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An investigation of maternal anaemia among HIV infected pregnant women on antiretroviral treatment in Johannesburg, South Africa

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Abstract

Introduction: maternal anaemia is a major public health problem in developing countries. Data suggests that anaemia contributes to the progression of Human Immunodeficiency Virus (HIV)-infection. The aim of this study was to investigate if pregnancy was an aggravating factor for anaemia among HIV-positive women on anti-retroviral treatment (ART). Methods: we analyzed data of all HIV-positive women aged 18-49 years receiving ART at Themba Lethu Clinic, Helen Joseph Hospital, Johannesburg, South Africa, from 1st April 2004- 30th April 2011. HIV-positive pregnant women were matched with non-pregnant women using the year of initiation of treatment. The outcome of interest “anaemia” was defined as "no anaemia", “anaemia” and "moderate/severe anaemia". We fitted an ordered logistic regression model to predict the likelihood of having severe/moderate anaemia versus no anaemia. We included pregnancy status as a predictor of the outcome and controlled the effect of other covariates in the analysis. Results: the study included 236 HIV positive patients, of which half (n=118, 50%) were pregnant. At baseline, about (n=143, 60%) of patients were anaemic. The proportion of pregnant women classified as anaemic (anaemia, moderate/severe) differed significantly (p=0.02) from that of non-pregnant women. The following characteristics were significantly associated with anaemia at baseline: Body mass index (BMI) category (p=0.01); World Health Organization (WHO) stage (p=0.001) and CD4 count (p=0.001). Seven months after initiation of treatment, the proportion of HIV positive women with anaemia decreased significantly. Conclusion: anaemia is a significant risk factor for untoward health outcomes, especially among HIV-positive pregnant women. Early ART access might result in a significant decrease in anaemia in pregnancy.

Introduction

Anaemia occurs when the oxygen-carrying capacity of red blood cells is insufficient and fails to satisfy the physiologic demands of the body [1]. Most disability-adjusted life years (DALys) caused by anaemia occur in low-income countries, representing approximately 3.9% of DALYS in women in sub-Saharan Africa (SSA) [2]. Anaemia has become a common clinical manifestation in Human Immunodeficiency Virus (HIV) infection and is found in an estimated 30% of patients with HIV and as much as 75-80% of those diagnosed with Acquired Immunodeficiency Syndrome (AIDS) [3]. In 2017, the HIV prevalence in South Africa (SA) was 13.1% translating to approximately 7.9 million people [4].

The causes of anaemia in HIV infected patients are multifactorial and include the direct effects of the infection and its viral proteins, coupled with immune dysregulations in HIV infection, which can be responsible for bone marrow suppression, as well as the presence of pathogenic opportunistic infections that causes abnormalities in blood cell counts [5]. The potential causes of anaemia within the context of HIV include gastrointestinal blood loss, malignancies, bone marrow infections, deficiency of erythropoietin, immune system-mediated haemolysis and the HIV infection of the haematopoietic stem cell [6]. Independent of viral load and CD4 counts, the incidence of anaemia can influence the progression of HIV to AIDS whilst the recovery from anaemia significantly reduces the risk of death and improves the survival of those infected with HIV [6,7].

Anaemia is also common among pregnant women and studies in SA have reported an extremely high prevalence in HIV infected pregnant women with high rates of perinatal and maternal morbidity [2,7-9]. Existing evidence shows the significant difference in the incidence of anaemia among HIV infected pregnant women at booking compared to those who have already been on anti-retroviral treatment (ART) [8]. In SA, this is important since 30% of pregnant women
attending public health care facilities were reported to be HIV positive [10]. Anaemia during pregnancy is defined as a haemoglobin concentration of less than 11.0g/dl, while moderate and severe anaemia is when haemoglobin concentration less than 10.9g/dl and 7.0g/dl, respectively [11]. Globally, about 41.8% of pregnant women and 30.2% of non-pregnant women are anaemic, but in SSA, the proportion is higher with approximately 57% of women being anaemic [12,13]. The saving mothers report (2010-2013) states that 40% of maternal deaths in SA between 2010 and 2013 were related to anaemia [14]. Interventions to overcome or combat iron deficiency according to the South African National Department of Health Guidelines for Maternity Care (2015) recommend the oral intake of 200mg of ferrous sulphate tablets twice daily and 5mg of folic acid once daily [15].

Some recent studies conducted in Kwa-Zulu Natal, SA, demonstrated a much higher prevalence of anaemia in HIV positive pregnant women than in their HIV negative counterparts [6,8,16-22]. CD4 cell count remained a significant risk factor for anaemia among pregnant women pre and postnatally, after adjusting for age, ART regimen and gravidity [18]. The severity of the cases differed between studies, however most of the respondents presented with anaemia or moderate anaemia while the occurrence of severe anaemia was rare [8,16-22]. Anaemia during pregnancy can have negative maternal and neonatal outcomes such as increased risk of delivering low birth weight (LBW) infants as well as a higher risk of fetal anaemia [8,23]. In the mother, anaemia can cause decreased physical and mental capacity, reduced tolerance to infections and maternal mortality because of anaemic heart failure. In the context of HIV, iron deficiency and maternal anaemia are independent markers of disease progression and mortality [18,23-25]. Although studies to determine the incidence and predictors of anaemia in HIV infected patients receiving ART have been conducted at Themba Lethu Clinic, there is, however, limited data on anaemia in HIV infected pregnant women. This study is one of the few that have investigated maternal anaemia among the HIV infected pregnant women receiving treatment at the largest public sector HIV treatment site in SA.

Methods

Study design: we analyzed secondary data of all HIV-positive women receiving ART at Themba Lethu Clinic, Helen Joseph Hospital in Johannesburg, South Africa. We included data of patients enrolled between 1st April 2004 (baseline) to 30th April 2011 (end-line) and tracked anaemia among those patients seven months later. The Themba Lethu Clinic is the largest public sector ART site in the country [26].

Study population: our study included retrospective records for 236 HIV positive women aged 18-49 years on ART. Of these 118 were pregnant and 118 were non-pregnant HIV positive women. Patients included had the following parameters “baseline CD4 count, haemoglobin measurements and at least 7 months of follow-up time”. Both pregnant and non-pregnant study participants were offered similar first line ART regimens comprised of fixed dose combinations of stavudine (D4T), tenofovir (TDF), efavirenz (EFV), zidovudine (AZT), lamivudine (3TC) and nevirapine (NVP). Specific triple therapy regimens included D4T/3TC/NVP; D4T/3TC/EFV; 3TC/TDF/EFV; 3TC/TDF/NVP; and AZT/3TC/NVP.

Study variables: the outcome variable was anaemia. This was defined as the haemoglobin concentration as per the Demographic and Health Survey (DHS) [27]. Guidelines "severe anaemia (<7.0g/dl), moderate anaemia (7.0 - 9.9g/dl), anaemia (10.0 - 10.9g/dl)". This allowed us to have a more flexible definition that considers the pregnancy status of half of the study participants. We combined moderate and severe anaemia into one single category because there were very few patients classified as having severe anaemia; either at baseline (n=7) or at end-line (n=2). The analyses took into account the sociodemographic characteristics of the patients i.e. “age” (in
complete years), pregnancy status (Y/N), education (no education, primary or just literate, secondary and beyond), smoking status (Y/N), alcohol consumption (Y/N) and employment status (employed/unemployed). Clinical and anthropometric measurements included body weight and height, which were used to compute a body mass index (BMI). We categorized BMI into the following using the Centers for Disease Control and Prevention (CDC) cut-off points: underweight: BMI <18.5, normal: BMI 18.5-24.5, overweight: BMI 25-29.9, obese: BMI ≥30. Furthermore, we included patients’ “CD4 count at baseline WHO stage of HIV disease at baseline and 7 months follow-up time after initiation of treatment”.

**Statistical analysis:** we performed data analysis using STATA 15. Patient’s demographics and clinical characteristics at baseline and end-line were described using percentages and frequencies for all categorical data. Means and standard deviation were used for all continuous variables. Given that our primary exposure was pregnancy and the two groups were balanced with respect to age and year of ART initiation, we also created a dichotomous variable for anaemia coded 1 for patients with any type of anaemia and 0 if otherwise. We then used the McNemar’s test to investigate the association between overall anaemia status (dichotomous) between the two data points (baseline and exit). For other categorical variables with more than 2 categories, we used Pearson Chi-square test to document any association between those characteristics and different levels of anaemia. An analysis of variance (one-way ANOVA) was used to test for equality of mean ‘CD4 count at baseline and end-line’ across the three levels of anaemia. A small proportion of patients had item missing data on CD4 count (n=7 at baseline and n=49 at end-line) and BMI (n=22 at baseline, n=36 at end-line). These item-missing values were replaced by the mean values in the series to reduce non-response bias. A sensitivity analysis was conducted with complete case analysis and we found no evidence that the mean imputation introduced a bias to the estimates. We used an ordered logistic regression model to predict the likelihood of these patients being in the higher versus the lower category of anaemia levels as a function of selected covariates, using pregnancy as the primary exposure variable. The final model excluded 10 patients with unknown status on history of alcohol use. Results from Brant test showed no violation of the proportional odds assumption.

**Ethical approval:** ethical clearance was obtained from Monash University Human Research Ethics Committee (certificate number 2016-0696).

**Results**

At baseline, the two groups of HIV-positive female patients were similar in every aspect except for their age. Nearly 75% of the patients were in the age group (25-34 years); 80% and 68% of the pregnant and non-pregnant women respectively, being in this age category (Table 1). There were 143 HIV positive women classified as anaemic of which 56 had having moderate/severe anaemia and 87 had anaemia. Using McNemar test for marginal frequency, we found that the proportion of pregnant women classified as anaemic (anaemia, moderate/severe) at baseline (60.6%, n=143) was statistically different (p=0.001) from that of anaemic women at end-line (36.0%, n=85 patients). Of the 236 participants; 7(3.0%) were categorized as severe anaemia at baseline. These were combined with patients classified as having moderate anaemia and we found that more than half of the patients were either anaemic (36.9%) or had moderate/severe (23.7%) anaemia at baseline. Patients differed significantly at baseline with respect to the following characteristics: BMI categories (p=0. 01); WHO stage (p=0.001) and CD4 count (p-value: 0.001). For instance, at baseline the CD4 count of patients were relatively low among anaemic patients (mean: 122.7; sd: 95.9) whereas non-anemic patients had on average higher CD4 count (mean: 189.8; sd: 161.5). Anaemia was higher among underweight women and those in the normal BMI range as opposed to overweight and obese patients (Table 2).
At end-line (after seven months of treatment), the number of women with moderate/severe anaemia decreased significantly from 56 at baseline to 23. In addition, the number of HIV positive women with anaemia decreased from 87 at baseline to 62 seven months after starting treatment. Furthermore, the average CD4 count of women with moderate/severe anaemia increased from 124.1 to 287.3. However, across the three groups (non-anaemic, anaemia and moderate/severe anaemia) there was no statistically significant difference in CD4 count (Table 3). In the ordered logistic regression model, we found no association between anaemia status (anaemia or severely/moderately anaemic) and pregnancy status of the patients at baseline and seven months later. However, among patients with WHO stage 3 HIV disease at baseline, the odds of moderate/severe anaemia or anaemia versus no anaemia was 2.64 (95% C.I: 1.35-5.15) compared to patients with WHO stage 1. Among patients with secondary/tertiary education, the odds of moderate/severe anaemia versus anaemia or no anaemia was 0.47 (95% C.I: 0.22-1.00) lower than patients with no education. At end-line, there was no statistically significant factor associated with anaemia of any grade (Table 4).

Discussion

The prevalence of anaemia was 60.6% at baseline and 36.0% after seven months of follow-up, showing a decline in the proportion of anaemic status with treatment. Previous studies have shown that prevalence of anaemia was higher among ART naive HIV positive pregnant women and declined once ART was initiated [27-29]. Such findings highlight the indirect positive effect of ART on anaemia among HIV positive pregnant women due to among other things immune recovery and improved general wellbeing. This highlights the need for continued scale up of ART coverage among HIV positive women in the population. We aimed to document the effect of pregnancy on anaemia among HIV patients. Using a dummy definition of anaemia (‘1’ for any level of anaemia and ‘0’ for no anaemia), we found no evidence that pregnancy was an aggravating factor for anaemia among study participants at baseline (OR: 0.94; 95% C.I: 0.56-1.61). At end-line, there is evidence that pregnant women were less likely to have higher levels of anaemia. However, this observed difference was not statistical significant (OR: 1.11; 95%: 0.60-2.06). Other previous studies have however reported pregnancy as a risk factor for clinical anaemia and that pregnant women had a 1-3 fold increased risk of developing anaemia than their non-pregnant counterparts [30]. Data suggests that ART had a positive indirect effect on anaemia among HIV positive patients. Even though our data could not establish an association between anaemia in HIV positive pregnant versus HIV positive non-pregnant women, the observed absence of a relationship might be due to existing differences in age distribution of our study population.

The study Banerjee et al. referenced above had a much younger sample of women and predominantly of the age group of 10-20 years was significantly associated with the development of anaemia in pregnant women [30]. The age range of our participants was (18 - 49 years) however, approximately 75% of the patients in their middle ages (25 - 34 years). The relationship between a woman’s age and anaemia is widely reported and suggests that there is an association between anaemia and younger age particularly in adolescent girls and young women [31]. The risk of anaemia increases when adolescent girls and young women become pregnant [32]. Anaemia in this age group is often attributed to iron deficiency. Iron is essential in the development of a woman, especially during puberty, as this period of rapid growth often requires more iron to meet the physiological needs, coupled with blood loss during menstruation [33]. While it is well established that anaemia is a common disorder during pregnancy, our findings imply that pregnancy alone, as a determinant of anaemia is variable and that risk factors of anaemia are multifactorial. Therefore, a greater understanding of this association may play an important role in
the management of clinical anaemia in pregnancy especially among those who are HIV infected. At baseline, our study showed that WHO stage, BMI category, education and CD4 count were significant predictors of anaemia. Patients with advanced HIV disease (WHO stage >2) were more likely to be in the higher category of anaemia compared with patients who did not have advanced HIV disease. However, after seven months of treatment, this difference was nonexistent suggesting that the observed difference in the likelihood of developing anaemia existed regardless of WHO stage, as long as the patient was on ART.

This is consistent with similar studies that suggest ART helps reverse clinical anaemia among HIV positive patients [33,34]. Other studies have suggested that anaemia is also a frequent complication among patients with low vitamin D status, iron deficiency, helminth infection, malaria infection and co-infection with Tuberculosis (TB) [35-38]. Although our study attributes the resolution of anaemia to ART use, we did not determine whether the presence of anaemia was a result of iron deficiency or of haematinic supplements in pregnancy. As such, we could not rule out the possibility of the positive effect of haematinic supplementation contributing to the resolution of anaemia among HIV positive pregnant women. Education was a key predictor of anaemia at baseline. The relationship between education and women’s health has widely been documented [39].

Education improves maternal and child health outcomes through different catalytic pathways. Similarly, we found that women with higher education were less likely to have anaemia at any stage of HIV disease compared with women with no or low education. However, as previously reported women in urban areas, with higher education attainment, were more likely to have anaemia [40]. Our findings suggest a different relationship, especially because our study setting was in an urban area. Better-educated women in society are more likely to be employed and be of a higher socio-economic status, have economic means to have regular meals, sustain a balanced diet and have better access to health care. Other previous studies have demonstrated the way in which anaemia interacts with socio-economic class and have reported higher prevalence of anaemia in patients of low ‘socio-economic status, educational level’, reduced meal frequency and residing in impoverished rural locations [41-43]. Our study had one main limitation. We did not assess the effect of other medications that the patients took on anaemia; such other medications might have had an effect on haemoglobin concentration or might have positively or otherwise influenced the patient’s compliance to taking haematinic supplementation.

Conclusion

Our study has shown that ART use in HIV infected pregnant women with anaemia improves haemoglobin concentration regardless of baseline CD4 count or WHO stage of HIV disease within the first 7 months of ART usage. This supports the evidence that suggests that initiation and adherence to ART enhances the resolution of anaemia in HIV infected individuals regardless of the degree of immunosuppression at initiation. In view of the these findings it is important to: encourage universal access to ART among HIV positive patients, especially pregnant women; identify efforts to prevent anaemia in vulnerable people within the national HIV programs; have more frequent routine testing for anaemia in women especially those made vulnerable to the condition by HIV infection; enforce interventions and treatment efforts to prevent anaemia among those at higher risk by employing a multidisciplinary approach from a health care provider perspective including where possible, involving the services of a nutritionist.

What is known about this topic

- Anaemia among women of childbearing age is a common condition in developing countries and complicates outcomes of pregnancy;
The presence of anaemia among HIV infected individuals generally contributes to the progression of HIV infection to advanced disease.

What this study adds

- Anaemia is a significant risk factor for poor health outcomes, especially among HIV positive pregnant women;
- The proportion of HIV positive women with anaemia decreased significantly, as such, ART access certainly shows to have a significant impact in reducing the intensity of anaemia in pregnancy;
- Monitoring HIV positive pregnant women is crucial to ensure that they have access to ART at an early stage.

Competing interests

The authors declare no competing interests.

Authors' contributions

Jewelle Methazia and Peter Nyasulu conceived and designed the study; Emery Ngasamana analysed the data; Jewelle Methazia, Emery Ngasamana and Peter Nyasulu wrote the paper; Jewelle Methazia, Modupe Ogunrombi, Emery Ngasamana, Wells Utembe and Peter Nyasulu reviewed the paper for critical intellectual content. All the authors have read and agreed to the final manuscript.

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Tables

Table 1: baseline characteristics of participants, by pregnancy status
Table 2: baseline characteristics of the study participants
Table 3: end-line characteristics of study participants
Table 4: ordered logistic regression model for prediction of anaemia (no-anaemia, mild anaemia, moderate/severe anaemia)

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### Table 1: baseline characteristics of participants, by pregnancy status

| Characteristics         | Non-pregnant N=118 (n/N) % | Pregnant N=118 (n/N) % | p-value |
|-------------------------|----------------------------|------------------------|---------|
| **Age category**        |                            |                        |         |
| <25                     | 12 (10.2)                  | 14 (11.9)              | 0.008   |
| 25-34                   | 80 (67.8)                  | 95 (80.5)              |         |
| 35-49                   | 26 (22.0)                  | 9 (7.6)                |         |
| **BMI category**        |                            |                        |         |
| Underweight             | 17 (14.5)                  | 15 (12.7)              | 0.35    |
| Normal                  | 74 (63.3)                  | 68 (57.6)              |         |
| Overweight              | 19 (16.2)                  | 20 (17.0)              |         |
| Obese                   | 7 (6.0)                    | 15 (12.7)              |         |
| **Alcohol status**      |                            |                        |         |
| Non-consumer            | 102 (90.3)                 | 101 (89.4)             | 0.83    |
| Consumer                | 11 (9.7)                   | 12 (10.6)              |         |
| **WHO stage**           |                            |                        |         |
| 1                       | 51 (43.2)                  | 57 (48.3)              | 0.86    |
| 2                       | 24 (20.4)                  | 20 (17.0)              |         |
| 3                       | 32 (27.1)                  | 31 (26.3)              |         |
| 4                       | 11 (9.3)                   | 10 (8.4)               |         |
| **Education**           |                            |                        |         |
| No education            | 25 (21.2)                  | 17 (14.4)              | 0.16    |
| Primary or literate     | 7 (5.9)                    | 14 (11.9)              |         |
| Secondary and beyond    | 86 (72.9)                  | 87 (73.7)              |         |
| **Occupation**          |                            |                        |         |
| Disabled or unknown     | 8 (6.8)                    | 5 (4.2)                | 0.48    |
| Employed                | 52 (44.1)                  | 47 (39.9)              |         |
| Unemployed              | 58 (49.1)                  | 66 (55.9)              |         |
| **Smoking status**      |                            |                        |         |
| Non-smoker              | 112 (94.9)                 | 111 (94.1)             | 0.78    |
| Smokers                 | 6 (5.1)                    | 7 (5.9)                |         |
| CD4 count (mean, sd.)   | 142.26 (116.8)             | 156.72 (139.5)         | 0.39*   |

Note: p-value refer to Pearson Chi-square test, unless otherwise indicated; *p-value refers to independent t-test
Table 2: Baseline characteristics of the study participants

| Characteristics          | Severe/moderate anemia N=56 (n/N) % | Anemia N=87 (n/N) % | No anemia N=93 (n/N) % | p-value |
|--------------------------|------------------------------------|---------------------|------------------------|---------|
|                         |                                     |                     |                        |         |
| **Pregnancy status**     |                                     |                     |                        |         |
| Non-pregnant             | 30 (53.6)                           | 42 (48.3)           | 46 (49.5)              | 0.82    |
| Pregnant                 | 26 (46.4)                           | 45 (51.7)           | 47 (50.5)              |         |
| **Age category**         |                                     |                     |                        |         |
| <25                      | 4 (7.1)                             | 13 (15.0)           | 9 (9.7)                | 0.37    |
| 25 - 34                  | 41 (73.2)                           | 65 (74.7)           | 69 (74.2)              |         |
| 35 - 49                  | 11 (19.7)                           | 9 (10.3)            | 15 (16.1)              |         |
| **BMI category**         |                                     |                     |                        |         |
| Underweight              | 15 (27.8)                           | 12 (13.8)           | 5 (5.4)                | 0.01*   |
| Normal                   | 31 (55.4)                           | 56 (64.4)           | 55 (59.8)              |         |
| Overweight               | 6 (9.7)                             | 13 (14.9)           | 20 (21.7)              |         |
| Obese                    | 4 (7.1)                             | 6 (6.9)             | 12 (13.1)              |         |
| **Alcohol status**       |                                     |                     |                        |         |
| Non-consumer             | 51 (92.7)                           | 75 (91.5)           | 77 (86.5)              | 0.46*   |
| Consumer                 | 4 (7.3)                             | 7 (8.5)             | 12 (13.5)              |         |
| **WHO stage**            |                                     |                     |                        |         |
| 1                        | 14 (25.0)                           | 41 (47.1)           | 53 (57.0)              | 0.001***|
| 2                        | 8 (14.3)                            | 16 (18.4)           | 20 (21.5)              |         |
| 3                        | 25 (44.6)                           | 24 (27.6)           | 14 (15.0)              |         |
| 4                        | 9 (16.1)                            | 6 (6.9)             | 6 (6.5)                |         |
| **Education**            |                                     |                     |                        |         |
| No education             | 14 (25.0)                           | 15 (17.2)           | 13 (14.0)              | 0.40    |
| Primary or literate      | 4 (7.1)                             | 10 (11.5)           | 7 (7.5)                |         |
| Secondary & beyond       | 38 (67.9)                           | 62 (71.3)           | 73 (78.5)              |         |
| **Occupation**           |                                     |                     |                        |         |
| Disabled or unknown      | 1 (1.8)                             | 4 (4.6)             | 8 (8.6)                | 0.49*   |
| Employed                 | 23 (41.1)                           | 39 (44.8)           | 37 (39.8)              |         |
| Unemployed               | 32 (57.1)                           | 44 (50.6)           | 48 (51.6)              |         |
| **Smoking status**       |                                     |                     |                        |         |
| Non-smoker               | 53 (94.6)                           | 81 (93.1)           | 89 (95.7)              | 0.77*   |
| Smokers                  | 3 (5.4)                             | 6 (6.9)             | 4 (4.3)                |         |
| CD4 count (mean, sd.)    | 124.1 (90.1)                        | 122.7 (95.9)        | 189.8 (161.5)          | 0.001*** |

Note: p-values from Pearson Chi-square test, unless otherwise indicated. *p > 0.05. **p < 0.01. ***p < 0.001
Table 3: end-line characteristics of study participants

| Characteristics       | Anemia Status at end-line |          |          |          |          |          |
|-----------------------|----------------------------|----------|----------|----------|----------|----------|
|                       | Severe/moderate N=23 (n/N) % | Anemia N=62 (n/N) % | No anemia N=151 (n/N) % | p-value   |          |
| Pregnancy status      |                            |          |          |          |          |          |
| Non-pregnant          | 14 (60.9)                  | 30 (48.4) | 74 (49.0) | 0.55      |          |
| Pregnant              | 9 (39.1)                   | 32 (51.6) | 77 (51.0) |            |          |
| Age category          |                            |          |          |          |          |          |
| <25                   | 2 (8.7)                    | 10 (16.1) | 14 (9.3)  | 0.69\(^f\) |          |
| 25 - 34               | 18 (78.3)                  | 44 (67.4) | 114 (75.5) |            |          |
| 35 - 49               | 3 (13.0)                   | 9 (14.5)  | 23 (15.2) |            |          |
| BMI category          |                            |          |          |          |          |          |
| Underweight           | 7 (30.4)                   | 10 (16.1) | 15 (10.0) | 0.23\(^f\) |          |
| Normal                | 13 (56.5)                  | 35 (56.0) | 94 (62.7) |            |          |
| Overweight            | 2 (8.7)                    | 12 (19.4) | 25 (16.6) |            |          |
| Obese                 | 1 (4.4)                    | 5 (8.1)   | 16 (10.7) |            |          |
| Alcohol status        |                            |          |          |          |          |          |
| Non-consumer          | 21 (95.5)                  | 52 (89.7) | 130 (89.0) | 0.84\(^f\) |          |
| Consumer\(^1\)        | 1 (4.5)                    | 6 (10.3)  | 16 (11.0) |            |          |
| WHO stage             |                            |          |          |          |          |          |
| 1                     | 9 (39.1)                   | 30 (49.4) | 69 (45.7) | 0.63\(^f\) |          |
| 2                     | 6 (26.1)                   | 12 (19.3) | 26 (17.2) |            |          |
| 3                     | 5 (21.8)                   | 13 (20.0) | 45 (29.8) |            |          |
| 4                     | 3 (13.0)                   | 7 (11.3)  | 11 (7.3)  |            |          |
| Education             |                            |          |          |          |          |          |
| No education          | 4 (17.3)                   | 13 (21.0) | 25 (16.6) | 0.41\(^f\) |          |
| Primary or literate   | 2 (8.7)                    | 2 (3.2)   | 17 (11.2) |            |          |
| Secondary and beyond  | 17 (74.0)                  | 47 (75.8) | 109 (72.2) |            |          |
| Occupation            |                            |          |          |          |          |          |
| Disabled or unknown   | 0 (0.0)                    | 3 (4.8)   | 10 (6.6)  | 0.72      |          |
| Employed              | 9 (39.1)                   | 23 (37.1) | 67 (44.4) |            |          |
| Unemployed            | 14 (60.9)                  | 36 (58.1) | 74 (49.0) |            |          |
| Smoking status        |                            |          |          |          |          |          |
| Non-smoker            | 21 (91.3)                  | 58 (93.6) | 144 (95.4) | 0.68      |          |
| Smokers               | 2 (8.7)                    | 4 (6.4)   | 7 (4.6)   |            |          |
| CD4 count (mean, sd.) | 287.3 (89.0)               | 258.6 (120.5) | 280.6 (150.4) | 0.52      |          |

Note: p-values from Pearson Chi-square test, unless otherwise indicated. \(^f\): p-value from Fisher exact test. \(^p\): p-value from one-way Anova assuming equal variance. \(\dagger\): current or past

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Table 4: ordered logistic regression model for prediction of anaemia (no-anaemia, mild anaemia, moderate/severe anaemia)

| Characteristics     | Baseline model                          | End-line model                        |
|---------------------|-----------------------------------------|---------------------------------------|
|                     | Moderate/severe & anemia vs. no anemia  | moderate/severe & anemia vs. no anemia|
| Pregnancy status    |                                         |                                       |
| Non-pregnant        | Ref                                     | Ref                                   |
| Pregnant            | 0.94 (0.56 - 1.61)                      | 1.11 (0.60 - 2.06)                    |
| Age category        |                                         |                                       |
| <25                 | Ref                                     | Ref                                   |
| 25 - 34             | 1.26 (0.55 - 2.88)                      | 0.78 (0.34 - 1.70)                    |
| 35 - 49             | 0.99 (0.35 - 2.79)                      | 0.72 (0.25 - 2.08)                    |
| BMI category        |                                         |                                       |
| Underweight         | Ref                                     | Ref                                   |
| Normal              | 0.43 (0.19 - 0.96) *                    | 0.54 (0.17 - 1.70)                    |
| Overweight          | 0.22 (0.08 - 0.60 **)                   | 0.42 (0.14 - 1.32)                    |
| Obese               | 0.24 (0.08 - 0.76) *                    | 0.36 (0.10 - 1.33)                    |
| Alcohol status      |                                         |                                       |
| Non-consumer        | Ref                                     | Ref                                   |
| Consumer            | 0.54 (0.21 - 1.40)                      | 0.51 (0.18 - 1.47)                    |
| WHO stage           |                                         |                                       |
| 1                   | Ref                                     | Ref                                   |
| 2                   | 1.13 (0.55 - 2.33)                      | 1.78 (0.86 - 3.67)                    |
| 3                   | 2.64 (1.35 - 5.15) **                   | 0.86 (0.37 - 2.00)                    |
| 4                   | 2.54 (0.95 - 6.75)                      | 1.94 (0.62 - 6.11)                    |
| Education           |                                         |                                       |
| No education        | Ref                                     | Ref                                   |
| Primary or literate | 0.51 (0.17 - 1.49)                      | 0.40 (0.11 - 1.53)                    |
| Secondary and higher| 0.47 (0.22 - 1.00) *                   | 0.76 (0.34 - 1.66) *                  |
| Occupation          |                                         |                                       |
| Disabled or unknown | Ref                                     | Ref                                   |
| Employed            | 3.68 (0.72 - 18.85)                     | 3.30 (0.38 - 28.87)                   |
| Unemployed          | 3.28 (0.65 - 16.63)                     | 4.17 (0.48 - 35.80)                   |
| Smoking status      |                                         |                                       |
| Non-smoker          | Ref                                     | Ref                                   |
| Smoker              | 1.60 (0.48 - 5.27)                      | 1.95 (0.55 - 6.98)                    |
| CD4 Count (mean, sd.)| 1.00 (0.995 - 1.00)                  | 1.00 (1.00 - 1.001)                  |

†p.10. *p .05. **p.01. ***p.001