Folate and Vitamin B-12 status of anemic pregnant women and association to hemoglobin during antenatal care, 17-37 weeks in Ambo Hospital, Oromia, Ethiopia, a multi regression analysis of socio-economic and serum folate and Vitamin B-12.

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Abstract

Maternal health during pregnancy has been very critical and important for intergenerational health. According to WHO about half of anemia is believed to be due to iron deficiency anemia. In Ethiopia, the anemia prevalence was reported 22% in pregnant and believed due to IDA. Despite recommendation of iron-folic acid supplementation adherence is still low. The objective of the research was to determine the prevalence of folate and vitamin B-12 deficiency among pregnant and association to anemia during second and third trimester. A cross-sectional hospital-based design was conducted to complete this research. 104 pregnant women were selected based on the current altitude adjusted hemoglobin status after informed consent form is signed from each participant. Serum folates, vitamin B-12 were determined using ECLIA and C-reactive protein by Cobas Integra e411. Serum folate, vitamin B-12 and CRP were defined as deficient below 3 ng/mL, 150 pg/mL and greater than 5 mg/L respectively. Data were coded and analyzed using SPSS 22 version. About half of pregnant women were with formal education. 68.1% of pregnant women were anemic based on cutoff point (Hgb<10.9 g/dL). Study participants deficiencies were observed in 27.9% for folate, 26.9% for vitamin B-12 and 23.1% for CRP based on WHO cut-off point. There is a positive association between folate and hemoglobin, low awareness among elder pregnant but, no association between age and hemoglobin value. There is high prevalence of CRP in comparison to previous studies. Supplementation of vitamin B-12 will be recommended including further research on risk factors including rural residents to strength the finding.

Keywords: Anemia; Folate; Vitamin B-12; CRP; Pregnancy; Micronutrients

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Introduction

Maternal health during gestation has been very serious and imperative for intergenerational health. Anemia is a public and community health concerns that touching 2 billion society's worldwide [1]. Anemia affects suspiciously women in developing countries, and is self-determining factor for diminished quality of life, rise morbidity and mortality, particularly in the third trimester. According to WHO, about half of the anemia is supposed to be due to iron deficiency anemia (IDA). However, besides iron deficiency, defects in numerous other micronutrients including vitamin A, folate, Vitamin B-12 and possibly zinc also cause anemia [2,3]. In Ethiopia, the incidence of anemia amongst pregnant women was again reported to be 22% [4], which suggests that anemia is a moderate public health concern [5].

Nutrition in most developing countries is principally plant based with little consumption of animal source foods. This leads to an increased risk of multiple micronutrients deficiencies, especially at life style like pregnant when physiological demands are substantially increased [6,7]. Moreover, such plant-based diets contain significant amount of mineral absorption inhibitors like phytate and polyphenols that impair the bioavailability of micronutrients. Despite all this and WHO's recommendation that pregnant women take a prophylactic dose of 30-60 mg of Iron/Iron-folic acid supplementation, adherence to these recommendation, particularly in developing countries like Ethiopia have been very low [8]. Although anemia could also be due to other micronutrient deficiencies, multiple micronutrients supplementations are not yet proposed and not available for pregnant women.

The first thousand days, starting from conception to the child's second birthday, is a critical window of opportunity for nutritional interventions that aim to improve pregnancy, birth and developmental consequences. This is a period when a child's brain, organs and body is rapidly developing [9]. Anemia during pregnancy has been associated with complication in pregnancy outcomes and countless risk of low birth weight, which itself has been linked to adverse physical and cognitive growth. In Ethiopia, the leading cause for the poor adherence to iron supplementations was reported to beside effects [10,11]. Therefore, it is crucial to correct anemia during pregnancy. More importantly little is known about the etiology of anemia in most developing countries including Ethiopia. The

investigation of the etiology of anemia during third trimester pregnant women by quantifying serum folate and vitamin B-12 in addition to dietary intake is necessary to recognize nutritional deficiencies with inevitability [12,13]. The purpose of this study was to investigate the prevalence of folate and vitamin B-12 deficiency and dietary intake of anemic pregnant women attending antenatal care during second and third trimester in the Ambo Hospital of the west shows zone, Ethiopia.

Subject and Methods

The cross sectional Institutional based design was carried out between December 2014 and June 2015 in Ambo, a west Showa zone of Oromiyaa region, located at 115 km from Addis Ababa to determine the prevalence of anemia, serum folate and vitamin B-12 status of pregnant woman attending ANC during the second and third trimester. The study population was composed of pregnant women attending the regular pregnancy ANC during the second and third trimester. The study area is found at a longitude of 37° 32' to 38° 3' E, and latitude of 8° 47' to 9° 20' N and the altitude range is from 1900 to 2275 meters above sea level. The climatic condition of the area is 23% highland, 60% midland, and 17% lowland. It has an annual rainfall ranging from 800-1000 mm and temperature ranging from 20°C to 29°C.

Agriculture is the main occupation of the population of the area. The study population consisted of a sample of all second and third trimester pregnant women attending ANC at Ambo hospital. But, any women with pregnancy related complications such as history of diabetes mellitus, hypertension, those on iron supplements, history of blood transfusion within the last 3 months were excluded. The dependent variable is Hemoglobin level and Ecological, physiological and biochemical indices are considered as independent factors.

Sample size and sampling technique

Among 206 pregnant women screened for analysis during ANC service, 104 anemic pregnant women were selected for further biochemical analysis based on hemoglobin value in considering Hemo-dilution. Sample size was determined based on the EDHS 2011 [14] the prevalence of anemia among the women aged 15-49 in Oromiyaa region is 19.3%. The maximum permissible limit is 19.2% of "p" and standard normal value (z=1.96). Using the single population proportion formula with a 95% CI (d=0.95) and 5% margin of error sample size was calculated and obtained 238. But, the researcher has planned to collect sample from 250 pregnant women to increase accuracy of data. Within the specified period only 206 of pregnant women were registered with hemoglobin result of below 11.5 g/dl. Due to resource limitation (financial and time constraint) the principal investigator was forced to consider homo-dilution and accepted the anemic pregnant women hemoglobin value below 10.5 g/dl [15]. Based on the above consideration 104 pregnant women sample were selected for further investigation of micronutrient analysis.

Ethical considerations

The research was ethically cleared from Addis Ababa University Institutional Health and Research Ethical Review Board committee in College of Natural Science and further the research ethically approved regional Health Research Ethical Review Committee.

Sample collection

Socio-demographic characteristics: Verbal informed consent was obtained from all the participants and a semi-structured questionnaire was given in Afan Oromo language to gather information including age, time of previous gestation, and maternally related characteristic, present and past history in nutritional assessment of anemic pregnant women and dietary habit.

Management/handling of the experimental unit and sample collection process: Blood sample (ca. 4 mL) was drawn into vacutainer tubes without anticoagulants by medical laboratory technicians from the antecubital vein. Gestational ages were considered by the reported last menstrual period and examining of fundal height by experienced midwives at maternal and child health, MCH of Hospital [16,17].

Hemoglobin test and altitude adjustment: Hemoglobin tests were performed from the fresh blood sample before centrifugation. Separate vacutainer tube without EDTA was used for whole blood sample collection for the analysis of complete blood count (CBC) by ADVIA 2120i 5 Part Cell Counter (Siemens). A hemoglobin level was used to define anemia by adjusting for altitude according WHO Hb adjustment formula [18]. Hb adjustment=$-0.032 \times (\text{Altitude} \times 0.0032808) + 0.022 \times (\text{Altitude} \times 0.0032808)^2$. Considering hemo -dilution of pregnancy based on WHO and international guidelines, anemia was then defined as Hgb < 10.9 g/dl in pregnant women [19,20]. After correction, severe, moderate and mild anemia was defined as Hgb below 7gm/dL, 7-9.9 gm/dl and 9.9-10.9 gm/dl respectively [21]. The sample collected into the tube was allowed to clot and centrifuged at 3000 × g, for 15 min to extract serum for biochemical analysis and stored at -20°C until analyzed [22]. A sample collected into a tube was allowed to store and transported within a maximum of 3 days for biochemical analysis of serum folate and B-12.

Laboratory analysis

Determination of iron biomarkers: Serum vitamin B-12 and folate concentrations were measured using ECLIA [22]. Serum CRP was determined using the Nephelometry Assay method by Cobas Integrate 411 by using Liquid, ready-to-use reagents [23]. Based on the WHO cut-off point folate and B-12 were deficient considering Serum folate and B-12 deficiencies were defined as levels below 3 ng/mL and 150 pg/mL respectively. Inflammation in pregnant women was determined assuming serum CRP greater than 5 mg/L [24].

Statistical analysis

The data were coded and analyzed with the Statistical analyses using SPSS (version 22; SPSS Inc., Chicago, IL) software [22]. The Pearson correlation coefficient was used to measure the strength of the linear relationship between normally distributed variables. Descriptive analyses of categorical variables were reported and $p<0.05$ at $\alpha=0.05$ denoted a statistically significant difference in all statistical comparisons. To analyze

the differences between groups, the independent student t test was used for continuous variables, among mild and moderate anemic participant and between second and third trimester anemic pregnant women**.** Pearson's correlation was used to examine correlation between hemoglobin and serum folate and B-12 [21].

Results

Socio-demographic characteristics, correlation and regression analysis

Socio-demographic characteristics: Socio-demographic of all mothers (n=206) attended ANC during the study period is presented Table 1. The mean ± SD of gestational age was 23.3 ± 5.6 weeks, mean age of the pregnant women was 24.6±5.03. All the study participants were Christians and 95.6% were married. Only 57.8% had had their own latrine and 50% had formal education. Based on the socio-demographic data collected, 35 (17%) of pregnant women were infected with malaria at least once in the past two years.

Pregnancy history and complications in the current pregnancy: Based on the Table 2 †* report, previous pregnancy history only 1.9% were delivered into their babies within the interval of less than 2 years and 91 (44.2%) was pregnant for the first time. More than one-third of the study participants (34.5%) were received information about nutrition and about 12.6% of participants had faced blood loss during previous pregnancy. Based on the Table 3†* report, 36.5% of study participants were not taking any iron or IFA supplementation at all. Hence, 63.5% of the participants were taken either iron or iron-folic acid supplementation but not more than one month. Increased breathing, especially on routine activity 71 (34.5%) and headaches 57 (27.7%) were the two leading signs of symptoms

Table 1. *Selected ecological indicator characteristics of the pregnant woman attending ANC.*

Characteristics*	Mean ± SD	(N=206)	(%)
Age of Participant			
15-19	24.6 ± 5.03	82	39.8
20-25		50	24.3
26-30		52	25.2
31+		22	10.7
Marital Status			
Married		197	95.6
Not Married		9	4.4
Gestational Age			
14-26wks (2)	23.3 ± 5.6	137	66.5
27-37wks (3)		69	33.5
Occupation			
Farmer		62	30.1
House wife		113	54.9
Merchant		31	15
Educational Status			
Illiterate		75	36.4
Read and Write		28	13.6
With formal education		103	50
Owns Latrine			
Yes		119	57.8
No		86	42.2

* The socio-demographic characteristic includes all pregnant women attended ANC during 2nd and 3rd trimester (N=206)

Table 2. *Maternal pregnancy history (n=206)*

Characteristics*	Frequency	%
Previous deliveries History		
First pregnancy	91	44.2
Health Institution	50	24.3
Home	65	31.6
Information about Nutrition		
Yes	71	34.5
No	135	65.5
Blood Loss		
Yes	26	12.6
No	180	87.38
Interval between babies		
<2 year	2	1.9
>2 year	113	54.85

* The maternal pregnancy history includes all pregnant women attended ANC during 2nd and 3rd trimester (N=206)

Table 3. *Complications related to the current pregnancy (n=206)*

Characteristics*	Frequency	%
Use of Contraceptive*		
Yes	126	61.2
No		
Iron or Folate Supplementation		
Iron Supplementation	25†	23.6
Iron-folic acid (IFA) supplementation	41†	39.4
No Supplementation	38	36.5
Sign of Symptoms		
Persistent swelling of feet, hands or face	24	11.7
Increasing breathing, especially on routine activity	71	34.5
Headaches	57	27.7
Blurring of Vision	29	14.1
Fever temperature >38 C	6	2.9
High Colored urine in the past two weeks	10	4.9
No Symptoms	9	4.4

* Only data in use of contraceptive include all the 206 pregnant women in 2nd and 3rd trimester
† Out of 104 anemic pregnant women none of them took IFA supplementation more than one month

and 14.1% had blurred vision during the current pregnancy. This finding has also supported the statement of anemia during pregnancy has been attributed not only due to increased iron requirements during the second and third trimester of gestation, but also due to effects of physiological state as well as also described in a study of pregnant women in Ugandan [25].

Dietary assessment and nutritional status of respondents: Regarding food consumption and dietary pattern, 87.9% (n=181) of the participants was received at least three meals a day. Few (5.3%) of mothers were missing their breakfast during the fasting period. Animal source foods were consumed by less than half of the respondents (Table 4).

Prevalence of micronutrient deficiency (folate and vitamin B-12) deficiencies among pregnant women attending ANC

The prevalence of selected micronutrients was presented in Table 5 and the mean hemoglobin values of anemic pregnant women were 10.5 ± 1.2 g/dL. Mean ± SD of serum folate, serum vitamin B-12 and serum CRP were noted as 7.6 ±3.5 (ng/mL), 187.4 ± 53.7 (pg/mL) and 4.5 ± 1.6 (mg/L), respectively. Vitamin B-12 is prevalent in 26.9% (B-12 deficiency <150 pg/

mL), Folate is deficient in 27.9% (serum folate <6.8 ng/mL) and 23.08% had acute inflammation based on the serum CRP > 5 mg/L.

Prevalence of micronutrient deficiency and CRP infection anemic pregnant women

The mean and prevalence of micronutrient status of pregnant women categorized by the severity of anemia (mild or moderate) is presented in Table 6. From the data below, no statistically significant difference was observed between mild and moderate anemic pregnant women. Based on the category of GA as presented in Table 7**.** Except for CRP values, no significant difference was detected in serum folate and B-12 composition between the second and the third trimester.

Prevalence of anemia based on folate, vitamin B-12 and C-reactive protein of pregnant women attending ANC

Among the anemic pregnant women (n= 104), ~71% were in the 2nd trimester, whereas 29% in the 3rd trimester of pregnancy.

Table 4. *Reported food groups consumed during the period of current pregnancy (n=206)**

Characteristics*	Frequency	%
Frequency of meals per day		
2 per day	25	12.1
3 per day	181	87.9
Level of Fasting		
Deprivation of animal source foods except fish	30	14.6
Deprivation of all animal source foods	32	15.5
Deprivation of animal Source foods and no breakfast	11	5.3
No fasting	62	59.6
Drink alcohol during pregnancy		
Yes	51	24.8
No	155	75.24
* Data of food group includes all pregnant women attended ANC during 2nd and 3rd trimester		

Table 5. *Prevalence of micronutrient deficiencies among anemic pregnant women attending (n=104)*

Characteristics*	Frequency	%	Mean + SD
Altitude adjusted Hb	206		10.5± 1.2*
Hb (<10.9 g/dL)	104	50.48	9.7±0.66
Serum Folic acid	104		7.6±3.5
FAD (<6.8 ng/ml)	29	27.9	
Serum Vitamin B12			187.4±53.7
Vit B12 deficiency (<150 pg/mL)	28	26.9	
Serum CRP			4.5±1.6
Presence of inflammation CRP (>5.0 mg/L)	24	23.1	
Presence of inflammation CRP (<5.0 mg/L)	82	78.85	
Hb: hemoglobin, FAD: folic acid deficient, CRP: C-reactive protein * Only result of hemoglobin included all pregnant women			

Table 6. *Micronutrient status of pregnant women with mild and moderate anemia (n=104)*

Characteristics*	Types of anemia				p-value	95% CI of the difference	
	Moderate		Mild			lower	upper
	N	Mean +SD	N	Mean + SD			
Serum FA	59	7.9±3.7	45	7.2±3.4	0.296	-0.653	2.122
Serum B12	59	178.8±46.5	45	198.6±60.5	0.072	-41.41	1.838
Serum CRP	59	4.5±1.7	45	4.4±1.5	0.642	-0.487	0.786
* The mean difference is significant at the p≤ 0.05							

Folate and B-12 was severe in 17.31% and 26.92% respectively. The remaining 82.69% and 73.08% were under a risk of deficiency. But 24 (34%) had high inflammation based on serum CRP (Table 8).

Discussion

Socio-demographic characteristics

Maternal under-nutrition diminishes a woman's productivity, causing repercussions for this woman, her family, her community, and the broader society. Daba et al., [26] reported that educational level, monthly income and nutrition information during pregnancy were identified as important predictors of knowledge of women on nutrition during pregnancy among the study participants. Due to the third trimester covers the 28th week onwards till delivery the severity of anemia is directly proportional to the occurrence of complications in pregnancy. Etiologic pattern is often complex in second and third trimester [16,17]. Based on the previous pregnancy history delivery recorded there is no significant association due to the interval between babies, to use of contraceptive and history of blood loss to hemoglobin value. According to the study of Obse et al., [27] decreasing gap between previous birth, had caused an increase in the magnitude of anemia. Opposite to the Obse finding there was no association between previous birth and hemoglobin status in the current finding. This might be due to all the participant's history of birth interval was greater than two years and the study respondents are from the similar status (all are anemic).

Anemia and micronutrient status of pregnant women

For comparison of each micronutrient status categorized by age according to the present finding, severity of anemia has almost increased as the age of the respondent's increases; this finding also supported by Obse et al., [27]. Serum folate was significantly associated with serum B-12 (r=0.27; P=0.02), No significant relation was observed between moderately and mildly anemic mothers considering both folate and B-12 concentration. This indicated that folic acid deficiency and vitamin B-12 deficiency is not the only cause of anemia during pregnancy [16,17].

But, serum folic acid and CRP status were recorded lower in the older pregnant women. The study conducted by Kefiyalw et al., [28] stated that the prevalence of anemia was higher in pregnant women in the age group of 18-26. This might be due to Kefyalew's study included all status of pregnant women (anemia and non-anemic). According to Gibson et al., [24] the study conducted in Southern Ethiopia a high prevalence of protein and zinc deficiency was observed and two-third of pregnant women were anemic in the area were no cellular animal products consumed. This study might have an advantage over the previous study, but, due to all participants are from the same group (anemic) still no correlation and any prediction was obtained from the current data. Deficiency of vitamin B-12 in the current study also agreed with the deficiency that conducted in southern Ethiopia and in Nepal [2,27].

C-reactive protein inflammatory status and anemia

Regarding the infection of pregnant women based on serum CRP the prevalence of inflammation was very high in comparison to

Table 7. *Micronutrient status of pregnant women between second and third trimester anemic women (n=104)*

Characteristics*	Mean + SD			95% CI of the difference	
Gestational age	14-26wks (2nd trimester, n=74)	14-26wks (3rd trimester, n=30)	P-value	Lower	Upper
	Mean +SD	Mean + SD			
Hemoglobin	9.64+0.65	9.65+0.6	0.9	-0.29	0.25
Serum Folic acid	8.0+3.6	6.7+3.3	0.105	-0.263	2.75
Serum vitamin b12	182.3+45.54	200+69.1	0.206	-45.28	10.07
Serum CRP	4.2+1.4	5.0+2.0	.050*	-1.6	0
* The mean difference is significant at the p< 0.05					

Table 8. *Prevalence description of folate, vitamin B12 and C-reactive protein of pregnant women attending ANC.*

	Total	Sub Total			Moderate anemia, Hb<9.9	Mild anemia Hb<10.9
				Sum %	N (%)	N (%)
Gestational Age *	206	74	14-26wks	71.15	41 (69.5)	33 (73.3)
		30	27-37wks	28.85	16 (15.4)	4 (3.8)
Serum Folic acid	104	18	Severe	17.31	8 (7.7)	10 (9.6)
		11	Possible	10.58	5 (4.8)	6 (5.8)
		74	Normal	71.15	45 (43.3)	29 (27.9)
Serum Vitamin B12	104	28	Severe	26.92	18 (30.5)	10 (9.6)
		46	Moderate	44.23	29 (27.9)	17 (16.4)
		30	Normal	28.85	12 (11.5)	18 (17.3)
Serum CRP	104	80	sCRP<5	76.92	47 (45.2)	33 (31.7)
		24	sCRP>5	23.08	12 (11.5)	12 (11.5)
ND: Non-deficient, 14-26 wks = Second trimester; 27-37 wks = Third trimester *Gestational age data included for all the 206 participants						

study conducted in southern Ethiopia. In a study conducted by in Uganda similar result was reported. According to the CRP concentration was markedly raised and nearly the two-third of the women had elevated CRP [26,27]. In the current finding based on the CRP values Infection/inflammation was higher in the third trimester [29]. In Malawi also, the same result was recorded which agreed with the finding of the present study [30]. The contradiction of findings with might be due differences in study participants (only anemic pregnant included in this study). Iron deficiency and malaria are significantly interrelated and affecting developing world especially in pregnancy was explained in another finding and supported by this finding. Whereas anemia was associated with markers of infections in another research finding from Tanzania also and in another finding, anemia was significantly associated with elevated CRP, with an odds ratio of 2.74 [31].

Micronutrient interrelations

The Pearson correlation showed a significant negative association between the age of a woman and IFA supplementation (r=-0.20; p=0.04). The negative association of age of women and iron-folic acid supplementation indicated that the older women's awareness of IFA supplementation was low compared with the younger women. On the other hand, serum CRP was positively correlated with vitamin B-12 (r=0.26; p=0.01). In the correlation analysis of selected socio-demographic with prevalence of anemia with adequate iron store (anemic only based on Hb value) as a dependent variable there are not a significant correlation between selected socio-demographic data and biochemical analysis.

Correlation and regression analysis of Hb and B-12

Logistic regression analysis was used to assess the association between hemoglobin and folate. There was also a negative correlation between age of women and hemoglobin value (r=-0.26; p=0.04); whereas there was a positive correlation between IFA supplementation and Hb. This show that iron and FAD might be the causes for depletion of Hb. Surprised that there was a positive correlation between IFA supplementation and serum folic acid.

Factors affecting and predictors in severity of anemia

Positive correlation was found between serum CRP and serum B-12 (r=0.32; p=0.02) in moderately anemic pregnant women. According to Gebremedhin et al., [8] in level indicates infection/inflammation only 8.4% of the pregnant women had elevated serum CRP (CRP $\geq$ 6 mg/L). Among up to date finding conducted in the southern Ethiopia by Gibson et al., [24] showed that none of the biochemical indices of folate or vitamin B-12 status, were significant predictors of hemoglobin concentrations. This finding was not supported by this study. This might be due to the frequent consumption of folate rich fermented enset in southern Ethiopia. Because in Sidama Zone of Southern Ethiopia, maize (*Zea mays L.)* and fermented enset (*Enset ventricosum*) products are the major staple foods, contributing up to 90% of energy. In the current study the older the woman had the least hemoglobin (9.40 ± 0.94), serum folate (6.09 ± 2.97) and CRP (3.8 ± 0.8) [16,17,32].

Conclusion

The prevalence of anemia among third trimester anemic pregnant women in this study compared with second trimester anemic pregnant women was high. Study participants had high inflammation/infection based on serum CRP >5 mg/L. The older pregnant women were observed more deficient almost in all micronutrients than younger women. The high prevalence of vitamin B-12 deficiency more than expected might be due to less amount of B-12 required and the supplementation of folic

acid, which causes to increase in B-12 because they have the interrelation between them. The present study has shown that a clinical study of micronutrients iron biomarkers and vitamin deficiency is a hidden risk for pregnant women especially in second and third trimester.

In conclusion, this study helped us to understand the association between the level of anemia and the biosocial factors. Women in developing countries have a high prevalence of ID but also tend to be deficient in other micronutrients including vitamin B-12, folate and infection/inflammatory predictors. The current study has several advantages over previous Ethiopian studies that examined micronutrient status during pregnancy including study area.

Recommendations

The necessity for vitamin B-12 supplementation needs to be confirmed with prospective randomized trials from different regions of our country before the introduction of a fortification program for prevention of neural tube defect. The topical development of analytical means for examination of hepcidin in biological samples is providing an opportunity to include hepcidin as an innovative biomarker reflecting iron status and to that hepcidin concentration was positively correlated with ferritin and this test was recommended to be included in the future research. Further research on risk factors of anemia, which include rural residents, should be conducted to strengthen and broaden these findings.

Limitation of the study

This study has a few limitations, first, the nature of the study being a cross-sectional study design; it does not show risk factors in detail. Second, it is a hospital-based study with a relatively small sample size. Lack of comparison group (anemic with non-anemic). The pregnancy complications such as hypertension, blood sugar level and urinary albumin was not determined; even that study participants were not recalled their complications such as hypertension, blood sugar level and urinary albumin and no further interpretation of analysis was done in such complication factors. The current status of the mother somewhere else than Ambo health centers and Ambo hospital was not included. And lack of anthropometric measurement of participants and their infants are also other limitation.

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