Querying the value of indiscriminate iron supplementation in pre-eclampsia: a cross-sectional study

Vinogrin Dorsamy[1], Chauntelle Bagwandeen[1], and J Moodley[2]

[1]University of KwaZulu-Natal College of Health Sciences
[2]University of KwaZulu-Natal Nelson R Mandela School of Medicine

July 10, 2021

Abstract

Objective To determine the prevalence and type of anaemia and its association with pre-eclampsia (PE) in pregnant South Africans of African ancestry. Design Cross-sectional study design Setting A regional hospital in KwaZulu-Natal, South Africa. Sample 671 women seeking antenatal care. Methods Participants had haemoglobin(Hb), anthropometric measurements, HIV status, blood pressure levels(BP) and proteinuria measured to determine PE. Iron studies and transferrin receptor levels were assessed in a subset and chi-square tests of association between normotensive and pre-eclampsia sub-groups and blood parameters were conducted. Results No difference in Hb concentration amongst the 4 groups (F (3,621)=0.981, p< .001, η2=.014) was observed. A chi-square test of association (χ2(3)=6.674, p=.083) showed no associations between study groups and having anaemia. The severity of anaemia did not vary amongst study groups (χ2(12) =10.756, p=.550). Using ferritin, there was an association between the study groups having an iron deficiency, anaemia, both or neither (χ2(3)=12.559, p=.045) with a positive association between normotensive term and iron deficiency (adjusted residual(AR) 2.2) and positive association between early-onset PE(AR 2.4). Similar trends were found for transferrin and soluble transferrin receptor ferritin index. Twenty-two percent of the participants were not iron deficient. Conclusion Early-onset PE is associated with high iron status and not anaemia. Normotensive term pregnancies were associated with iron deficiency anaemia. Broad iron supplementation without adequate determination of iron deficiency in pregnant women needs to be revisited. Funding National Research Foundation (TTK170508230162), University of KwaZulu-Natal UCPD and Medical Research Council of South Africa(SIR Grant UNS14197).
Keywords Anaemia, iron deficiency, pre-eclampsia

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Vinogrin Dorsamy[1], Chauntelle Bagwandeen[2], Jagidesa Moodley[3]

[1]Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa

[2]Department of Public Health Medicine, School of Nursing and Public Health, University of KwaZulu-Natal, South Africa

[3]Women's Health and HIV, School of Clinical Medicine, University of KwaZulu-Natal, South Africa

Corresponding Author : Vinogrin Dorsamy

Address : Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, South Africa

Email Address : dorsamyv1@ukzn.ac.za

Email addresses of authors

Chauntelle Bagwandeen (Bagwandeenc@ukzn.ac.za)

Jagidesa Moodley (jmog @ukzn.ac.za)

Disclosure of interests

The authors declare no competing interests.

Abstract

Objective

To determine the prevalence and type of anaemia and its association with pre-eclampsia (PE) in pregnant South Africans of African ancestry.

Design

Cross sectional study

Setting

A regional hospital in KwaZulu-Natal, South Africa.

Sample

671 women seeking antenatal care.

Methods

Participants had haemoglobin(Hb), anthropometric measurements, HIV status, blood pressure levels(BP) and proteinuria measured to determine PE. Iron studies and transferrin receptor levels were assessed in a subset and chi-square tests of association between normotensive and pre-eclampsia sub-groups and blood parameters were conducted.

Results

No difference in Hb concentration amongst the 4 groups (F (3,621)=0.981, p< .001, η2=.014) was observed. A chi-square test of association ($\chi^2(3)$=6.674, p =.083) showed no associations between study groups and having anaemia. The severity of anaemia did not vary amongst study groups ($\chi^2(12)$ =10.756, p =.550). Using ferritin, there was an association between the study groups having an iron deficiency, anaemia, both or neither ($\chi^2(3)$=12.559, p =.045) with a positive association between normotensive term and iron deficiency (adjusted residual(AR) 2.2) and positive association between early-onset PE(AR 2.4). Similar trends were found for transferrin and soluble transferrin receptor ferritin index. Twenty-two percent of the participants were not iron deficient.

Conclusion

Early-onset PE is associated with high iron status and not anaemia. Normotensive term pregnancies were associated with iron deficiency anaemia. Broad iron supplementation without adequate determination of iron deficiency in pregnant women needs to be revisited.

Funding

National Research Foundation (TTK170508230162), University of KwaZulu-Natal UCPD and Medical Research Council of South Africa(SIR Grant UNS14197).

Keywords

Anaemia, iron deficiency, pre-eclampsia

Tweetable abstract

Iron deficiency is not associated with early onset pre-eclampsia and must be established before supplementation given

Introduction

The importance of addressing anaemia during pregnancy is well established[1]. Iron supplementation is routinely prescribed during antenatal care to prevent adverse maternal and perinatal outcomes[2]. While iron deficiency is the commonest cause of anaemia, and anaemia is associated with poor pregnancy outcomes, it is incorrect to assume that such associated outcomes are all related to iron deficiency[3–5]. Increasing evidence points to the contrary: iron overload or raised levels of the universal marker for anaemia, haemoglobin (Hb), may also be responsible. Such outcomes may also be associated with tuberculosis, HIV, sexually transmitted infections, parasitic infections and obesity, which also play a role in the aetiology of anaemia[6–9].

Current antenatal care, especially in low and middle income countries (LMIC's), mandates iron supplementation without establishing iron deficiency or type of anaemia[10]. Indiscriminate supplementation in pregnant women who may be iron replete, should be questioned since such practice may not only be wasteful, but harmful[3,5,11]. Iron is toxic and its regulation is carefully controlled to ensure that it is contained at all times, as increases in circulating and intracellular iron induces reactive oxidative species, cellular damage and ferroptosis[5]. In South Africa(SA), a LMIC, with a high burden of the above-mentioned conditions, the commonest direct cause of morbidity and mortality in pregnant women is pre-eclampsia(PE)[12], and like anaemia, is linked to preterm birth and small for gestational age(SGA) babies. While PE has been shown to be associated with severity of anaemia[13], increased Hb is also a risk factor for these outcomes, suggestive of a 'Goldilocks' range for Hb levels: neither too high nor too low[3,7,14]. Haemoglobin levels alone may not reveal an association with PE, which may be surreptitiously masked within a subtype of anaemia.

It is important that Hb and iron status be used to differentiate the type of anaemia present. The aim of this study was to delineate the prevalence and type of anaemia and its association with the spectrum of PE in SA, a country burdened by its high prevalence and concurrent epidemics of infectious and lifestyle diseases, using Hb levels in conjunction with iron markers. We delineated anaemia type by iron deficiency or anaemia of inflammation using markers such as ferritin, transferrin and its cognate receptor, in order to measure the association between the type of anaemia and the type of PE (early and late onset PE). We further established the burden of HIV and obesity using body mass index (BMI) and their associations with PE.

Materials and Methods

Study design and setting

This study was nested in a large, single-centre, prospective, population-based study that recruited pregnant women from a regional hospital providing maternity care services in Durban, South Africa. Participant recruitment occurred from May 2017 to February 2020. Pregnant women attending the hospital or its affiliated clinic for routine antenatal investigations[10] or those presenting to the labour ward for a scheduled delivery or due to complications were requested to enrol in the study. The standard of care followed that described in the South African maternal care guidelines. In particular, as related to this study, Hb determination was made at the visit and iron supplementation was prescribed throughout pregnancy[10]. The sampling was purposive and non-probabilistic as participants were recruited into four groups based on the subtypes of PE, gestational age and timing of their antenatal visit.

Preeclampsia was diagnosed as sustained systolic blood pressure of [?] 140mmHg and a diastolic blood pressure of 90 mm Hg taken on two occasions at least six hours apart; in addition, the diagnosis depended on the presence of one or more of the following: New onset proteinuria (1+ on a urine dipstix) or >30mg/dl urine protein concentration or abnormal renal, and hepatic indices. Preeclampsia was also divided into i) early onset PE (EOPE) as PE occurring before 34 weeks gestation and ii) late onset (LOPE) as that occurring [?] 34 weeks of gestation[15].

The HIV status was recorded if known or a rapid HIV test was done as standard practice.

Inclusion and exclusion criteria

South African pregnant women of African ancestry, 18 years of age or older were eligible for inclusion. Participants who had any pre-existing conditions or who were on any medications for hypertension or anti-inflammatory conditions that could skew possible presentation of PE were excluded, as were those who agreed to participate but who were lost to follow-up, and where critical outcome data was not available despite tracing attempts. Due to challenges experienced with follow-up and delivery at sites other than the research site and due to the lockdown measures to combat the Covid-19 pandemic, instituted in March 2020, there was an unexpected attrition that was difficult to address, given the inability to conduct face to face visits. Attempts to account for loss to follow up were made by reconciling birth data from hospital chart data with labour ward birth data records, where available.

Outcome measures

The main outcomes of interest were normotensive pregnancy, early and late onset PE.

Exposure measures .

Haemoglobin concentration, mean cell volume (MCV), serum iron, ferritin, transferrin saturation, and serum transferrin receptor, as well as other haematological parameters were used as exposure variables.

Haemoglobin levels were determined by use of a haemoglobinometer or full blood count reports from patients' charts. Iron studies were analysed from serum samples using the Abbott Architect Ci 8200 chemiluminescent microparticle immunoassay (Abbott Diagnostics, Abbott Park, IL, USA) and soluble transferrin was determined from serum using the Roche Cobas 6000 electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany).

Anaemia was defined according to the WHO classification of <11g/dL[1]. Anaemia severity was grouped as: no anaemia raised Hb > 13.2 g/dL; no anaemia: [?]11 g/dL; mild anaemia: 11g/dl < Hb [?] 9.9g/dL; moderate anaemia: 9.9g/dL <Hb < 7.0; severe anaemia [?] 7.0 g/dL. Haematological parameters were grouped according to their cut-points for determination of iron deficiency or iron deficiency anaemia. Ferritin levels in pregnancy were grouped as per Daru et al.[16] and more recently Auerbach et al.[17] where ferritin < 30 ng/mL with Hb <11 g/dL is considered iron deficiency, anaemia while ferritin below 15ng/ml in non-anaemic participants are classified as iron deficient. Similarly a cut-point of >3 g/L for transferrin or 37.68 μmol/L was used to define iron deficiency[18,19]. A raised C-reactive protein (CRP) was determined by cut-point > 10 g/dL[20]. Serum transferrin receptor/ log (ferritin) index used a cut-point of 1.4[21].

Statistics

The number of participants in the study was validated using *a priori* power calculations with conservative effect size estimate of $\rho = 0.02$, an α error of 0.05 and a power (1-β) of 0.90[22].

Statistical analysis was conducted using SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). For each parameter evaluated, tests for normality were conducted using z-values of skewness and kurtosis and where these values fell within the range -1.96 to +1.96, these were indicative of normal distribution. Where categorical variables were present χ^2 tests were used and p < .10 was used to detect asymptotic significance. One-way ANOVAs and a Tukey's HSD post hoc test were used to identify between-group differences. A *p*- value < .05 was considered statistically significant. Where data for certain parameters were missing, statistical analyses for these parameters were conducted using available data and noted as such in the (n) and degrees of freedom in the results.

Patient and public involvement (PPI)

There were no patient and public involvement strategies used in the study.

Results

General Characteristics

Approximately 1600 women were approached for enrolment. Six hundred and seventy-one (n=671) participants who met the inclusion criteria agreed to participate (Figure 1), with 410 women (61.1%) falling into the normotensive group. The average age of the participants were 28.85 years old (SD 6.22).

Two hundred and sixty-one (38.9%) participants presented with either EOPE (n=141) or LOPE (n=120). The prevalence of anaemia was 35.5%, with haemoglobin values missing for 6.9%. One hundred and thirty-five women (20.1%) were mildly anaemic, 99 (14.8%) were moderately anaemic and 4 (0.6%) were severely anaemic. The prevalence of HIV infection was 46.1% (n=309). Table 1 summarises other relevant statistics.

Maternal factors and Haematological indices

There was no statistically significant difference in Hb concentrations amongst the four groups (F (3,621) =0.981, $p < .001$, $\eta^2 = .014$). A chi-square test of association ($\chi^2(3) = 6.674$, $p = .083$) showed no significant associations between the study groups and anaemia. The severity of anaemia did not vary amongst the groups (χ^2 (12) = 10.756, $p = .550$).

The chi-square test using anaemia/no anaemia and hypertensive/normotensive 2 x 2 table revealed χ^2 (3) = 3.471, $p = .064$ Cramer's V strength of association of 0.075 indicating a near significant association.

Ferritin levels

Using Hb cut-off and serum ferritin as a marker for iron deficiency in both anaemic and non-anaemic participants, out of 209 participants, 97 (46.4%) had no anaemia and were not iron deficient, 24 (11.5%) had no anaemia with iron deficiency and 61 (29.2%) had iron deficiency anaemia, indicating that 27 women (12.9%) were anaemic without signs of iron deficiency. Using a Fisher exact test comparing the study groups with the anaemia by ferritin group, χ^2 (3) = 12.559,$p = .045$ there was a significant association between the study groups having iron deficiency, anaemia, both or neither. The adjusted residuals (AR) showed a negative association of the TermNT group with the non-iron deficient anaemia category (AR -2.2), and a negative association of the EOPE group with iron deficiency anaemia category (AR -2.2) but a stronger positive association with the non-iron deficient anaemia category (AR 2.4). As Figure 2 depicts, both the hypertensive groups had significantly higher frequencies of non-anaemic, non-iron deficient participants.

Transferrin

Similarly, using the combination of serum transferrin and Hb as a marker (n=559), 352 (63%) were not anaemic, 51 (9.1%) were anaemic without iron deficiency and 156 (27.9%) had iron deficiency anaemia. A Pearson chi-square test for association between the term pregnancy groups and being either non-anaemic or anaemia with or without iron deficiency, classified by transferrin level (either less or greater than 36.7 μmol/L showed that the groups were not independent from each other $\chi(4) = 18.205$, $p = .001$, Cramer's V 0.155. Adjusted residuals were indicative of a significantly negative (AR -2.8) association of the EOPE group with iron deficiency anaemia and a negative association of non-iron deficiency anaemia with the TermNT group. There were no other associations between the other groups.

Soluble transferrin receptor ferritin (STfR) index

The soluble transferrin receptor (STfR)/log(ferritin) index was calculated for 151 participants, resulting in 118 (78.1%) classified as iron deficient regardless of anaemia status. 45 (31.9%) were both anaemic and iron deficient and 65 (46.1 %) were iron deficient and not anaemic. Eighteen (32.1%) and 9 (17%) participants were not iron deficient in the EOPE and LOPE groups respectively. A chi-square test of association between anaemia/iron deficiency and term normotensive and both PE groups shows significant associations ((χ^2 (12, N=114) = 13.195, $p = .040$, Cramer's V=0.241), with adjusted residuals showing statistically significant positive associations between being anaemic without iron deficiency and the EOPE group (AR 2.3), with a negative trend of association between EOPE and iron deficiency anaemia (AR -1.5: not significant); being both anaemic and iron deficient and LOPE (AR 2.1); and iron deficiency with no anaemia and the normotensive group (AR 2.5); These results (Table 3) should be treated with caution as there was underrepresentation in the normotensive term group with only 5 cases analysed.

When cross tabulating the index with severity of anaemia (anaemia level group), there were no dependent associations found ((χ^2 (3, N=141) = 2.585, p = .467, Cramer's V= 0.135).

Transferrin Saturation

A chi-square test of association between anaemia/iron deficiency and TermNT and both PE groups shows significant associations ((χ2 (6, N=387) = 24.376, p < .001, Cramer's V=0.177). Adjusted residuals are provided in table 4 and indicate significant positive association of iron overload with EOPE and iron insufficiency in TermNT with a negative association between EOPE and reduced transferrin saturation.

Body Mass Index

BMI was statistically significantly different across all four groups F(3,583)=20.256, p < 001, η2 = .094) with means reported in Table 2. Post hoc analysis revealed that the FirstVisitNT group had a significantly lower BMI than the other groups (p<.001 for all), and that there was no significant difference between the TermNT group and EOPE group (p=.577) despite a difference in mean gestational age. There was a significant difference between the TermNT and LOPE - BMI was 3.3 kg/m^2 lower (p =.002) - but there was no difference between EOPE and LOPE (p = .086).

HIV

A chi-square test of association between HIV and TermNT and both PE groups shows significant associations (χ2 (2, N=425) = 9.086, p = .011, Cramer's V=0.146). Adjusted residuals are provided in table 5. HIV infection in pregnancy was associated with EOPE and being HIV negative was more strongly associated with LOPE with no significance of HIV in normotensive pregnancies.

Discussion

Main findings

This study highlights the importance of identifying the cause of anaemia in pregnant populations as negative associations were found with iron deficiency, positive with anaemia unrelated to iron deficiency, and pre-eclampsia. The prevalence of anaemia was 35.5% with 20.1% being mildly anaemic, 14.8% moderately anaemic and 0.6% severely anaemic, with no difference between the normotensive and PE pregnancy groups There was no association between PE and severity of anaemia. While 350 participants were not anaemic (52.2%) , 37 (5.5%) had a raised Hb (>13.2g/dl). When evaluating the type of anaemia using a combination of ferritin and Hb level, iron deficiency anaemia was positively associated with normotensive term pregnancy, while EOPE was not associated with iron deficiency, independent of anaemia. Using iron markers such as transferrin and transferrin saturation showed similar trends, with a significant association between above normal saturation and PE. In the subset analysis of STfR-ferritin index, iron deficiency was found to be present in 118/151 participants (78.1%) and iron deficiency anaemia in 31.9%. Importantly, almost 22% of this subset was not iron deficient while 32.1% and 17% of the sample had no deficiency in the early and late pre-eclamptic groups respectively. Further investigation into iron saturation or overload revealed an association with EOPE. HIV prevalence was 46.1% and obesity was 50.3% and presence of both was significantly associated with PE.

Strengths and limitations

This is the first study, as far as we are aware, that distinguishes early and late onset PE and the type of anaemia in our population. Sampling is representative of the South African majority who share a similar socioeconomic status and burden of disease profile, and may be relevant to LMIC's regionally and globally. Although extrapolation to an affluent population might be limited, the conclusion and recommendations are equally applicable. The cross-sectional study design, normotensive early gestation participants lost to follow up, lack of maternal mortality statistics and limited birth outcome data impedes inference of cause and consequence. This study limits its definitions of types of anaemia to iron deficiency distinct from other causes collectively without exploring those in detail.

Interpretation

Haemoglobin levels showed no significant association with either the presence or severity of anaemia. However, when using iron study markers such as ferritin and sTfR-ferritin index, associations were found with EOPE and either not being anaemic or iron deficient while normotensive pregnancy was associated with iron deficiency. The observed frequency of non-anaemia and hyperferritinaemia was significantly larger than the expected frequency in the EOPE group. Women with no anaemia and no iron deficiency were more frequently found in the EOPE group while anaemic women without iron deficiency were less associated with being normotensive and delivering at term. The LOPE group was significantly associated with anaemia regardless of iron deficiency, similarly evident when using the sTfR-ferritin index.

Our results that there is a hyperferritinaemia in PE are similar to other studies[23–25]. Raman et al.[26]showed higher mean ferritin levels in pregnant women with pregnancy induced hypertension and PE, concluding that hyperferritinaemia may mask iron deficiency. Ferritin is an acute phase reactant[27], and where there are infectious co-morbidities, iron deficiency may be hidden due to raised ferritin. van den Broek et al.[18], when studying how well ferritin levels predicts bone marrow haemosiderin and true iron deficiency, found a 90% sensitivity and 85.1% specificity using ferritin regardless of infection. We found a significant association related to the timing of PE as the association is strongest in the EOPE group, while no statistical variance in the means of CRP between the groups were noted. Thus, as Milman[28] recommends, rather than blanket supplementation with iron, individual iron prophylaxis should be adjusted to serum ferritin levels in early pregnancy and suggest a potential benefit to measuring ferritin as an early marker of PE. Similarly, we found the use of transferrin confirmed the association found with ferritin that iron deficiency was negatively associated with the EOPE group.

A significant association between EOPE and having non-iron deficient anaemia was found in a smaller subset of participants using the sTfR-ferritin index, while LOPE was associated with anaemia and iron deficiency. The index is more sensitive for iron deficiency when there is concomitant inflammation[21] , and it detected that 20% of the sample had no iron deficiency.

Due to limited sampling of TermNT, we were unable to determine that association. Nevertheless, the results confirm the trends reported here and elsewhere[21,26,29] for serum ferritin levels.

The prevalence of HIV in our study population was 46.1% and 50.3% of women were obese, an independent risk factor for PE. Both of these conditions are associated with anaemia of inflammation[30], which if sustained, will lead to an iron deficient phenotype due to withholding of iron from what the body deems an infective insult, releasing Interleukin-6, which stimulates hepcidin release. This removes iron from circulation, thereby reducing efficient erythropoiesis[23,31]. Effectively, iron is then denied to the growing placenta and fetus which may impact on their growth[5,23,32]. The body storage capacity for iron is limited and sustained inflammatory conditions increase the risk of iron overload, especially when women are iron replete and are taking oral supplements[5]. This is toxic and has been shown to be associated with adverse birth outcomes[3,5]. Our study shows that both serum iron and transferrin saturation were increased in EOPE, indicating an association between iron overload and PE.

Iron overload has been associated with ferroptosis involving other organ systems[5], notably those implicated in the pathophysiology of PE[25,33–35], as well as gestational diabetes (GDM), a condition also associated with adverse birth outcomes[29,36–38]. While women who have anaemia should be treated, without understanding the cause of anaemia we risk subjecting women to iron overload. Using both ferritin and sTfr-ferritin index, iron overload was strongly associated with EOPE, while anaemia or iron deficiency was consistently associated with TermNT pregnancies, hence better birth outcomes. Poor birth outcomes is as associated with increased iron or raised haemoglobin as iron deficiency[3,14,39] and iron overload or anaemia of inflammation is associated with PE[5]. Of concern is that when using the index, while 77.8 % of the participants were iron deficient, by implication 22.2% were not. Hence a fifth of the population is being treated for a condition they do not have, with a potentially toxic substance[5,33–35].

Conclusion and recommendations

Our findings justify the need to revisit the present standard of determining anaemia (Hb alone), to ensure that we manage patients appropriately, with treatment dependent on cause rather than ubiquitous prescription of iron. Iron markers such as serum ferritin, at the least, may provide an insight into the true association between being non-anaemic and non-iron deficient and having EOPE. Further investigation is warranted where iron markers for storage, transport or use is measured to corroborate our findings. While the initial outlay of using such tests in resource-constrained settings may be baulked at, this will be outweighed by possible caesarean delivery and intensive care costs related to iatrogenic preterm delivery and distal therapeutic costs for long-term sequelae of iron overload.

Iron supplementation should be approached with caution in women at risk of PE and standard practise guidelines for antenatal care should consider targeted iron regulation based on the evidence presented in these and other studies. Health promotion and education on managing obesity, addressing the burden of infective causes of anaemia and mass treatment at the individual and population level to reduce the burden of parasitic infestations should be elevated as a health priority to reduce maternal and infant morbidity indices.

Acknowledgements

The authors wish to thank all participants who agreed to enrol in this study. We wish to acknowledge Melissa Bengu for data handling and processing for the study and Boikhutso Tlou for statistical analysis.

Disclosure of interests

The authors declare no competing interests.

Contribution to authorship

All authors (VD, CB, JM) contributed to the design of the study. VD and CB analysed and interpreted the quantitative data and all authors (VD, CB and JM) interpreted the data. VD drafted the first version of the article. All authors (VD, CB and JM) commented on drafts of the article and have read and approved the final version for publication.

Detail of ethical approval

The study received full ethical clearance from the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (BREC552/16) in February 2016 and from the KwaZulu-Natal Department of Health and hospital management at the research site. The purpose of the study was explained to all pregnant women recruited who gave written informed consent to participate.

Funding

This study was funded by the South African National Research Foundation (NRF) Thuthuka grant (TTK170508230162) and the South African Medical Research Council (SIR Grant UNS14197), which included external peer review. The funders played no role in the conducting or research or writing of the manuscript.

Table figure caption list

Figure 1. Study groups: participants grouped according to the time of hospital visit, hypertensive state and gestational age.

Figure 2. Frequencies of anaemia coupled with iron deficiency as determined by specific cut-points of ferritin and Hb levels.

Table 1. Characteristics and parameter frequencies of all pregnant women enrolled in study

Table 2. Maternal variables and haematological indices per study group

Table 3. Cross tabulation of Anaemia and soluble Transferrin receptor ferritin index vs term and pre-eclamptic groups

Table 4. Cross tabulation of transferrin saturation vs TermNT and Pre-eclampsia groups

Table 5. Cross tabulation of HIV vs normotensive term and PE Groups

Data availability

The data that support the findings of this study is part of a larger data set that is still being processed for further study. The data is not publicly available due to privacy or ethical restrictions.

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Table 1. Characteristics and parameter frequencies of all pregnant women enrolled in study

Characteristic	Feature			
	Normotensive	*Hypertensive*		
Hypertensive status n (%)	**410 (61.1)**	**261 (38.9)**		
	18-28	*29-39*	*>=40*	
Age (year group)- n (%)	**352 (52.5)**	**293 (43.7)**	**26 (3.9)**	
	Primigravidae	*Multigravidae*		
Gravidity	**142 (21.2)**	**505 (75.3)**		
	First VisitNT	*TermNT*	*EOPE*	*LOPE*
PE group – n (%)	**246 (36.7)**	**164 (24.4)**	**141 (21)**	**120 (17**
	No Anaemia ≥11g/dL	*Anaemia < 11.0 g/dL*	*Missing*	
Anaemia Total- n (%)	**387 (57.7)**	**238 (35.5)**	**46 (6.9)**	
	No Anaemia Raised Hb >13.2g/dL	*No Anaemia >11 g/dL*	*Mild [?]10.9 g/dL*	*Moderat*
Anaemia level Group- n (%)	**37 (5.5)**	**350 (52.2)**	**135 (20.1)**	**99 (14.8**
BMI	*<18.5*	*<25*	*<30*	*[?]40*
BMI group- n (%)	**77 (11.5)**	**70 (10.4)**	**160 (23.8)**	**256 (38**
	Negative	*Positive*		
HIV- n (%)	**362 (53.9)**	**309 (46.1))**		

Values reported are frequencies and percentage. Where values do not add to 100% the missing percentage indicates where data was not available. PE -Pre-eclampsia, First VisitNT - First visit normotensive, TermNT – Term normotensive EOPE Early onset pre-eclampsia, LOPE - Late onset pre-eclampsia, Anaemia defined according to WHO <11g/dL, BMI - Body mass index reported as raw weight / (height)^2 , C/S- Caesarean section, NVD -Normal vaginal delivery, IOL -Induction of labour, GA -gestational age, SGA -Small for gestational age, LGA -Large for gestational age

Table 2. Maternal variables and haematological indices per study group

		First VisitNT	**TermNT**	**EOPE**	**LOPE**	**Total**	*p*
Age	**N**	246	164	141	120	671	.098
	Mean	27.87	28.59	29.47	28.83	28.55	
	Std. Deviation	5.755	6.264	6.433	6.722	6.220	

		First VisitNT	TermNT	EOPE	LOPE	Total	p
BMI (kg/m^2)	**N**	200	146	138	103	587	.000*
	Mean	30.0885	33.1955	34.3007	36.5398	32.9836	
	Std. Deviation	6.73127	6.16876	8.55735	7.90375	7.63541	
Haemoglobin (g/dL) at recruitment	**N**	215	163	138	109	625	.437
	Mean	11.154	11.245	11.388	11.330	11.260	
	Std. Deviation	1.5079	1.3871	1.2725	1.2919	1.3900	
Maternal Hb at birth	**N**	188	61	65	68	382	.972
	Mean	10.579	10.564	10.548	10.528	10.562	
	Std. Deviation	0.8087	0.7253	0.8035	0.8162	0.7936	
Mean Cell volume (fL)	**N**	202	157	130	107	596	.963
	Mean	89.129	88.774	89.089	88.931	88.991	
	Std. Deviation	6.9548	6.6766	6.2479	6.9563	6.7181	
ESR (mm/hr)	**N**	203	148	122	98	571	.009*
	Mean	34.55	35.16	40.17	42.04	37.19	
	Std. Deviation	22.187	21.028	21.830	19.912	21.601	
CRP (mg/L)	**N**	153	143	117	87	500	.467
	Mean	11.35	10.31	13.53	13.13	11.87	
	Std. Deviation	24.147	15.494	15.553	12.684	18.210	
RDW	**N**	159	154	124	105	542	.373
	Mean	14.741	15.177	14.827	14.938	14.923	
	Std. Deviation	2.1557	2.6740	2.3265	1.7761	2.2905	
Mean Cell Hb Concentration	**N**	159	154	125	105	543	.499
	Mean	33.255	33.483	33.604	33.455	33.439	
	Std. Deviation	1.5442	3.0385	1.0240	1.2105	1.9594	

		First VisitNT	TermNT	EOPE	LOPE	Total	p
Serum Transferrin Receptor (mg/L)	**N**	35	8	66	64	173	.514
	Mean	3.909	3.288	3.359	3.553	3.539	
	Std. Deviation	2.0434	0.9583	1.8863	1.6239	1.7935	
Serum Iron	**N**	216	155	134	111	616	.000*
	Mean	14.1292	14.2329	18.6638	17.9153	15.8239	
	Std. Deviation	7.72026	8.28140	13.61558	9.59026	9.93705	
Transferrin umol/L	**N**	191	144	129	108	572	.000*
	Mean	38.67	46.98	39.016	42.39	41.54	
	Std. Deviation	7.06	9.39	8.77	8.79	9.06	
Transferrin Saturation %	**N**	190	143	131	113	577	.000*
	Mean	19.24	15.94	26.01	22.07	20.52	
	Std. Deviation	12.58	10.02	21.89	15.07	15.56	
Ferritin g/dL	**N**	52	32	79	82	245	.000*
	Mean	34.38	28.63	75.23	38.84	48.29	
	Std. Deviation	25.574	19.272	98.063	43.400	65.116	
STrFerrRatio	**N**	33	5	57	56	151	.021
	Mean	3.2866	2.1309	2.0801	2.7248	2.5846	
	Std. Deviation	2.49118	0.55494	1.30197	1.84298	1.85195	
AnaemiaGroup	**N**	215	163	138	109	625	.083
	Mean	0.45	0.36	0.32	0.36	0.38	
	Std. Deviation	0.498	0.482	0.468	0.482	0.486	
BMI Group	**N**	239	162	133	110	644	.000*
	Mean	2.90	3.43	3.62	3.58	3.30	
	Std. Deviation	1.211	1.108	0.950	1.252	1.183	
Anaemia Severity Group	**N**	215	163	138	109	625	.173
	Mean	2.58	2.49	2.38	2.47	2.49	

		First VisitNT	TermNT	EOPE	LOPE	Total	p
	Std. Deviation	0.882	0.856	0.785	0.856	0.851	

***indicates significance. ESR-Erythrocyte sedimentation rate, CRP – C-reactive protein, BMI -Body mass index, RDW – Red cell distribution width, sTrFerrRatio -Soluble Transferrin/log(ferritin) index**

Table 3. Cross tabulation of Anaemia and soluble transferrin receptor/ferritin index vs term and pre-eclamptic groups.

		Normotensive N	Normotensive %	Normotensive AR	EOPE N	EOPE %	EOPE AR	LOPE N	LOPE %	LOPE AR	Total N	T %
Anaemia sTfRFerrIndex	Anaemia-Fe Def-	0	0.0%	-1.0	11	19.6%	.8	8	15.1%	-.4	19	1
	Anaemia+Fe Def-	0	0.0%	-.6	7	12.5%	2.3*	1	1.9%	-2.0*	8	7
	Anaemia-Fe Def+	5	100.0%	2.5*	25	44.6%	-.4	23	43.4%	-.6	53	4
	Anaemia+Fe Def+	0	0.0%	-1.5	13	23.2%	-1.5	21	39.6%	2.1*	34	2
Total	Total	5	100.0%		56	100.0%		53	100.0%		114	1

Significant residuals are marked with an asterisk and shaded. AR -Adjusted residuals; EOPE-Early onset preeclampsia; LOPE – Late onset pre-eclampsia. The left column indicates the type of anaemia and + means condition present and – means absent

Table 4. Cross tabulation of transferrin saturation vs TermNT and pre-eclampsia groups **Table 4. Cross t**

PE Group	TermNT
	EOPE
	LOPE
Total	Total

Significant residuals are marked with an asterisk and shaded. AR -Adjusted residuals; EOPE-Early onset preeclampsia; LOPE – Late onset pre-eclampsia. The left column indicates the type of anaemia and + means condition present and – means absent

Table 5. Cross tabulation of HIV vs normotensive term and PE Groups

		HIV- N	HIV- %	HIV- AR	HIV+ N	HIV+ %	HIV+ AR	Total N	Total %
PE Group	TermNT	85	37.1%	-.7	79	40.3%	.7	164	38.6%

	EOPE	66	28.8%	-2.1*	75	38.3%	2.1*	141	33.2%
	LOPE	78	34.1%	2.9*	42	21.4%	-2.9	120	28.2%
Total	Total	229	100.0%		196	100.0%		425	100.0%

Significant residuals are marked with an asterisk and shaded. Minus (-) means negative magnitude. AR -Adjusted residuals; EOPE- Early onset preeclampsia; LOPE – Late onset preeclampsia.

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