

## ORIGINAL RESEARCH ARTICLE

# Prevalence and Correlates of Maternal Anemia in Rural Sidama, Southern Ethiopia

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## Abstract

In order to assess the prevalence and correlates of prenatal anemia, a survey was conducted among 700 randomly selected pregnant women in rural Sidama, Southern Ethiopia. The prevalences of anemia, Iron Deficiency (ID) and ID anemia were 31.6%, 17.4% and 8.7%, respectively. The burden of anemia was significantly high among illiterates, women devoid of self-income, lowlanders, multiparas and women aged 25-34 years. Women who weren't on iron-folate supplementation had 1.90 (95% Confidence Interval (CI): 1.14-3.19) times increased odds of anemia. Anemia was associated with ID, zinc deficiency and elevated C-reactive protein with odds ratio of 2.46 (95% CI: 1.63-3.73), 2.29 (95% CI: 1.62-3.23) and 1.98 (95% CI: 1.12-3.47) respectively; however, it was not associated with vitamin A deficiency. Though ID was a significant correlate of anemia, only 11.8% of anemia was attributable to it. Zinc, iron and vitamin A deficiencies did not show synergistic interaction in associating with anemia. (*Afr J Reprod Health* 2014; 18[1]: 44-53).

**Keywords:** Maternal nutrition, maternal anemia, anemia in pregnancy

## Résumé

Afin d'évaluer la prévalence et les corrélats de l'anémie prénatale, une enquête a été menée auprès de 700 femmes enceintes sélectionnées au hasard à Sidama rural, au sud de l'Éthiopie. Les taux de prévalence de l'anémie, la carence en fer (CF) et l'anémie de CF étaient de 31,6 %, 17,4 % et 8,7%, respectivement. La charge de l'anémie était significativement élevée chez les analphabètes, les femmes dépourvues de revenu de soi, des plaines, multipares et les femmes âgés de 25-34 ans. Les femmes qui ne prennent pas des suppléments en fer et en folate avaient 1,90 (95 % intervalle de confiance (IC) : 1.14 à 3.19) fois des risques accrus d'anémie. L'anémie a été associée à une CF, le zinc et la protéine C-réactive avec un rapport de cotes de 2,46 (IC à 95% : 1,63 à 3,73), 2,29 (IC 95% : 1,62 à 3,23) et de 1,98 (IC 95% : 1,12 à 3,47) respectivement, mais il n'était pas associée à la carence en vitamine A. Bien que la CF soit un corrélat important de l'anémie, on ne pouvait lui en attribuer que 11,8%. Les carences en zinc, en fer et en vitamine A n'ont pas montré d'interaction synergique en s'associant à une anémie. (*Afr J Reprod Health* 2014; 18[1]: 44-53).

**Mots-clés:** nutrition maternelle, anémie maternelle, anémie pendant la grossesse

## Introduction

Anaemia is a disorder characterized by a decline in the concentration of haemoglobin (Hgb) or circulating erythrocytes and a concomitant impairment in the capacity of oxygen transportation<sup>1</sup>. Despite having diverse causes, nutritional deficiency is the prominent origin of anemia<sup>2</sup>. Iron Deficiency (ID) alone contributes to half of its burden<sup>2</sup>. Other micronutrients of significance include vitamin A, vitamin B<sub>12</sub> and folate<sup>2</sup>.

Anaemia is a global public health problem affecting two billion people worldwide.

Particularly preschool-age children and women of reproductive age take the disproportionate burden. Globally, almost half of all preschool children (47.4%) and pregnant women (41.8%) and close to one-third of non-pregnant women (30.2%) are anemic<sup>2,3</sup>. Consequences of anaemia include increased maternal and perinatal mortality, impaired physical and cognitive development of children and reduced work productivity<sup>2</sup>.

Many studies have documented the adverse effects of maternal anemia<sup>5</sup>. According to World Health Organization (WHO) 12.8% and 3.7% of maternal mortality in Asia and Africa respectively are directly attributable to anemia<sup>4</sup>. Further, ID is

the underlying cause for 22% of maternal death worldwide<sup>6</sup>. A meta-analysis showed that the risk of maternal mortality can be reduced by 20% for each 1 g/dl increase in Hgb concentration<sup>6</sup>. Other consequences of maternal anaemia include prematurity and low birth weight<sup>5</sup>.

In Ethiopia, reasonable number of national level surveys determined the prevalence of anaemia in women of reproductive age and came up with figures ranging from 16.6 to 30.4%<sup>7-10</sup>. Concerning pregnant women, two large scale surveys reported 30.6%<sup>8</sup> and 18.4%<sup>11</sup> prevalences. Small scale studies in Awassa<sup>12</sup> and Jimma<sup>13</sup> towns also found 15.1% and 38.2% prevalences. All the studies consistently witnessed the public health significance of anaemia in the country.

In the developing world several studies attempted to identify risk factors of maternal anemia<sup>14-19</sup>. Yet they end up in divergent and equivocal conclusions. Thus, this study was conducted to assess the prevalence and potential correlates of maternal anaemia in Rural Sidama, Southern Nations, Nationalities and Peoples Region (SNNPR), Ethiopia, where a previous study reported a seriously high prevalence of anemia<sup>20</sup>.

## Method

### Study design

The study used a community based, cross-sectional, quantitative design with descriptive and analytic components.

### Setting

The study was carried out between January and February 2011 in 18 rural kebeles of Sidama zone, which is one of the 15 zones of SNNPR<sup>21</sup>. A kebele is the smallest administrative unit in Ethiopia that comprises roughly 1000 households. In Sidama, of the total population nearing three million, 95% dwells in rural areas<sup>21</sup>. Nearly half of the population lives in the midlands (1750 to 2300 m above sea level (ASL)); whereas 30% and 20% dwell in the highlands (>2300 m ASL) and lowlands (< 1750 m ASL), respectively<sup>22</sup>. About 85% of the population depends upon subsistent

farming<sup>16</sup>. Principal crops grown are *Enset* (*Enset ventricosum*), coffee and maize<sup>22</sup>.

### Sample size

In order to determine the prevalence of anaemia, an adequate sample size of 696 pregnant women was computed using single proportion formula. The computation was made at 95% confidence level, 5% margin of error, 29% expected prevalence of anemia<sup>20</sup>, 10% non-response rate and design effect of 2. However, with the intention of maximizing the sample size for the analytic component of the study, 750 pregnant women were recruited.

Using double proportion sample size formula, the available sample size (221 anaemic and 479 normal subjects) was judged to be sufficient for identifying key correlates of anaemia. The computation was made using the Epi Info statistical package with the inputs of 95% confidence level, 80% power and 1:2 ratio between anaemic and normal subjects. Expected prevalences of exposures in non-anaemic subjects and Odds Ratio (OR) for the association between the exposures and anaemia were taken from studies conducted in the country<sup>24</sup> and elsewhere<sup>25</sup>.

### Sampling technique

Initially all kebeles in the zone were listed and stratified into the three agro-ecological zones: lowlands, midlands and highlands. The total sample size was allotted to the strata proportionally to their population size (20%, 50% and 30% respectively). From each stratum, 6 kebeles were selected at random. In every stratum, the sample size was distributed to the kebeles proportional to their population size. Eventually 750 subjects were selected using systematic random sampling technique. The sampling frame for pregnant women was developed through a rapid house-to-house enumeration. Presumptive symptoms of pregnancy (amenorrhea and/or change in the size of the uterus) with subsequent urine test were used to diagnose pregnancy.

### Data collection method

A structured and pretested questionnaire was used for assessing the potential correlates of anaemia.

The part of the questionnaire on Dietary Diversity (DD) was adopted from a standard tool<sup>26</sup>. Other sections were developed by the Principal Investigators (PIs). The content validity of the questionnaire was assessed based on the conceptual framework of the study; whereas, its reliability was checked via test-retest method. Three trained and experienced data collectors gathered the data at the nearby health posts. Altitude was measured at the centres most locations of the kebeles using Magilan® Global Positioning System.

The DD was assessed using 24 hours recall method. Respondents were asked whether they had taken any food from predefined 12 food categories on the preceding day. Accordingly, the level of Dietary Diversity Score (DDS) was computed out of 12. Consistent with the recommendation of Food and Agriculture Organization (FAO) of the United Nations<sup>27</sup>, DD was classified as high (DDS  $\geq 6$ ), optimal (DDS = 4 or 5) and low (DDS  $\leq 3$ ).

#### ***Blood sample collection and laboratory analysis***

Venous blood was collected using plain Monovette® system and stainless steel needles. Hgb was determined using HemoCue Hb 301® instantly after sample collection. The whole-blood was clotted for 20 minutes, centrifuged at 3000×g for 10 minutes and serum was extracted following standard procedures. The samples were transported in icebox and stored frozen at -20°C until analysis. Serum zinc, retinol and ferritin concentrations were determined at Ethiopian Health and Nutrition Research Institute (EHNRI) using Varian SpectrAA® Flame Atomic Absorption Spectrometer, Shimadzu® High Performance Liquid Chromatography and Roche-Elecsys® Chemistry Analyzer, respectively. C-Reactive Protein level (CRP) was determined qualitatively using latex HumaTex®.

#### ***Data analysis***

Data entry, screening and analysis were carried out by the PIs using Statistical Package for Social Sciences (SPSS) 19.0. Serum ferritin exhibited positively skewed distribution and it was converted to the normal distribution using

logarithmic transformation. Hgb values were adjusted for altitude according to the recommendation of Centres for Disease Prevention and Control (CDC)<sup>28</sup>. Independent t-test and one-way Analysis of Variance (ANOVA) were used to compare Hgb concentration across categories of the independent variables.

Principal Component Analysis (PCA) was applied in the computation of wealth index. Wealth index was calculated as a composite indicator of living standard based on 23 variables related to ownership of selected household assets, size of agricultural land, quantity of livestock, materials used for housing construction, and ownership of improved water and sanitation facilities. The analysis yielded a summary score that explained 61.1% of variability of the data and the score was ultimately divided into 5 equal wealth quintiles (poorest, poorer, middle, richer and richest). PCA was also used for the reduction of data related to maternal workload.

Logistic regression was used to assess the association between the dependent and independent variables and to control confounders. Linear regression model was not favoured as its r-squared value was extremely low. In the regression analysis, the independent variables were entered in three distinct models – distal, intermediate and proximate. The distal model comprised agro-ecology and socio-demographic factors. The intermediate model encompassed various dietary and health care related factors; whereas, the proximate model includes micronutrient deficiencies, Middle Upper Arm Circumference (MUAC), gestational age and CRP level. Independent variables that turned out to be significant ( $P < 0.05$ ) in simple regression models were considered for the multivariate analysis. Model fitness was assessed using the Hosmer-Lemeshow statistic. The major assumptions of t-test, ANOVA, PCA and logistic regression were checked to be satisfied.

Potential interaction of micronutrients in associating with anaemia was measured on additive scale. Additive scale is preferred as it is known to have better correlation with biological interaction<sup>29,30</sup>. As suggested by Kalilani and Atashili<sup>31</sup>, Synergy Index (SI) was calculated by substituting OR for the risk ratio in the original

Rothman's formula. The 95% CI for SI was computed according to the recommendation of Hosmer and Lemeshow<sup>32</sup>.

### Ethical Considerations

The study was conducted in confirmation of national and international ethical guidelines for biomedical research involving human subjects. Ethical clearance was secured from the institutional review board of Addis Ababa University. Informed written consent was taken from the study subjects. Nutrition education was provided to all subjects and anaemic women were given iron-folate supplements.

## Results

### Background information about study subjects

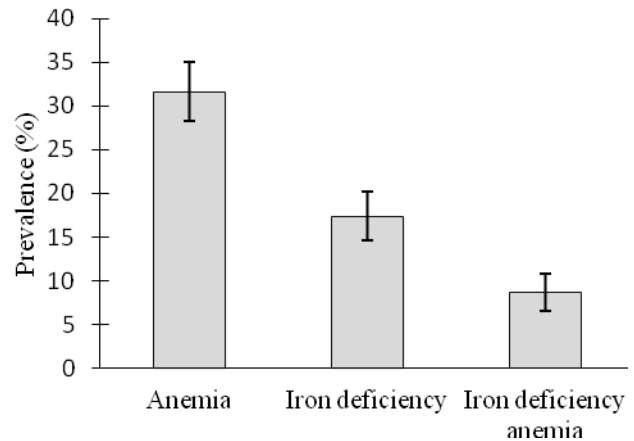
Of 750 pregnant women recruited for the study, 700 (93.1%) volunteered to participate. The altitude of the kebeles included in the study ranged from 1584 to 2763 meters ASL. The mean age ( $\pm$  standard deviation) of the respondents was 28.5 ( $\pm$ 5.5) years. Regarding gestational age, 50 (7.2%), 347 (49.6%) and 303 (43.3%) were in the first, second and third trimesters, respectively. About 462 (66.0%) of the respondents were illiterates and 561 (80.1%) were housewives. The vast majority – 679 (97.0%) and 596 (85.1%) – were Sidama in ethnicity and Protestant Christians in religion, respectively.

### Prevalence of anaemia

The mean Hgb concentration was 11.4 ( $\pm$ 1.2) g/dl (95% CI: 11.3-11.5 g/dl). The concentrations for the first, second and third trimesters were 11.8 ( $\pm$ 1.4), 11.4 ( $\pm$ 1.2) and 11.3 ( $\pm$ 1.2) g/dl respectively ( $F=4.686$ ,  $P=0.010$ ). In overall, the prevalence of anaemia (Hgb < 11.0 g/dl in the first or third trimester or < 10.5 g/dl in the second trimester<sup>33</sup>) was 31.6% (95% CI: 28.2-35.0%). The prevalences of mild, moderate (Hgb < 9.9 g/dl<sup>33</sup>) and severe (Hgb < 7.0 g/dl<sup>33</sup>) anaemia were 20.4, 10.4 and 0.7%, respectively.

The median serum ferritin concentration was 29.0 mg/l. The concentration for the first, second and third trimesters were 37.3, 31.4, 26.6 mg/l,

respectively. The variation was significant ( $F=6.922$ ,  $P=0.001$ ). About 17.4% (95% CI: 14.6-20.2%) of the subjects had ID (serum ferritin < 15 mg/l<sup>34</sup>) and 8.7% (95% CI: 6.6-10.8%) had iron deficiency anaemia (anaemia with serum ferritin < 15 mg/l<sup>35</sup>) (Figure 1).



**Figure 1:** Prevalences (with 95% CI) of anaemia, iron deficiency and iron deficiency anaemia among pregnant women in Rural Sidama, Southern Ethiopia.

### Correlates of anaemia

#### Socio-demographic correlates of anaemia

Mean Hgb levels for women aged 15-24, 25-34 and 35-49 years were 11.7 ( $\pm$ 1.1), 11.3 ( $\pm$ 1.2) and 11.4 ( $\pm$ 1.2) g/dl, respectively. The global difference was significant and post-hoc test identified significantly lower level in the middle age category compared to the youngest group ( $F=6.561$ ,  $P=0.002$ ). Likewise, in the logistic model the odds of anaemia in women aged 25-34 years were 1.82 (95% CI: 1.14-2.90) times higher than the youngest group.

Low maternal socio-economic status was deleteriously associated with Hgb status. The Hgb in illiterates (11.3  $\pm$ 1.2 g/dl) was significantly lower than literates (11.5  $\pm$ 1.2 g/dl) ( $t=2.177$ ,  $P=0.030$ ). The odds of anaemia were also 1.68 (95% CI: 1.14-2.48) times raised in the earlier group. Similarly, the concentration in women devoid of self-income (11.3  $\pm$ 1.2 g/dl) was lower than their counterparts (11.6  $\pm$ 1.2 g/dl) ( $t=2.531$ ,  $P=0.012$ ). After adjusting for statistically

significant socio-demographic variables, the odds were 1.60 times increased (95% CI: 1.08-2.37) in women devoid of self-income. Nevertheless, Hgb level and the odds of anaemia were comparable across the wealth index categories ( $F=1.136$ ,  $P=0.339$ ).

As altitude increases, the odds of anaemia tend to decline. The Hgb levels (adjusted for altitude) among respondents from the highlands, midlands and lowlands were 11.5 ( $\pm 1.2$ ), 11.4 ( $\pm 1.1$ ) and 11.1 ( $\pm 1.3$ ) g/dl, respectively. The variation was significant ( $F=7.111$ ,  $P=0.001$ ). In the multivariate model, the odds were 2.03 (95% CI: 1.27-3.24) times elevated in the lowlands compared to the highlands.

#### ***Dietary, parity and health care related correlates of anemia***

There was a significant negative association between parity and mean Hgb concentration ( $F=3.832$ ,  $P=0.010$ ). The Hgb concentration declined from 11.7 ( $\pm 1.3$ ) g/dl in nulliparas to 11.2 ( $\pm 1.3$ ) g/dl in grand multiparas. Compared to nulliparas, women with parity of 1-2, 3-4 and 5 or more had 2.17 (95% CI: 1.24-3.78), 2.26 (95% CI: 1.28-3.98) and 2.83 (95% CI: 1.43-5.59) times increased odds of anaemia.

The association between DD and risk of anaemia was assessed. Taking high DD group as a

reference, the odds were 1.94 (95% CI: 1.15-3.27) times elevated in the low DD category. Likewise, those who did not consume animal source foods in the preceding day of the survey had 1.64 (95% CI: 1.07-2.51) times increased odds. In the bivariate analysis, women from *enset* staple diet category, compared to maize staple diet, had better Hgb status and reduced odds of anaemia. However, in multivariate model the association failed to be significant.

About 140 (20.0%) of the respondents took iron-folate (Fe-Fol) supplement (150 mg ferrous sulphate and 500  $\mu$ g folate per day) at least once in the preceding four weeks of the survey. However, only 105 (15.0%) reported full compliance with the supplement in the reference period. Yet Fe-Fol supplementation demonstrated a beneficial effect in reducing the burden of anaemia. The Hgb in respondents who reported full compliance (11.7 $\pm$ 1.1 g/dl) was significantly superior to those who did not take any supplement (11.3 $\pm$ 1.2 g/dl) ( $t=2.912$ ,  $P=0.004$ ). The odds of anaemia were 1.90 (95% CI: 1.14-3.19) times raised in the latter group.

The Hgb level and odds of anaemia were not statistically different across variables like receiving nutrition education during pregnancy, distance from the nearby health facility, frequency of coffee intake and maternal workload during the preceding one week of the study (Table 1).

**Table 1:** Hemoglobin status across various dietary, reproductive and health care related factors of pregnant women in Rural Sidama, Southern Ethiopia

Variables	Hgb in g/dl mean ( $\pm$ sd)	Anaemia		Crude OR (95% CI)	Adjusted OR (95% CI) **
		+	-		
Parity					
0	11.7 ( $\pm 1.2$ )	22	79	1 <sup>r</sup>	1 <sup>r</sup>
1-2	11.4 ( $\pm 1.2$ )	79	167	2.32 (1.35-4.00)*	2.17 (1.24-3.78)*
3-4	11.3 ( $\pm 1.2$ )	77	128	2.59 (1.49-4.50)*	2.26 (1.28-3.98)*
$\geq 5$	11.2 ( $\pm 1.3$ )	29	40	3.06 (1.57-5.95)*	2.83 (1.43-5.59)*
Staple diet					
Enset based	11.4 ( $\pm 1.2$ )	120	279	1 <sup>r</sup>	1 <sup>r</sup>
Maize based	11.2 ( $\pm 1.2$ )	87	135	1.42 (1.02-1.98)*	1.35 (0.93-1.95)
Dietary diversity					
Low	11.2 ( $\pm 1.3$ )	91	127	2.63 (1.62-4.29)*	1.94 (1.15-3.27)*
Optimal	11.4 ( $\pm 1.2$ )	93	192	1.71 (1.06-2.77)*	1.53 (0.94-2.51)
High	11.7 ( $\pm 1.1$ )	23	95	1 <sup>r</sup>	1 <sup>r</sup>
Consumed animal source foods					
No	11.3 ( $\pm 1.2$ )	171	291	1.89 (1.27-2.80)*	1.64 (1.07-2.51)*
Yes	11.6 ( $\pm 1.2$ )	36	123	1 <sup>r</sup>	1 <sup>r</sup>
Prenatal Fe-Fol supplementation					

Yes	11.7 (±1.1)	21	75	1 <sup>f</sup>	1 <sup>f</sup>
No	11.3 (±1.2)	178	318	1.92 (1.16-3.17)*	1.90 (1.14-3.19)*
Nutrition education in pregnancy					
Yes	11.4 (±1.2)	51	107	1 <sup>f</sup>	-
No	11.4 (±1.2)	156	307	1.10 (0.76-1.59)	-
Frequency of coffee intake per day					
≤ 3 coffee cups	11.4 (±1.2)	105	215	0.95 (0.69-1.31)	-
> 3 coffee cups	11.4 (±1.2)	102	199	1 <sup>f</sup>	-
One-way walking distance from nearby health facility					
0-30 minutes	11.4 (±1.1)	172	368	1 <sup>f</sup>	1 <sup>f</sup>
Longer than 30 minutes	11.1 (±1.5)	35	46	1.56 (1.01-2.41)*	1.40 (0.89-2.21)
Maternal workload					
Lowest	11.4 (±1.2)	42	82	1 <sup>f</sup>	-
Lower	11.4 (±1.1)	34	92	0.74 (0.46-1.25)	-
Middle	11.3 (±1.1)	48	78	1.24 (0.75-2.03)	-
Higher	11.5 (±1.4)	41	83	0.99 (0.60-1.63)	-
Highest	11.3 (±1.2)	42	79	1.10 (0.69-1.82)	-

1<sup>f</sup> set as reference, \* Significant association at 95% confidence level, \*\* adjusted for all significant variables in the table

### Association of anemia with zinc, vitamin A and iron deficiencies

Hgb and the log transformed serum ferritin showed a positive correlation ( $r=0.230$ ,  $P=0.000$ ). The Hgb in iron deficient ( $10.8\pm 1.2$  g/dl) and normal ( $11.5\pm 1.2$  g/dl) subjects significantly vary ( $t=5.979$ ,  $P=0.000$ ). In the logistic model the odds of anaemia were 2.46 (95% CI: 1.63-3.73) times elevated in iron deficient subjects. Yet, among anaemic pregnant women only 27.1% (95% CI: 21.2-33.0%) had concomitant ID. Based on population fraction, merely 11.8% (95% CI: 7.5-16.0%) of anaemia was attributable to ID. Hence the aforementioned share of anaemia in the populations can be alleviated through eliminating ID.

There was also a positive correlation between Hgb and serum zinc ( $r=0.209$ ,  $P=0.000$ ). The Hgb in zinc deficient (serum zinc < 56 µg/dl in the first trimester, or < 50 µg/dl in the second or third trimester<sup>36</sup>) subjects ( $11.1\pm 1.2$  g/dl) was significantly lower than their counterparts

( $11.7\pm 1.1$  g/dl) ( $t=5.990$ ,  $P=0.000$ ). In the logistic model zinc deficiency and anaemia were associated to each other with AOR of 2.29 (95% CI: 1.62-3.23).

Likewise, correlation analysis identified a significant but weaker association between Hgb and serum retinol concentrations ( $r=0.086$ ,  $P=0.023$ ). The Hgb concentration of vitamin A deficient (serum retinol < 0.7 µmol/l<sup>37</sup>) subjects ( $11.3\pm 1.2$  g/dl) was inferior to the level found in normal subjects ( $11.5\pm 1.2$  g/dl) ( $t=2.280$ ,  $P=0.023$ ). Yet in the logistic model in which the effects of other independent variables were controlled, the association turned out to be insignificant.

Anaemia and Severe Acute Malnutrition (SAM) (MUAC < 220 mm<sup>38</sup>) tend to occur together. The correlation between MUAC and Hgb level was positive and significant ( $r=0.173$ ,  $P=0.000$ ). Pregnant women with SAM had a significantly lower Hgb ( $t=3.411$ ,  $P=0.001$ ) and 1.90 (95% CI: 1.33-2.73) times increased odds of anaemia (Table 2).

**Table 2:** Association between anaemia and various nutritional deficiencies among pregnant women in Rural Sidama, Southern Ethiopia

Variables	Hgb in g/dl mean (±sd)	Anaemia		Crude OR (95% CI)	Adjusted OR (95% CI) **
		+	-		
Zinc deficiency					
Deficient	11.1 (±1.2)	149	222	2.40 (1.72-3.35)*	2.27 (1.61-3.20)*
Normal	11.7 (±1.1)	72	257	1 <sup>f</sup>	1 <sup>f</sup>
Vitamin A deficiency					

Deficient	11.4 ( $\pm$ 1.2)	104	185	1.41 (1.02-1.95)*	1.14 (0.81-1.61)
Normal	11.3 ( $\pm$ 1.2)	117	294	1 <sup>r</sup>	1 <sup>r</sup>
Iron deficiency					
Deficient	10.8 ( $\pm$ 1.2)	60	62	2.51 (1.68-3.74)*	2.42 (1.59-3.66)*
Normal	11.5 ( $\pm$ 1.2)	161	417	1 <sup>r</sup>	1 <sup>r</sup>
Severe acute malnutrition					
Yes	11.1 ( $\pm$ 1.4)	84	108	2.11 (1.49-2.98)*	1.90 (1.33-2.73)*
No	11.5 ( $\pm$ 1.2)	137	371	1 <sup>r</sup>	1 <sup>r</sup>

1<sup>r</sup> set as reference, \* Significant association at 95% confidence level, \*\* adjusted for all variables in the table, CRP status and gestational age

### Interaction of micronutrients in associating with anaemia

The potential existence of interaction among the aforementioned three micronutrients (iron, zinc and vitamin A) in associating with anaemia was investigated. Nevertheless, synergistic interactions were not witnessed across the three possible pairs of micronutrients; zinc and iron deficiencies; vitamin A and zinc deficiencies; and vitamin A and iron deficiencies with SI of 1.19 (95% CI: 0.41-3.42), 0.80 (95% CI: 0.36-1.75) and 1.15 (95% CI: 0.30-4.37), respectively.

### Anaemia and CRP status

About 8.4% of the pregnant women had elevated serum CRP (CRP  $\geq$ 6 mg/l) level indicative of infection/inflammation. Hgb level significantly declined by 4.4% from 11.4 ( $\pm$ 1.2) g/dl in CRP negative subjects to 10.9 ( $\pm$ 1.3) g/dl in CRP positive subjects ( $t=3.061$ ,  $P=0.002$ ). In the logistic model adjusted for ferritin, zinc, vitamin A, SAM status and gestational age, elevated CRP was associated to anemia with AOR of 1.98 (95% CI: 1.12-3.47). Based on the attributable population fraction, about 4.6% (95% CI: 1.8-7.4%) of anemia was Population attributable infection.

## Discussion

WHO recommended various cutoff points to rank the public health significance of anemia in a population<sup>39</sup>. Based on this criterion, with 31% prevalence, anemia has moderate public health significance in the area.

A previous study in the locality found 29% and 13% prevalences of anemia and iron deficiency anemia, respectively<sup>20</sup>. The figures are comparable to the current study (31% and 9%) in the sense that the confidence intervals of the estimates overlap.

Women aged 25-34 years had the highest odds of anemia. This might be as a result of the fertility intensive nature of the age group. Other local studies witnessed parallel findings. According to a study based on the Ethiopia Demographic Health Survey (DHS) 2005 data, the risk of anemia was 1.3 to 1.6 times higher in women aged 30-39 years compared to those aged 15-19 years<sup>24</sup>. Another national level study found relatively higher prevalence of anaemia; iron deficiency anemia and ID amongst women aged 31-35 years<sup>9</sup>.

The involvement of women in income generating activities was associated with reduced risk of anemia. This might be due to the reason that in rural Ethiopia women's income is usually spent to cover household food and related expenditures. On the other hand, the fact that household wealth status was measured based on a relative rather than an absolute scale might explain the unanticipated null association between anemia and household wealth status.

Maternal education showed positive influence on Hgb irrespective of the economic status indicators. Several studies in pregnant and non-pregnant women concluded likewise<sup>40-42</sup>. Maternal education might have reduced susceptibility to anemia through enhancing awareness and practice of good nutrition prior to and during pregnancy.

The odds of anemia were observed to rise as parity advances. This is consistent with the understanding that repeated pregnancies deplete maternal nutrition store. A previous study witnessed that the risk of anemia was 1.47, 1.51 and 1.86 times elevated in women with 1-2, 3-5 and more than 5 previous births, as compared to nullparas<sup>24</sup>. Nevertheless, many other studies concluded equivocally<sup>14,19,43-45</sup>.

Severe under nutrition and multiple micronutrients can lead to nutritional anemia. In the current study SAM, iron, vitamin A and zinc status indicators

were considered as potential predictors of anemia. Nonetheless, as other relevant nutrients like folate and vitamin B12 had not been considered, the comprehensiveness of the model might have been compromised.

Iron deficiency demonstrated a significant association with anemia. Similarly, prenatal iron supplementation showed a beneficial effect in reducing the burden of anemia. These are tangible evidences of the importance of iron in the etiology of anemia. Nevertheless, in the current study only a quarter of anemic subjects had concomitant ID and merely 11.8% of anemia was attributable to ID. The finding is against the prevailing assumption that 50% of anemia is attributable to ID<sup>2,46</sup>. This might be potential evidence that, at least in the locality, the contribution of ID to anemia might not be as huge as previously assumed. Studies conducted among school children in Thailand<sup>47</sup> and Sri Lanka<sup>48</sup> concluded the same.

Zinc deficiency showed a significant association with anemia. A previous study in the locality also concluded likewise. According to the study, in a regression model consisting plasma zinc, CRP, gravidity and ferritin as covariates, zinc emerged as the strongest predictor of prenatal anemia<sup>20</sup>. Studies in Sudan<sup>25</sup>, Iran<sup>49</sup> and Nepal<sup>50</sup> and also found significant positive correlation between zinc and Hgb levels. As the studies are cross-sectional it is not viable to implicate causal inference. Nevertheless, as zinc is known to participate in multiple metabolic pathways, it might have a causal contribution to anemia. Potential biological mechanisms have been discussed before<sup>51</sup>.

Vitamin A is believed to be involved in the pathogenesis of anemia through diverse biological mechanisms including enhancement of growth and differentiation of erythrocyte progenitor cells and mobilization of iron stores from tissues<sup>52</sup>. Nonetheless in the current study vitamin A status was not associated with anemia. The finding potentially signifies the negligible contribution of vitamin A deficiency to the burden of anemia in the locality. Previous studies conducted among pregnant women reported conflicting conclusions<sup>52</sup>. Many indicators of micronutrient status including zinc, retinol and ferritin artificially

change during infection/inflammation secondary to acute phase response<sup>53</sup>. Nevertheless, Hgb has not been identified as an acute phase reactant parameter<sup>54</sup>. Accordingly, the association witnessed between CRP and Hgb is taken as a valid relation. The statistically significant 4.4% decline in the Hgb level among subjects with elevated CRP signifies the importance of infection as a secondary risk factor of anemia. Previous study in the locality<sup>20</sup> and studies in Sudan<sup>25</sup>, Tanzania<sup>55</sup> and Ghana<sup>19</sup> also identified elevated CRP as a significant predictor of Hgb.

In general the major strengths of the study are the fact that adequate and representative samples were taken from the locality. The study also assessed synergistic relation between multiple nutritional deficiencies in associating with anemia. Such endeavors have been somehow overlooked so far. On the other hand, in few of the reported associations “egg or chicken” dilemma might not be excluded due to the cross-sectional nature of the study. In addition, the reported prevalence figures of anemia, ID and iron deficiency anemia are prone to seasonality bias.

## Conclusion

The prevalences anaemia, ID and iron deficiency anaemia were 31.6, 17.4 and 8.7%, respectively. Anaemia is of public health concern in the locality.

Advanced parity, maternal age of 25-34 years, substandard maternal socio-economic status, reliance on poorly diversified and plant source based diet and acute infection/inflammation were the prominent negative correlates of anaemia. Prenatal iron-folate supplementation showed beneficial effect in reducing the burden of anaemia yet its coverage is exceedingly low. Though ID was a significant correlate of anaemia, only a quarter of anaemic women had concomitant ID and merely 11.8% of anaemia was attributable to ID. Anaemia was associated with zinc deficiency and severe acute malnutrition but not with vitamin A deficiency. The study did not witness synergistic interaction among iron, zinc and vitamin A in associating with anaemia.

The exceedingly high burden of anaemia should be alleviated through enhancing maternal socio-economic status, birth control, prevention of illnesses, advancement of dietary diversity and



expansion of prenatal iron-folate supplementation. The observed positive association between zinc deficiency and anaemia should be investigated further through controlled trials.

## Contribution of Authors

All the authors participated in the designing, data collection, analysis and write-up of the study. The authors have read and approved the final manuscript.

## Acknowledgements

We are grateful for all study subjects who took part in the study. The authors acknowledge Addis Ababa University, School of Public Health for funding the study and EHNRI for conducting the laboratory analyses.

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