ASSOCIATION OF SERUM C-REACTIVE PROTEIN CONCENTRATIONS WITH SEVERITY OF PREECLAMPSIA AND IMMINENT ECLAMPSIA IN PREGNANT WOMEN IN ZIMBABWE

BY

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Abstract

Preeclampsia is a hypertensive disorder in 3-7% of all pregnant women characterised by elevated blood pressure and proteinuria. It is a chief source of morbidity and mortality worldwide. The health of both the preeclamptic woman and her infant are dependent on how long she carries the foetus. To date, delivery of the child is the only cure for preeclampsia. C-reactive protein (CRP) is part of the innate immune system. It scavenges for chromatin released by dead cells during the acute phase, activates complement and acts as an opsonin for various pathogens. Elevation of CRP is still considered the beacon of the acute-phase response. It has been shown that C-reactive protein concentration is elevated in preeclampsia. Measuring the CRP concentration in preeclamptic women could help in understanding the best course of action for the pregnant woman and the foetus, especially if there is an association between CRP levels and severity of preeclampsia.

Objectives:
To determine the levels of C-reactive protein in Zimbabwean women who have preeclampsia during singleton pregnancy.
To determine the association of CRP concentration and blood pressure in pregnant women.

Materials and Methods: This was a cross sectional study conducted at Mbuya Nehanda Maternity Hospital Antenatal Ward and Antenatal Clinic including pregnant women with singleton pregnancy. Blood was collected to yield serum for the CRP assay, a particle enhanced turbidimetric immunoassay. Uric acid and blood pressure were also measured.

Results:
There was a positive correlation between CRP and mean arterial pressure (p=0.0137) and also between CRP and uric acid (p=0.0095).

Conclusion:
In conjunction with other biomarkers and clinical signs, CRP can help to give a fuller picture of the state of the pregnant women who has preeclampsia. The aetiology of preeclampsia is not well established but having more information about the condition will help in the monitoring and treatment of the pregnant woman to ensure she and her baby are well.
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>4-AAP</td>
<td>4-Aminoantipyrine</td>
</tr>
<tr>
<td>ANC</td>
<td>Antenatal Clinic</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>DCHBS</td>
<td>3,5-Dichloro-2-hydroxybenzene sulfonate</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated Intravascular Coagulation</td>
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<tr>
<td>G</td>
<td>Gram</td>
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<td>HELLP</td>
<td>Haemolysis, Elevated Liver enzymes and Low Platelets</td>
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<td>HIV</td>
<td>Human Immunodeficiency virus</td>
</tr>
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<td>IFCC</td>
<td>International Federation of Clinical Chemistry and Laboratory Medicine</td>
</tr>
<tr>
<td>µmol/L</td>
<td>Micromoles per litre</td>
</tr>
<tr>
<td>mg/L</td>
<td>Milligrams per litre</td>
</tr>
<tr>
<td>ml/L</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mmHg</td>
<td>Millimetres of mercury</td>
</tr>
<tr>
<td>PIH</td>
<td>Pregnancy induced hypertension</td>
</tr>
<tr>
<td>PROM</td>
<td>Premature rupture of membranes</td>
</tr>
<tr>
<td>Nm</td>
<td>Nanometres</td>
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1. Introduction

1.1 Background

Preeclampsia is a hypertensive disorder in 3-7% of all pregnant women characterised by elevated blood pressure and proteinuria. It is a chief source of maternal morbidity and mortality worldwide (1). To date, delivery of the child is the only cure for preeclampsia.

Preeclampsia is so named because it was originally identified as a disorder preceding eclampsia. Eclampsia is when the pregnant woman experiences one or more seizures. Now we know that eclamptic seizures are only one of various possible complications of preeclampsia. Although an eclamptic seizure typically occurs as a complication of severe preeclampsia, it can also show up without any prior signs of severe disease (2).

C-reactive protein (CRP) is an acute-phase protein produced by the liver in response to injury, infection and other inflammatory conditions. It has been used most often as a biomarker in cardiovascular disease where its concentration rises many times above baseline in an acute attack (3). This study aims to investigate if CRP could be a good biomarker for the severity of preeclampsia in Zimbabwean women. A good biomarker is one that is easily measured, can be used to measure the presence or progress of disease, and also used to monitor the disease/condition as it resolves. CRP concentration has been shown to increase in women with preeclampsia (4) (5). The CRP assay is simple, quick and affordable. CRP is easily measurable and not too invasive, requiring a simple blood draw for the assay. Its half-life is 19 hours so it is cleared from the bloodstream fairly quickly as preeclampsia resolves (6). For these reasons, we saw that CRP is a good candidate for a biomarker of the severity of preeclampsia.
2.1 Research Question
What is the association, if any, between serum C-reactive protein concentration and the severity of preeclampsia and imminent eclampsia in pregnant women in Zimbabwe?
2 Literature Review

2.1 The Dangers of Preeclampsia

Dubbed the “disease of theories” because of its enigmatic aetiology (7), preeclampsia remains one of the dangers of pregnancy where delivery of the child is the only cure. There are several risk factors for preeclampsia including pre-existing hypertension, Diabetes mellitus and history of preeclampsia (8). It has been found that pregnant young women under 17 years and also pregnant women over 40 are at higher risk of preeclampsia than those in their 20-30s. Having children by one man then changing partner to have a pregnancy by a different man also increases risk of preeclampsia as it seems there is a protective mechanism from repeated exposure to the same sperm (9).

The health of both the preeclamptic woman and the infant are dependent on how long she carries the foetus. Delivery of the baby needs to be at the best time and in the best way to benefit both mother and child. This is because preeclampsia can have a variety of outcomes like stroke, renal failure, pulmonary oedema, cardiac failure, Disseminated Intravascular Coagulation (DIC) (10) and eclampsia which threatens the mother’s life with convulsions and coma if she continues the pregnancy. On the other hand, the infant’s well-being is at risk if the mother delivers early because preterm infants are not as physiologically and metabolically mature as term infants. For example; the thymus of infants born to preeclamptic mothers is smaller than in those from normal pregnancy (11) and the birth weight is lower than in normal pregnancy (12).
**Risk Factors for Preeclampsia include:**

- Diabetes Mellitus
- Pre-existing hypertension
- Under 17 years old
- Personal/family history of preeclampsia
- Over 40 years old
- Excessive amniotic fluid
- Obesity
- Pregnant by different partner
- Multiple gestations
- 1st Pregnancy

**Complications of Preeclampsia can include:**

- Eclampsia (seizures)
- Stroke
- Foetus - Small for gestational age
- Pulmonary oedema
- Restricted intrauterine growth
- Disseminated Intravascular Coagulation (DIC)
- Cardiac failure
- Haemolysis, elevated liver enzymes, low platelets (HELLP)
- Renal failure

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**Figure 1- Preeclampsia Risk Factors and Complications**

**2.1 C-Reactive Protein**

It has been shown that C-reactive protein (CRP) concentration is elevated in preeclampsia (4), (13), (14). Measuring the serum CRP concentration in preeclamptic women could help in
understanding the best course of action for the woman and the foetus, especially if there is an association between CRP levels and severity of preeclampsia. 

Pentameric C-reactive protein (CRP) has a molecular weight of 25039 and consists of 5 non-covalently associated sub-units of 206 amino acids each (15).

![Image: The pentameric structure of C-reactive protein]

**Figure 2 - The pentameric structure of C-reactive protein**

CRP was discovered in 1930 by Tillet and Francis during their studies of patients with acute pneumonia. They utilised serum from febrile patients. CRP is found at high levels in "acute-phase" sera such as from these feverish patients. They found that when this serum was mixed with a cell-wall component of pneumococci that they called ‘Fraction C’, a precipitate formed due to reactivity of CRP with a polysaccharide in the pneumococcal cell wall (16).

The CRP gene is located on chromosome 1 and has 2300 base pairs (15). The inflammatory cytokines interleukin-6 and tumour necrosis factor alpha (TNF-α) are the chief inducers of the secretion of C-reactive protein in the liver. It has been shown that a small amount of CRP
is produced by some lymphocytes and is expressed on the surface of these cells (17); but it is the liver CRP that contributes to the acute phase CRP serum levels.

C-reactive protein was the first of many acute-phase reactants later discovered, but today elevation of CRP is still considered the beacon of the acute-phase response. The acute phase is a number of physiologic changes that happen soon after the beginning of an infection or other inflammatory process. It involves an increase in the blood level of various proteins (such as C-reactive protein), fever, and other metabolic changes (18). For many decades CRP has been used as a marker of injury or tissue damage as its concentration can rise many times above baseline in a 24-hour period in the acute phase. CRP is made in the hepatocytes thus liver failure impairs its production, but no other pathologies and very few drugs reduce CRP values. Its half-life is 19 hours and this half-life is constant whether in health or disease, so that the only determinant of circulating CRP concentration is the rate of synthesis (6). C-reactive protein is part of the innate immune system. CRP binds to damaged tissue, to nuclear antigens and to certain pathogenic organisms. It scavenges for chromatin released by dead cells during the acute phase (19), activates complement and acts as an opsonin for various pathogens. CRP recognises transformed self and foreign molecules and is thought to act as a surveillance molecule leading to a pro-inflammatory signal and activation of the humoural immune system (20).

Although sometimes referred to as an acute-phase protein, CRP accompanies both acute and chronic inflammatory disorders. CRP has been studied extensively in cardiovascular disease (3), metabolic syndrome (21) and HIV infection (22). There are several conditions that can cause an increase in plasma CRP concentration during pregnancy besides preeclampsia. For example, many women who deliver preterm babies were prone to intrauterine, kidney, bladder or vaginal infection where the pathogen triggered an inflammatory response that led to increased CRP concentration (23), (24).
2.3 Preeclampsia and Gestational Hypertension

Preeclampsia occurs during the second half of pregnancy (over 20 weeks) or sometimes even in the days immediately after delivery of the baby, leading to life threatening health complications for the new mother (25). This study focused on the women with preeclampsia before delivery.

Medical Dictionaries have the following definitions:

*Gestational hypertension (pregnancy-induced hypertension-PIH): “Hypertension during pregnancy in a previously normotensive woman or aggravation of hypertension during pregnancy in a hypertensive woman.”* (26)

*Preeclampsia: “A disorder occurring during late pregnancy or immediately following parturition, characterised by hypertension, oedema, and proteinuria. Also called toxaemia of pregnancy.”* (18)

*Eclampsia: “Convulsions and coma, rarely coma alone, occurring in a pregnant or puerperal woman, and associated with hypertension, oedema, and/or proteinuria.”* (27)

Preeclampsia is a complex, multisystem disorder that is believed to start from a placental problem like a failure of the normal invasion of trophoblast cells, leading to maladaptation of maternal spiral arterioles (28) or excessive development of the placenta in disorders such as hydatidiform mole (placental tumour). In molar pregnancy, the woman develops a placenta without a foetus, frequently developing severe preeclampsia in such cases, therefore, it is practical to accept that the placenta plays a central role in the pathogenesis of the preeclampsia (49). In preeclampsia, the failure of trophoblast invasion of the placenta results in a shortage of blood supply to the placenta (50), that is, placental ischemia. The ischemia in turn, results in endothelial dysfunction of the maternal blood circulation as the body attempts
to compensate for the lack of blood. Granger et al cite various proteins and cytokines that affect the renal system that have increased or decreased levels in preeclampsia in rats; and possibly lead to hypertension; such as tumour necrosis factor-α (TNF-α), angiotensin II and the vasoconstrictor endothelin (49). Nitrous oxide is a vasodilator that is sometimes found to be deficient in preeclampsia in rats which also triggers the haemodynamic effects found in hypertension (50). The placenta is not the only factor in preeclampsia. There may be renal involvement too (29) and Xia and Kellems hypothesize that “preeclampsia is a pregnancy-induced autoimmune condition characterized by the presence of disease-causing angiotensin receptor activating autoantibodies, AT1-AAs” (30). The latest theory of the aetiology of preeclampsia is that it is the foetus that initiates the problem. When there is a high oxygen demand from the growing foetus, the mother’s body attempts to adapt by channelling more oxygen to the baby but it causes her to have hypertension as she needs to increase systolic output to cope with the ever increasing demand for oxygen (31).

Since some women always have preeclampsia with each pregnancy and others never do, it was suspected that there was a genetic cause and indeed preeclampsia has been shown to have a genetic link (32) (33). The daughter of a woman who had preeclampsia has 23% likelihood of having preeclampsia herself compared to a daughter-in-law (10%) married to the son of a woman who had preeclampsia (32). In a Zimbabwean study, women whose mother or sister had a history of pregnancy-induced hypertension had an increased risk of preeclampsia or eclampsia (OR = 2.3 and 2.6, respectively) (34). Mittendorf et al. even found that being black was a risk factor for preeclampsia (35). More recently, two variants of the CRP gene seem to be associated with preeclampsia in an American Indian population, further supporting a possible role for CRP in preeclampsia (36). However, the most important risk factors of the prevalence of preeclampsia were still chronic hypertension, Diabetes mellitus and previous preeclampsia, respectively (37).
The diagnosis of preeclampsia is at least two readings of systolic blood pressure over 140 mmHg or diastolic blood pressure over 90 mmHg and 24-hour proteinuria equal to or more than 0.3 g after the 20th week of pregnancy (2). Oedema is another sign used for making the differential diagnosis, although most women experience some level of oedema in pregnancy. Physicians also use uric acid concentration to help in distinguishing preeclampsia from gestational hypertension (38). In severe cases preeclampsia is accompanied by the HELLP syndrome (Haemolysis, Elevated Liver enzymes and Low Platelets) (30). Clinicians also order biochemistry tests to check for creatinine and urea because those are typically low in cases of preeclampsia, whereas uric acid will be high (39). Prothrombin time and activated partial thromboplastin time are useful in finding out if the patient has developed Disseminated Intravascular Coagulation (DIC) which sometimes results from preeclampsia. DIC is triggered by endothelial damage from preeclampsia which results in haemostatic changes like increased clotting factors (10). Conjunctival redness and epigastric pain can also signal severity of preeclampsia in a patient whose blood pressure is not indicative of severe preeclampsia.

Pregnancy-induced hypertension is when a woman who is usually normotensive has blood pressure readings of over 140 mmHg or diastolic blood pressure over 90 mmHg when she is pregnant; or when hypertension is aggravated in pregnancy in a woman who previously had hypertension (18). Both systolic and diastolic fluctuate depending on the time of day, activity, stress etc. but systolic BP generally varies more than diastolic. The elevated diastolic blood pressure reveals more about the individual’s heart and blood vessel pressure since it is a measure of the blood pressure when the heart muscle is relaxed. Some physicians also calculate the mean arterial pressure (MAP) for differential diagnosis in prediction of preeclampsia (40). This is because systolic BP can fluctuate a lot and diastolic BP bears more weight in the overall management of patients. MAP becomes more informative than readings
of systolic and diastolic alone as it takes into account both numbers simultaneously and is weighted towards the diastolic BP (41). MAP gives a single number that can be used to compare blood pressure readings in the same patient or between patients.

Uric acid has been known to be elevated in women with preeclampsia since the late 19th century and elevated uric acid concentration has been shown to be predictive of preeclampsia (42), (38). Uric acid is the end product of purine metabolism and results in hyperuricaemia in high concentrations in the blood. Normal concentrations for women are under 360µmol/L. Johnson et al. explain how even uric acid concentration of 309µmol/L was accurate and sensitive in distinguishing preeclampsia and gestational hypertension (38). In this study, uric acid concentration was used to help differentiate gestational hypertension and preeclampsia at 360µmol/L.

The emphasis of this study is to investigate the CRP as a biomarker for the severity of preeclampsia. This is important because, although the severity of preeclampsia is not a determinant as to whether or not it develops into eclampsia, (43) the severity does have implications for the time of delivery of the baby. It is harmful to the mother when her blood pressure is extremely high so delivery of the baby as soon as possible would be advantageous for her. However, early delivery is risky for the infant which may not be mature enough yet to survive outside the uterus. Since preeclampsia only resolves after delivery of the baby, current healthcare has been focused on management of the condition until then. Management of preeclampsia is with antihypertensive therapy to reduce the blood pressure to within BP levels that are likely to be protective against maternal acute adverse cerebrovascular or cardiovascular events. The recommended drugs are those shown to be safe past the first trimester such as Methyldopa and the beta blocker Labetalol as a second line drug in severe cases because although it has low incidence of adverse effects, Labetalol can cause foetal growth restriction (44). Magnesium sulphate is used to prevent convulsions, fluid restriction
and diuretic therapy are key to preventing pulmonary oedema (45) and there are on-going studies on calcium as another option for reducing the risk of developing preeclampsia (46). Corticosteroids can also be prescribed to improve the mother’s liver and platelet function to prolong the pregnancy and to help the baby’s lungs mature if preterm delivery is necessary (47).

2.4 Rationale

The rationale behind this study stems from the evidence that in Zimbabwe, eclampsia was the leading cause of maternal death at 24.6% in urban areas (48). Duley concluded that maternal deaths associated with hypertensive disorders of pregnancy may be the most difficult to prevent (1) compared to other pregnancy complications like haemorrhage, sepsis, and ectopic pregnancy. There is also an increased risk of preeclampsia in HIV-infected women who are on antiretroviral therapy (49). Zimbabwe has one of the world’s highest rates of HIV infection; and more and more people are being initiated on antiretroviral therapy. These facts show that there needs to be more research to better understand preeclampsia and eclampsia in Zimbabwe. This study could help in understanding if CRP concentration can be a useful biomarker of the severity of preeclampsia for women in this country to help health professionals know the best course of action to take.

2.5 Specific Objectives

a. To determine the levels of C-reactive protein in Zimbabwean women who have preeclampsia during singleton pregnancy.

b. To determine the association of CRP concentration and blood pressure in pregnant women.
3 Research Methods

3.1 Study Design and Study Population

This was a cross sectional study. Study participants were recruited from the Antenatal Clinic (ANC), the Antenatal Ward and the Labour Ward at Mbuya Nehanda Maternity Hospital. This included women who were diagnosed with preeclampsia or pregnancy induced hypertension (PIH) in singleton pregnancy as well as women who had normotensive singleton pregnancy. Mbuya Nehanda Maternity Hospital is a member of the Parirenyatwa group of hospitals, and it is a referral centre serving patients from all areas of Zimbabwe’s capital Harare and from district and provincial hospitals, both low and high density areas, from e.g. Tafara, Norton, Mt. Pleasant and Chegutu etc. The Antenatal Ward at Mbuya Nehanda Maternity Hospital is a 28-bed ward that is sometimes full, even overflowing to the extension ward and at other times only partially filled with patients who are constantly being admitted, taken to the Labour Ward or discharged. During the study there was an average of 8 (~35%) women with gestational hypertension in the ward at any one time, making gestational hypertension one of the major reasons for hospital stay. Other reasons for hospital admission included infection such as pneumonia and malaria during pregnancy, premature rupture of membranes (PROM), postdates and physical trauma such as abuse or a fall during pregnancy.

3.2 Ethical Considerations

Approval was obtained from the Joint Research Ethics Committee of the University Of Zimbabwe College Of Health Sciences and Parirenyatwa Hospital (Appendix 1) and the Medical Research Council of Zimbabwe (Appendix 3).

Consenting participants gave written consent (English or Shona) after getting all the information about potential benefits and potential harm from the study.
It was a non-invasive study therefore had minimal harm to the participants. Some felt slight pain or discomfort at the site of venepuncture.

The protection of the identities of the participants was ensured by assigning identity numbers to their data sheets and samples.

All test results are available to the participants through their clinicians.

**3.3 Inclusion criteria**

Only pregnant women presenting themselves for antenatal care were included since the study investigated a condition found in pregnancy. Preeclampsia can occur a few days post-delivery but the focus of this study is antenatal.

The participants had to be willing to sign a consent form in accordance with international and national ethics regulations.

They also completed a questionnaire or had a brief interview outlining their medical history after a physical exam by the clinician. Vital information about previous pregnancies as well as weight, smoking, infections, diabetes, blood pressure, age and height was noted.

**3.4 Exclusion Criteria**

No multiple gestations. The study was for singleton pregnancy.

The participants had to have no liver disease or neoplasm. This is because CRP is made in the liver. Liver disease could result in a reduction in production, and sometimes neoplasm could result in an increase due to ectopic production of CRP.

Figure 3 below shows how this study was conducted, outlining the various steps taken to ensure efficiency.
3.6 Questionnaire and Data Collection

When a potential study participant agreed to sign the consent form, the next step was to complete a questionnaire in which bio-demographical information was obtained such as weight, age, number of pregnancies, history of pre-pregnancy hypertension etc. (see Appendix 4). If the participant was from the antenatal clinic, her blood pressure from that day’s visit was noted. If she was from the ward, the last recorded blood pressure was noted, which would be from that very morning. The Mbuya Nehanda Maternity Hospital staff checks the blood pressure of all the women every morning. The antenatal clinic and wards at Mbuya Nehanda Maternity Hospital use Mindray VS-800 digital BP meter.

3.7 Blood Sampling

Blood samples were collected into 4ml pro-coagulation tubes at the time of routine antenatal visits in the case of the Antenatal Clinic or during the morning rounds in the Wards. Within 90 minutes, the blood was centrifuged to yield serum and promptly frozen at -20°C. The
serum was kept chilled between freezer gel packs during the 10km drive from Parirenyatwa Hospital to Harare Central Hospital where the CRP assay was performed.

3.8 The Biochemical Assay for C - reactive protein

Serum CRP was analysed on the Siemens Dimension Automated Analyser (Siemens Healthcare Diagnostics Inc., Newark, DE, USA) using the C-Reactive Protein Extended Range method. This is a particle enhanced turbidimetric immunoassay (PETIA). Synthetic particles that are coated with antibody to C-reactive protein aggregate in the presence of C-reactive protein and cause the sample to become cloudy. The increase in turbidity is proportional to the concentration of CRP in the sample. The CRP-antibody aggregate absorbs spectrophotometrically at 340nm.

Haemolysis, icterus or lipaemia can interfere in this assay, increasing CRP results by ~30%. The manufacturer reports bias of less than 10% for interferents and that the CRP results should not be corrected based on this bias. The manufacturer utilises mg/dL as the units for CRP measurement but notes that a simple multiplication factor of 10 will bring it to the standard mg/L (50).

The expected normal CRP value for healthy individuals is around 3mg/L. The assay’s analytical measurement range is 0.5-250.0 mg/L and the reference range in use at Harare Central Hospital Biochemistry laboratory for the Zimbabwean population is 0.0-5.0 mg/L. For the purposes of this study, 0.0-5.0mg/L was reported as normal, anything over 5.0mg/L was reported as a high CRP level and anything over 10.0 mg/L was a very high CRP result.
Figure 4- Siemens Dimensions Xpand Chemistry Analyser

Following the instruction manual for the Siemens Dimension system (shown above), the instrument was calibrated using the Siemens Dimension RCRP CAL for 5 levels which are traceable to IFCC reference material certification. The control sera were BioRad Liquichek Elevated CRP Control. These were measured before and after the test samples for levels 1 and 2 as shown below:

<table>
<thead>
<tr>
<th>BioRad Liquichek</th>
<th>Level 1 Control</th>
<th>Level 2 Control</th>
<th>Units</th>
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<tr>
<td></td>
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<td>Range</td>
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<tr>
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<td>83.6</td>
</tr>
<tr>
<td>Measured Post-run</td>
<td>14.2</td>
<td></td>
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</table>

Table 1: CRP Controls

Table 1 shows that the Siemens Dimensions Xpand Chemistry Analyser performed well by maintaining the CRP control tests within the expected ranges.
3.9 Biochemical Assay for Uric Acid

The uric acid assay was run on the Beckman Coulter CX5Δ Chemistry analyser using the Uric Acid timed-endpoint method. The reaction goes according to the following equations:

\[
\text{Uric acid} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{uricase}} \text{allantoin} + \text{H}_2\text{O}_2 + \text{CO}_2 \quad (\text{equation 1})
\]

\[
\text{H}_2\text{O}_2 + 4\text{-AAP} + \text{DCHBS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + \text{H}_2\text{O} \quad (\text{equation 2})
\]

4-AAP = 4-aminoantipyrine

DCHBS = 3,5-dichloro-2-hydroxybenzene sulfonate

Uricase oxidises uric acid to give allantoin and hydrogen peroxide. This, with the reagents 4-AAP and DCHBS, in the presence of horseradish peroxidase, results in a coloured product called quinoneimine which is what is detected by the spectrophotometer at 520nm. The amount of quinoneimine dye, that is, the colour intensity at 520nm, is proportional to the amount of uric acid originally in the sample.

The analyser was calibrated using the Beckman Coulter CX Multi Calibrator and 3 levels of controls were run before the samples, midway and after the samples.

<table>
<thead>
<tr>
<th></th>
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<td>Pre-run</td>
<td>142</td>
<td>137.6-184.4</td>
<td>388</td>
<td>363.6-458.4</td>
<td>615</td>
<td>595.6-702.4</td>
<td>µmol/L</td>
</tr>
<tr>
<td>Mid-run</td>
<td>153</td>
<td>137.6-184.4</td>
<td>419</td>
<td>363.6-458.4</td>
<td>661</td>
<td>595.6-702.4</td>
<td>µmol/L</td>
</tr>
<tr>
<td>Post-run</td>
<td>156</td>
<td>137.6-184.4</td>
<td>423</td>
<td>363.6-458.4</td>
<td>672</td>
<td>595.6-702.4</td>
<td>µmol/L</td>
</tr>
</tbody>
</table>

Table 2: Uric Acid Controls

Table 2 shows that the Beckman Coulter CX5Δ performed well by maintaining the Uric acid control tests within the expected ranges.
3.10 Statistical Analyses

A cross sectional study is descriptive in nature, providing a snapshot of the population at a certain time. However, cause and effect of the disease may not be distinguishable but it is a great way to quickly and affordably assess frequency of the disease (e.g. syndrome of preeclampsia) and if a trend is evident.

Data analysis was done using Microsoft Office 2010 Excel, SPSS 16.0 and R version 2.15.1 statistical software tools.

A brief survey of the Mbuya Nehanda Antenatal Ward over 30 days showed that on average, 35.4% of the women in that ward were there because of a diagnosis of gestational hypertension/preeclampsia (range 23-57%). [See appendix 5a]
4 Results

34 women were included in the study. Bio-demographical data and preliminary analyses showed these results for mean/ mode averages:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (var): age of woman</td>
<td>29.8</td>
<td>19-41</td>
<td>years</td>
</tr>
<tr>
<td>Mean (var): gestational age of foetus</td>
<td>31.85</td>
<td>11-40</td>
<td>weeks</td>
</tr>
<tr>
<td>Mode: number of pregnancies/woman</td>
<td>3</td>
<td>1-6</td>
<td></td>
</tr>
<tr>
<td>Mean(var):MAP (mean arterial pressure)</td>
<td>96.11</td>
<td>64.3-124.3</td>
<td></td>
</tr>
<tr>
<td>Mean(var): Uric acid concentration</td>
<td>288.8</td>
<td>167-562</td>
<td>µmol/L</td>
</tr>
<tr>
<td>Mean(var): CRP concentration</td>
<td>8.859</td>
<td>0.4-43.6</td>
<td>mg/L</td>
</tr>
</tbody>
</table>

Table 3: Preliminary Analyses

Table 3 gives a general overview of the bio-demographical nature of the study participants who were finally included in the study. It is a group of women of child-bearing age, with most women’s age being in their mid-twenties to thirties and on their third pregnancy as the most frequent number of pregnancy. When we use blood pressure of 120/80 as normal, the MAP is 93.33. The average MAP for these study participants is higher at 96.11 but that is expected because many of the women have high blood pressure, the highest being MAP=124.3 which was calculated from very high BP measured at 198/92. Since preeclampsia is diagnosed after the 20th week of pregnancy, the mean gestational age was well within the window expected for preeclampsia to manifest. Normal CRP concentration is 5mg/L or less so the mean was high at 8.859mg/L, whereas, the mean for uric acid is below the cut-off number 360µmol/L.

The age of the pregnant woman is a risk factor for preeclampsia if she is under 17 years or over 40 years old (8). In this study there were none under 17 years and only one over 40 years therefore age was not statistically significant in this study (p=0.837).
At first, we wanted to find the association of CRP concentration and systolic and diastolic blood pressure. This was calculated using Pearson’s product moment correlation.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson's product-moment correlation</th>
<th>P-value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP and Diastolic BP</td>
<td>0.44</td>
<td>0.03</td>
<td>Moderate positive linear correlation</td>
</tr>
<tr>
<td>CRP and Systolic BP</td>
<td>0.25</td>
<td>0.20</td>
<td>No linear correlation</td>
</tr>
</tbody>
</table>

Table 4: CRP and Blood pressure

The Diastolic BP showed significance (p=0.03) whereas the systolic BP did not (p=0.2). This led to using mean arterial pressure (MAP) as a measure of hypertension because it gives a single number that can be used to compare two participants’ BP status. MAP was calculated and plotted against CRP concentration.

The equation commonly used for MAP is:

\[ MAP \approx \frac{2}{3}(DP) + \frac{1}{3}(SP) \] (equation 3)

where DP=diastolic blood pressure and SP=systolic blood pressure

![Graph showing the relationship between C-reactive protein and mean arterial pressure](image)

**Figure 5: CRP and MAP**
There was a positive correlation between CRP and MAP. The coefficient of correlation, r, is 0.5433. The coefficient of determination, R^2, is 0.2952. Since r is positive (positive slope of the graph line), this means there is a positive correlation. That is; as MAP increases, so does CRP.

**Checking Model Assumptions**

1. Normality: The response variable of interest CRP was log transformed. The log transformation of CRP gave a better fit of normality.

2. Linearity: There should exist a linear relationship between log CRP and the explanatory variable for the explanatory variable to be included in the final model. We performed univariate analysis using simple linear regression to check this assumption.

**Univariate analysis (log CRP)**

Univariate analysis was carried out using simple linear regression model $Y = \beta_0 + \beta_1 X$ where $Y$ is the dependent variable, $X$ is the explanatory variable. $\beta_0$ is the intercept and $\beta_1$ is the slope. The slope measures the relationship between $X$ and $Y$, that is, in this case, it gives the expected rate of change of the log transformed CRP given a unit increase of MAP, uric acid or age. For the variable of ‘number of pregnancy’, the slope measures a change of log transformed CRP for a given number of pregnancies. Table 5 below presents the slope that we get from the univariate analysis, and their respective p-values to measure the statistical significance of the relationship between explanatory variables and CRP.

From the p-values, using 0.05 significance, MAP and Uric acid showed significance in their relationship to CRP concentration. Although other studies have highlighted that very young mothers and very mature mothers are at risk for preeclampsia (51), the age of the women was not significant in this study.
### Table 5: Univariate Analysis of log (CRP)

We conclude that the Variables to be included in multiple regression analysis that predict log CRP are MAP and Uric acid using a cut-off of p-value < 0.05. The number of pregnancy showed high significance for the first pregnancy as shown in literature (52), that is; how many pregnancies the woman has had; but this could not also be included because of the relatively small sample size to cater for the number of levels for this variable.

#### Multiple Regression Analysis:

The general multiple regression equation for this data is

\[
\log \text{CRP} = \beta_1 \text{MAP} + \beta_2 \text{Uric acid}
\]  

where \( \beta_i \) is the coefficient estimate for the variable \( i \).

There was not a significant interaction between MAP and Uric acid in explaining a change in CRP (p>0.05) hence we proceeded with a simpler model of additive effects only. Table 6

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>Slope</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>( e^{0.036} = 1.037 )</td>
<td>0.0137*</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>( e^{0.005} = 1.005 )</td>
<td>0.0095**</td>
</tr>
<tr>
<td>Age</td>
<td>( e^{-0.007} = 0.993 )</td>
<td>0.837</td>
</tr>
<tr>
<td>Number of Pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>( e^{2.34} = 10.382 )</td>
<td>0.0002***</td>
</tr>
<tr>
<td>2</td>
<td>( e^{-0.23} = 0.794 )</td>
<td>0.7299</td>
</tr>
<tr>
<td>3</td>
<td>( e^{-0.97} = 0.379 )</td>
<td>0.1525</td>
</tr>
<tr>
<td>4</td>
<td>( e^{-0.80} = 0.449 )</td>
<td>0.2575</td>
</tr>
<tr>
<td>5</td>
<td>( e^{-1.50} = 0.223 )</td>
<td>0.0849.</td>
</tr>
<tr>
<td>6</td>
<td>( e^{-0.45} = 0.638 )</td>
<td>0.6412</td>
</tr>
</tbody>
</table>

**KEY**

* significant  
** moderately significant  
*** highly significant  
. boundary significance
below presents the slope that we get from the multiple regression analysis, and their respective p-values to measure the statistical significance of the relationship between explaining variables and CRP.

Final model:

<table>
<thead>
<tr>
<th>Explaining Variable</th>
<th>Slope</th>
<th>Standard Error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>$e^{0.026} = 1.026$</td>
<td>0.014</td>
<td>0.0694.</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>$e^{0.004} = 1.004$</td>
<td>0.002</td>
<td>0.0499*</td>
</tr>
</tbody>
</table>

Table 6: MAP and Uric Acid significance

$\beta_i$ need to de-transform using the exponent because they were estimated using log CRP. Substituting the estimates into equation 1 and taking the exponent on both sides gives:

$$CRP = e^{(0.026MAP + 0.004uric acid)}$$

(equation 5)

as the final model. It shows that a unit increase in MAP and a unit increase in the uric acid concentration result in an increase in CRP concentration by 1.03mg/L and 1.00mg/L respectively. The coefficient of correlation, $r$ ($r=0.5226$), is positive but low, showing that the relationship between the independent and dependent variables is not very strong but there still is a positive correlation. The coefficient of determination, $r^2$ ($r^2=0.2731$), is also low indicating that 27% of the dependent variable is explained by the independent variable, however 73% is unexplained indicating that there is likely some other unknown or hidden variable that will also explain the CRP concentrations in women with preeclampsia. In other words, the data shows that the MAP and uric acid can only explain 27% in the variation of CRP therefore there are other factors that also influence CRP concentration in pregnant women in Zimbabwe.
5 Discussion and Conclusions

5.1 Evaluation of the Study: Limitations and Recommendations

The investigation set out as a cross-sectional study into the possible association of maternal serum C-reactive protein levels and the severity of preeclampsia in Zimbabwean women. It was to ascertain the CRP concentrations found in pregnant Zimbabwean women with singleton pregnancy and to see if there is an association between CRP concentration and blood pressure in these women. The goal was to investigate if CRP can potentially be used as a biomarker for severity of preeclampsia. The study included normotensive women and women with any form of gestational hypertension; then used systolic BP over 140mmHg or diastolic BP over 90mmHg and uric acid over 360µmol/L to distinguish the more serious condition of preeclampsia from gestational hypertension. We successfully performed the assays and established that there is a positive correlation between CRP concentration, blood pressure (MAP) and uric acid in Zimbabwean women at Mbuya Nehanda Maternity Hospital. Clinicians made diagnoses of pregnancy-induced hypertension/gestational hypertension in those women who had borderline blood pressure that was not elevated enough to put them directly in the preeclampsia category. However, they kept monitoring these women using other clinical signs, symptoms and tests like proteinuria, oedema, epigastric pain or vision problems that could indicate a worsening of the condition to become preeclampsia or eclampsia.

Limitations of the study are as follows: There is no post-partum data firstly because this study focused on the time before delivery. Secondly because it was difficult to know when the women would go into labour, it was difficult to track the women after they went to the labour ward and most women were discharged soon after delivery. Since preeclampsia can occur
post-partum, future recommendation would be to monitor the women for up to two weeks after delivery, taking blood samples for CRP and uric acid and checking blood pressure regularly as indicators of onset of preeclampsia. However, this study focused on the antenatal phase with single antenatal time-point measurements.

Although the half-life of CRP is 19 hours and elevated CRP would have still been observed the next day, it would have been interesting to get blood samples of the participants before and after they started taking antihypertensive medication. The women from the Antenatal Ward with gestational hypertension or preeclampsia had been started on antihypertensive medication at least 12-20 hours before they were recruited into the study. Most of them would have been admitted to the ward in the late afternoon or previous night and immediately initiated on antihypertensives before being recruited into the study the following morning. A follow up study can investigate the effect of antihypertensive medication on the serum CRP concentrations by comparing before and after medication readings. We would recommend two time-points for women who are prescribed antihypertensives; one sample to be taken immediately after admission to the ward before initiation of the medication and one sample taken several hours after taking the medication.

We did not administer any treatment during this study and made no attempt to interfere with treatment (if any) that the study participants were on; but only observed and measured CRP concentrations at the time the women presented themselves at the clinic or when in the ward. As such, there is no ‘pre’ or ‘post’ treatment data.

This study included a fairly small number of study participants due to time and resource constraints. A larger sample size would be recommended to enable one to assess the effect of the number of pregnancies a women has had as a risk factor for preeclampsia. Higher rates of preeclampsia have been shown to occur in first pregnancies (12).
It is important to note that CRP is a marker of general inflammation and is not specific to preeclampsia. Other conditions could cause high concentrations of CRP. This means other conditions that can cause CRP concentrations to increase need to be taken into account when using CRP as a preeclampsia biomarker, making sure confounding factors are eliminated. In this study, the survey administered to the study participants attempted to capture information about possible inflammatory conditions as exclusion criteria. We excluded women with a history of liver disease, cardiovascular disease or neoplasm that could have caused high CRP levels. However, the survey was limited to the more common conditions and neglected rare conditions like systemic lupus erythematosus or osteomyelitis. In the future, a more comprehensive medical history and examination is recommended.

This study aimed to see if CRP can be used as a biomarker of severity of preeclampsia. In conjunction with other biomarkers and clinical signs, CRP can help to give a fuller picture of the state of the pregnant women who has preeclampsia. Recall that the aetiology of preeclampsia is not well established but having more information about the condition will help in the monitoring and treatment of the pregnant women to ensure she and her baby are well.

5.2 Conclusions from Data and evidence

Preeclampsia is a hypertensive disease of pregnancy that is still without definite known cause or cure- the only way to resolve it being delivery of the infant. At Mbuya Nehanda Maternity Hospital in Harare, this usually means induction of labour with Cytotec (misoprostol), a synthetic prostaglandin which causes uterine contractions, to ensure the wellbeing of the mother and infant. C-reactive protein has already been found to be elevated in preeclampsia (14) and it was also established that CRP concentration positively correlates to increasing blood pressure when Ustun et al. showed that there was significant correlation between MAP
and CRP ($r = 0.515$, $p = 0.0001$) (13). The data of this study concurs with these findings. A similar assay for highly sensitive C-reactive protein (hs-CRP) showed that hs-CRP could be used for identifying pregnant women at risk for preeclampsia (53). That information agrees with the findings of this study that show that in women at Mbuya Nehanda Maternity Hospital, a unit increase in mean arterial pressure or uric acid concentration, results in a unit increase in CRP concentration. C-reactive protein with uric acid and mean arterial pressure are potentially useful in assessing the severity of preeclampsia when elevated BP is used for diagnosis.

5.3 This Study in a Wider Context

This study is a good preliminary study into possible biomarkers of preeclampsia in Zimbabwe. These findings can be used to set expected values of CRP concentration in pregnant Zimbabwean women. A future study should include data on the results of other laboratory tests like creatinine, urea, urine albumin and liver enzymes. This will give greater detail of the biochemical state of the preeclamptic woman. In a longitudinal study, it would also be expedient to draw blood at several time points to compare the CRP levels before and after antihypertensive medication, before and after delivery of the baby and also at set times like at 30 weeks then at 35 weeks of gestation for easier comparison of the results.

It would be good to compare CRP, as a biomarker, with other potential biomarkers which have all been shown to be elevated in preeclampsia like neopterin (54), fibrinogen (13), interleukin-8 (55), cancer antigen 125 (CA-125) (5) and plasminogen activator inhibitor-1 (PAI-1) (56).

In cardiovascular studies, statins were used to reduce CRP concentrations which led to better prognoses for cardiovascular patients (57), (58). Reducing inflammation generally had an
effect on reducing atherothrombosis. Perhaps further studies may show that reducing CRP has an effect on the processes involved in preeclampsia.

Best et al. (36) have already shown that variants of the CRP gene affect CRP concentration in preeclampsia. The CRP gene has 85 single nucleotide polymorphisms (15) so another possibility for further study is to determine what the other various genetic and epigenetic aspects of CRP concentration are and how they relate to preeclampsia. This could help in even better diagnosis and assessment of severity of preeclampsia based on individualised molecular medicine.

5.4 Conclusion

High mean arterial pressure and uric acid are known indicators of severity of preeclampsia. The results show that the mean arterial pressure and uric acid concentration have positive correlation and an additive effect for predicting CRP concentration in women with preeclampsia in Zimbabwe. We aimed to investigate if CRP can be a biomarker for severity of preeclampsia for Zimbabwean women. The results of this study give an indication to the association between CRP concentration and the severity of preeclampsia as a good starting point. However, MAP and uric acid cannot account for all the causes of elevated CRP seen in preeclampsia. There are still other factors that seem to affect concentration of CRP in pregnant women with gestational hypertension/preeclampsia. To fully understand the effect of CRP on the severity of preeclampsia, these other factors need to be elucidated. In the meantime, CRP can be used in a panel of other tests for better clinical management of pregnant patients.
Bibliography


50. Siemens Healthcare Diagnostics Inc. RCRP. C-Reactive Protein Extended Range. . DF34 Flex Reagent Cartridge Insert. . Newark, DE : s.n., 2010.


### Appendices

<table>
<thead>
<tr>
<th>Appendix 1</th>
<th>Letter of Approval to carry out a research study from Joint Research Ethics committee of the University of Zimbabwe, College of Health Sciences and Parirenyatwa Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix 2</td>
<td>Endorsement from Institutional Ethical review Committee for approval of the study to the Medical Research Council of Zimbabwe</td>
</tr>
<tr>
<td>Appendix 3</td>
<td>Letter of Approval to carry out a research study from Medical Research Council of Zimbabwe</td>
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<tr>
<td>Appendix 4</td>
<td>Questionnaire used in data collection</td>
</tr>
<tr>
<td>Appendix 5</td>
<td>Tables of raw data</td>
</tr>
<tr>
<td></td>
<td>(a) Data from Mbuya Nehanda Antenatal Ward</td>
</tr>
<tr>
<td></td>
<td>(b) Raw data of all women in the study</td>
</tr>
<tr>
<td></td>
<td>(c) Pregnancy History</td>
</tr>
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