CHAPTER 7
C - reactive protein profiling in clinical isolates of malaria in pregnancy and healthy pregnant subjects.

Malaria is pervasive in tropical regions, caused mainly by the protozoan parasites *P. falciparum* and *P. vivax*, accounts for 515 million clinical cases (419) and 1 to 3 million deaths per year (420) *P. vivax*, the most rampant parasite causing human malaria, accounts for about 130-435 million infections annually and is the prime cause of malaria in most of Asia and Latin America (421). Although *P. vivax* infection is commonly considered to be much more benign than *P. falciparum* infection, data do suggests notable mortality associated with *P. vivax* malaria in the pre antimalarial era (422) and death caused by *P. vivax* malaria has been alarmingly recognized over the past few years (421,423). CRP is an acute phase reactant and is one of the most widely used acute phase inflammatory proteins due to its early rise and rapid
dynamics. Assessment of CRP can be useful in understanding the aetiology of severe malaria (424). The protein is synthesized in the liver and is ordinarily present at concentrations of less than 10 mg/L in the blood. The level of CRP is alarmingly elevated during infectious or inflammatory diseases states, within the first 6 to 8 hours and extreme at levels of up to 350-400 mg/L after 48 hours (425-429).

CRP adhere to phosphocholine expressed on the surface of mutilated cells, as well as to polysaccharides and peptosaccharides present on bacteria, parasites and fungi (430). This binding stimulates the classical complement cascade of the immune system and regulates the activity of phagocytic cells, reinforcing the role played by CRP in the opsonization of infectious agents and dead or dying cells (425,430). After the resolution of inflammation or tissue destruction, CRP levels decline, fabricating its role as a valuable marker for tracking disease activity (426,429). Quantification of CRP is mostly done by using ELISA, immunoturbidimetry, or antibody-based nephelometric assays which are typically sensitive to concentrations of 5-20 mg/L. Nevertheless, in women CRP concentrations seem to be elevated in late pregnancy (429). Recently, some acute phase reactants have appeared as biomarkers in malaria infection in addition to chemokines and cytokines. Specifically, CRP and NO have been apprehended as an important inflammatory biomarker (431,432). In case of malaria, the secretion of CRP is promoted by pro-inflammatory cytokines that are secreted by host mononuclear cells and a strong positive coalitions have been noted between CRP levels and parasitaemia (433). In fact, CRP levels have been documented valuable in monitoring the severity of malaria among pregnant women and as a prognostic marker in due course of response to treatment (434).

Quantification of serum C-reactive protein is customarily used for the assessment of injury in the body tissue or for identifying inflammation in
the body. C reactive protein seem to play a pathogenic role in malaria infections. C-reactive protein adheres to infected erythrocytes and channelize its effective clearance. This augmentation of the immune system towards infected RBCs also results in various harmful expositions. Also, CRP trigger complement pathway and platelet activation resulting in various unpredictable effects. Thus, assaying CRP can be effective in interpreting the pathogenesis of severe malaria (424). Although CRP is classically reviewed as a key regulator of the innate immune system and a supreme mediator of the acute-phase response (435) it is also correlated to various chronic inflammatory processes, such as certain rheumatologic conditions (436). This study was precursory venture to support the prudent use of C reactive protein lab measurements, in the light of available scientific evidence and on the clinical experience of physicians. Accurate evaluation of severity of disease is the need to channelize proper therapy and avoid the complications (424) Specific data on the CRP levels in the pathology of malaria among pregnant women at this understudied district have not been documented in this regard. Therefore, the present study focusses to investigate the level of CRP in clinical isolates of MIP subject and HPW which could serve as a valuable prognostic marker in assessing disease severity.

Materials and methods: -

Screening and enrollment: -

A cross sectional study was conducted at Sadar Hospital, Hazaribag involving 68 malaria in pregnancy subjects who were confirmed to be infected, with either P. vivax or P. falciparum constituted the study population and 50 pregnant healthy women were enrolled as the control group. Out of 68 participants enrolled in the current investigation, 3 had P. falciparum, 59 had P. vivax infection and 6 had mixed infection. Inclusion
in the study protocol involved no history of hereditary diseases or known severe disease at the time of conceiving, participants who consented in the study protocol, no immediate illness due to other infectious disease or malaria during present pregnancy.

Exclusion from the study protocol involved refusal to give signed consent or unwilling for sampling, clinically suspected or identified cases of HIV and hepatitis B infection. About 3ml of peripheral venous blood was collected from the study participants for assaying C reactive protein.

**Ethical clearance:**

Written informed consent were taken from the study participants under protocol approved by the Institutional Ethics Committee of the Vinoba Bhave University, Hazaribag, Jharkhand and human ethical guidelines as contemplated in the guidelines of the Medical Ethics Committee, Ministry of Health, Govt. of India.

**Statistical Analysis:**

The data were collected, recorded and analysed statistically to ascertain the significance of means of various groups by using SPSS software version 21. Continuous variables were expressed as mean ±S.D. Post-hoc tukey’s test was performed for multiple comparisons between means of various group. A value of P<0.05 was considered as statistically significant.

**Result:**

A significant correlation was found between CRP levels and parasitaemia. A statistically significant difference was observed between *P. falciparum* infected subjects (21±0.816 mg/l) and healthy pregnant women (11.52±0.499 mg/l) p<.0001. Significant difference have also been accorded between the CRP level of *P. vivax* infected pregnant subject
(22.084±1.093) and Healthy pregnant women (11.52±0.499) and mixed infection (23.5±4.03) and Healthy pregnant women (p<.0001).

**Discussions:**

An interesting finding of the current investigation is an elevated level of CRP in malaria in pregnancy subjects as compared to its healthy pregnant counterparts (p<.0001). Strikingly, no significant difference between CRP levels in *P. falciparum* and *P. vivax* have been demonstrated (p=.101). Our finding corroborates with the study of Paul et al (424) who documented a significant peak value in CRP in malaria infected subjects compared to its healthy counterparts but predicated no significant difference between *P. falciparum* and *P. vivax* cases. Since, our study is a single time point study, it’s hard to infer precise assessment of the course of the disease.

Quantification of serum CRP concentrations can render a simple measurement of disease severity and could serve as a prognostic marker in malaria.
Graph 8: - Concentration of C-reactive Protein in Malaria in pregnancy -Pf and Healthy pregnant women
This Graph shows the concentration of C-reactive Protein in MIP-Mixed Infection and HPW. p-value calculated was <0.0001 by unpaired t-test using Graphpad Software.

**Graph 9: - Concentration of C-reactive Protein in Malaria in pregnancy -mixed infection and Healthy pregnant women**
Graph 10: Concentration of C-reactive Protein in Malaria in pregnancy -Pv and Healthy pregnant women

This Graph shows the concentration of C-reactive Protein in MIP-Pv and HPW. The p-value calculated was <0.0001 by unpaired t-test using Graphpad Software.