorical data are presented as frequency counts (percent) and compared using the chi-square or Fisher's exact statistic as appropriate. Continuous data are presented as means (± standard error) and compared using the t-test or analysis of variance as appropriate. Risk factors for either *P. falciparum* or *P. vivax* parasitemia were evaluated by univariate analysis and then adjusted for significant predictors in multivariate analysis. Simple and multiple logistic regressions were used to analyze potential risk factors associated. Similar strategies were followed for factors associated with haemoglobin and anaemia during pregnancy and malaria in pregnancy; risks were assessed using haemoglobin or anaemia as dependent variables and all other factors as independent variables. The differences were considered statistically significant when the *P* value obtained was <0.05.

RESULTS

❖The prevalence of MIP was 5.4% and 4.3% at ANC and DU, and there were 13.2% malaria in women without pregnancy.

❖Interestingly, majority were infected with *P.vivax* (over 85%) and asymptomatic (extremely lower positive predictive value and non significant association of sign and symptoms in multivariate analysis) cases at ANC and DU.

❖Peripheral parasitemia was significantly associated with fever within past week, rural origin of subjects and first/second pregnancies in multivariate analysis, with the highest risk factor associated with fever followed by rural residence in pregnant women.

❖Strikingly, anaemia was prevalent in 86% at ANC cohort (N=1270) compared to 72% at DU cohort (N=870); whereas severe anaemia was 13.6% and 7.8%, respectively.

❖Even more anaemia prevalence were observed in MIP (88% and 89% at ANC and DU); whereas severe anaemia was 23% and 21%, respectively.

Association of Asymptomatic Infection with Malaria during Pregnancy at ANC and DU

We interestingly observed in our study, that 70.6% (48/68) of the positive cases of malaria in pregnancy subjects at ANC were asymptomatic with peripheral parasitemia compared to 29.4% symptomatic MIP cases.

In case of infection during malaria in pregnancy at DU, we observed 75.7% asymptomatic cases with peripheral parasitemia compared to 24.3% symptomatic cases.

Based on the data collected on sign and symptoms from the pregnant women attendee at ANC and DU subjects, we performed positive predictive value (PPV)

Sign/Symptoms At ANC	N	Observed Value (OV) (%)	Positive Predictive Value (PPV) (%)	95% CI in Proportion of PPV (%)
Fever	54	4.2	26	23.5-28.4
History of Fever	167	13.1	45	42.2-47.7
Headache	114	8.9	32	29.4-34.5
Body Pain	15	1.2	18	15.8-20.1
Dizziness	29	2.3	21	18.7-23.2
Vomiting	22	1.7	23	20.3-25.3
Convulsions	13	1.1	12	10.2-13.7
Sign/Symptoms At DU				
Fever	43	4.9	36	32.8-39.1
History of Fever	93	10.6	47	43.6-50.3
Headache	172	19.7	33	29.8-36.1
Body Pain	23	2.6	26	23.1-28.9
Dizziness	19	2.2	19	16.3-21.6
Vomiting	31	3.5	27	24.1-29.9
Convulsions	14	1.6	21	18.2-23.7

Table-1 Positive Predictive Value (PPV) of clinical signs and symptom for *Plasmodium vivax* infection

Almost all the predictive value for respective symptoms were observed to be very much low except for history of fever; which is relatively higher than the others only, though highest among all both at ANC and DU. However, the positive predictive value for history of fever at DU was slightly higher than ANC. None of the predictive value for any sign and symptoms was not even nearly or above 50%.

Further, in multivariate model, we analysed; any symptoms, fever, history of fever, headache, dizziness and vomiting at ANC and DU

Sign/Symptoms At ANC		n/N (%)	OR (95%CI)	*P* value
Any Symptoms	No	937/1203 (77.8)	1	
	Yes	20/68(29.4)	1.3 (0.9-1.9)	0.14
Fever	No	1138/1203 (94.5)	1	
	Yes	11/68 (16.1)	2.9 (1.6-5.4)	0.0003
History of Fever	No	1031/1203 (85.7)	1	
	Yes	14/68 (20.5)	1.4(0.8-2.3)	0.14
Headache	No	1076/1203 (89.4)	1	
	Yes	11/68 (16.1)	1.5(0.8-2.6)	0.13
Dizziness	No	1168/1203 (97.1)	1	
	Yes	4/68 (10.1)	1.9(0.7-5.5)	0.16
Vomiting	No	1178/1203 (98)	1	
	Yes	3/68 (4.4)	2.1(0.6-6.8)	0.21
Sign/Symptoms At DU				
Any Symptoms	No	623/833(74.7)	1	
	Yes	9/37 (24.3)	0.9 (0.5-1.7)	0.9
Fever	No	784/833 (94.1)	1	
	Yes	5/37 (13.5)	2.7 (1.2-6.1)	0.01
History of Fever	No	770/833 (98.6)	1	
	Yes	7/37 (18.9)	2.5 (1.2-5.1)	0.01
Headache	No	655/833 (98.6)	1	
	Yes	6/37 (16.2)	0.7 (0.3-1.5)	0.46
Dizziness	No	792/833 (95.1)	1	
	Yes	4/37 (10.8)	2.1 (0.8-5.6)	0.12
Vomiting	No	775/833 (93.1)	1	
	Yes	5/37 (13.5)	1.9 (0.8-4.5)	0.12

n=observed, N=total considered subjects, OR= odd ratio

Table-2 Association between signs/symptoms and malaria infection using multivariate analysis

❖We, observed that presence of any symptoms, history of fever, headache, dizziness and vomiting were not significantly associated with incidence of malaria during pregnancy at ANC; whereas only fever was found to be significantly associated at ANC.

❖In case of DU subjects all the symptoms were not significantly associated except fever and history of fever were significantly associated with incidence of malaria.

❖Based on the observation and analysis, we can infer that majority of the sign and symptoms have not shown or trended to be significantly associated, except fever and/or history of fever have some degree of significant association with malaria in pregnancy at ANA and DU in multivariate analysis.

Association of Haemoglobin with Asymptomatic *P. vivax* Infection during Pregnancy and at ANC and DU

❖We observed alarmingly higher prevalence of anaemia in MIP and in overall cohort both at ANC and DU.
❖ We also observed sizable prevalence of severe anaemia both at ANC and DU.
❖Based on the observation and analysis, we can infer that majority of the sign and symptoms have not shown or trended to be significantly associated, except fever and/or history of fever have some degree of significant association with malaria in pregnancy at ANA and DU in multivariate analysis.
❖Multivariate logistic regression showed that malaria infection; ferritin, iron, haemoglobin, and formal education were significantly associated with a higher risk of anaemia in overall cohort (N=1271 at ANC and N=870 at DU) as well as in malaria in pregnancy at ANC (N=68) and DU (N=37) subjects.

	Antenatal clinics n=1271 N (%)	Delivery units n=870 N (%)
Peripheral Parasitaemia		
Overall	68(5.4)	37(4.3)
Falciparum	3(0.23)	2(0.22)
Vivax	59(4.6)	32(3.67)
Mixed	6(0.47)	3(0.34)
By gravidity		
Primigravid	21/423(4.9)	11/338(3.2)
Secundigravid	38/578(6.6)	15/209(7.1)
Multigravid	9/270(3.3)	11/323(3.4)
Report of fever within 1 week	167(13.1)	93(10.6)
Anaemia	1093(86)	626(72)
Severe anaemia	148/1093(13.6)	49/626(7.8)

Table-3 Parasitaemia, reported fever, and anaemia among pregnant women attending antenatal clinics and delivery units

Risk Factors Associated with Anaemia in Overall Cohort and Malaria in Pregnancy at ANC and DU

❖We found highest (adjusted odd ratio in multivariate analysis) risk factor associated with anaemia were observed with haemoglobin level; followed by presence of malaria infection in both malaria in pregnancy and overall study cohort at ANC and DU.
❖However, ANC subjects have shown relatively higher risk ratio association of anaemia with haemoglobin and malaria compared to DU.

Factors at ANC	PW No.	PW Crude OR (95%CI)	PW *P*	PW Adjusted OR (95%CI)	PW *P*	MIP No.	MIP Crude OR (95%CI)	MIP *P*	MIP Adjusted OR (95%CI)	MIP *P*
Malaria[¥]										
No	1203	1		1		9*	1		1	
Yes	68	2.7 (1.4-3.8)	0.0001	2.8 (1.2-3.4)	0.0002	59[$]	3.1(1.7-5.3)	0.0001	3.4 (1.9-6.5)	0.0001
Ferritin (ng/ml)										
<50	117	1		1		5	1		1	
≥50	1154	1.8(1.3-2.3)	0.002	2.2 (1.6-3.5)	0.001	63	2.1 (1.6-3.3)	0.0001	2.4(1.7-4.1)	0.0004
Iron (µg/dl)										
≥40	236	1		1		10	1		1	
<40	1035	1.7(1.6-2.7)	0.006	1.9 (1.8-3.4)	0.003	58	2.2 (1.5-3.3)	0.001	2.3(1.2-2.7)	0.0001
Haemoglobin (Hb) (g/dl)										
Hb>11	217	1		1	0.0003	19	1		1	
Hb<11	1054	3.8(2.3-8.7)	0.0001	4.2(2.1-8.8)	0.0002	49	4.8 (1.7-8.1)	0.0001	5.4(1.6-8.6)	0.0002
Education										
NFE	357	1		1		13	1		1	
FE	914	2.1(1.4-2.9)	0.003	2.3(1.7-3.9)	0.001	55	2.2(1.6-3.5)	0.0001	2.6(1.3-3.4)	0.0001
Factors at DU										
Malaria[¥]										
No	833	1		1		5*	1		1	
Yes	37	2.1(1.2-2.5)	0.0001	2.4(1.6-3.8)	0.0002	32[$]	2.2(1.4-3.1)	0.001	2.8(1.7-4.7)	0.0001
Ferritin (ng/ml)										
<50	205	1		1		7	1		1	
≥50	665	1.4(1.2-1.7)	0.004	1.7(1.4-2.3)	0.0003	30	2.1(1.8-3.8)	0.0001	2.3(1.5-3.5)	0.0002
Iron (µg/dl)										
<40	683	1		1		31	1		1	
≥40	187	1.9(1.6-3.1)	0.002	2.2(1.3-2.9)	0.0002	6	2.3(1.5-3.5)	0.006	2.5(1.3-3.3)	0.0003
Haemoglobin (Hb) (g/dl)										
Hb>11	173	1		1		8	1		1	
Hb<11	697	3.1(1.8-5.6)	0.0001	3.6(2.1-7.6)	0.0001	29	4.2 (1.9-7.8)	0.001	4.8 (1.6-7.7)	0.0001
Education[#]										
NFE	321	1		1		11	1		1	
FE	549	1.6(1.1-1.8)	0.004	1.8(1.4-2.6)	0.0002	26	1.7 (1.5-2.6)	0.002	2.1(1.7-3.6)	0.0001

PW= pregnant women, MIP= malaria in pregnancy, ANC= antenatal care unit, DU= delivery unit; [#]NFE= no formal education, FE=formal education; [¥]=In case of MIP the comparison is between *P.vivax* vs *P.falciparum* and Mix infection; [$]=*P.falciparum*+ Mix Infection, [*]= *P.vivax*

Table-4
Risk factors for anaemia in pregnant women (PW) and malaria during pregnancy (MIP) using univariate and multivariate analysis

Prevalence of Anaemia during *P.vivax* infection at ANC and DU

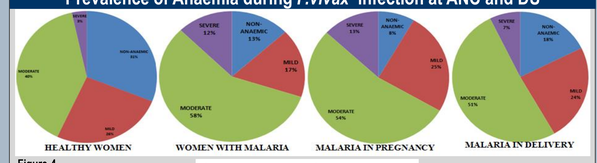


Figure-4

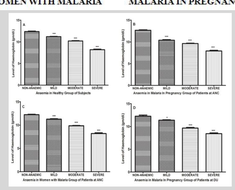


Figure-5A-D
Level of haemoglobin as classified anaemia in malaria infected subjects screened at antenatal care (ANC) unit and delivery unit (DU) in stratified group

Seminal Observations of Present Investigation

❖**High prevalence of anaemia during pregnancy and in delivering women in the malaria endemic population of Hazaribag, Jharkhand.**
❖**Prevalence of anaemia is significantly associated with *Plasmodium vivax* infection during pregnancy and in delivering women.**
❖**Most significant observation was the high prevalence of asymptomatic *P.vivax* infection at both ANC and DU.**

Suggestive Inference of Present Investigation for Policy Reorientation

❖**There is an urgent need to enhance the ITN availability, use and awareness both in population as well as health worker.**
❖**Distribution of ITNs at first ANC visit will be lucrative alternative for preventive strategy.**
❖**In view of the asymptomatic prevalence of co-infection, we need to further strengthen and emphasize the robust screening strategies, curative attention and safe treatment facilities at the community level health centres for pregnant and delivering women.**

TAKE AWAY MESSAGE

Our findings highlights the region specific priority consideration of early case detection and pressing need of enhanced management of asymptomatic pregnant women in an integrated manner both at the community health centres and also through restructuring the provision of active and passive surveillance strategy in perennial transmission and endemic zone.

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Contact information

Queries, clarifications and questions are welcome in absence of presenter. Constructive suggestions, academic inputs, scientific collaborations and comparative-cum-interactive investigations on similar area of research interest from other investigating groups were welcome in absence of presenter but can be approached to Dr. Mohammad Sohail, E-mail- soh.khan@hotmail.com Mobile- 0091-9798685959.