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## ORIGINAL ARTICLE

# DETERMINANTS OF MEGALOBLASTIC ANEMIA RELATED HEMATOLOGIC DISORDER AMONG PREGNANT MOTHERS ATTENDING ANTENATAL CARE IN EASTERN SHOA ZONE: A CASE CONTROL STUDY

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## ABSTRACT

***Introduction:*** *Unusual increase in the number of mothers referred to Tikur Anbassa Specialized Hospital from Eastern Shoa and Arsi zones of Oromia region of Ethiopia, with problems of severe anemia and thrombocytopenia, has been observed. Further investigation in Tikur Anbassa Specialized Hospital revealed features of megaloblastic bone marrow changes.*

***Objective:*** *The aim of the study was to identify potential risk factors associated with the disease in the study area.*

***Methods****: A case control study was conducted on 50 cases and 156 controls from mothers attending ANC in Adama and Assala Hospitals. .*

***Result:*** *Fifty cases and 156 controls participated in the study with mean (SD) age of 25.8 (±5.2) and 25.1 (±4.5), respectively**.** Cases were more exposed to fertilizers (AOR 7.3, 95% CI 3.6-15) and pesticides (AOR 6.2, 95% CI 2.8-13.5) than controls although specific chemicals were not identified in this study. Increased adverse maternal outcomes, including maternal deaths and perinatal mortality were observed among cases than controls.*

***Conclusion and recommendation:*** *Cases were more frequently exposed to pesticides and fertilizers and had poor pregnancy outcome compared to controls. This calls for further largescale study to explore potential environmental exposures, including those identified in this study and to further unravel the cause of this fatal and significant public health problem in this highly populated and industrial region of the country.*

***Key words:*** *Hematological disorders, pregnancy, Risk factors, Megaloblastic anemia.*

## INTRODUCTION

According to the United Nations (UN) estimates, approximately half of pregnant women suffer from anemia worldwide (1). · Among health care status indicators, prevalence of anemia during pregnancy is one of the key indicators. But still it has remained a challenge and a burden to health system, especially in the developing countries (2).

Pregnancy, being a state of increased metabolic demand due to the growing fetus and the changes in maternal physiology, requires increment in daily intake of all the nutrients. Plasma volume expansion, one of the physiological change of pregnancy, will lead to a drop in hemoglobin (Hgb), but not below 10.5gm/dl (3,4). When the drop in Hgb is below 10.5 gm/dl, nutritional iron deficiency is the main cause of anemia during pregnancy. But folate deficiency anemia and anemia due to deficiency of other micronutrients is also reported during pregnancy (5–8).

Seventeen percent of Ethiopian women, aged 15-49 years, are anemic. Thirteen percent having mild, 3% moderate and 1% severe anemia.

A higher proportion of pregnant women are anemic (22%) than women who are breastfeeding (19%) and women who are neither pregnant nor breastfeeding (15%). A higher proportion of women in rural Ethiopia are anemic (18%) than those in urban areas (11%) (9).

Similar to the finding of anemia in pregnancy, thrombocytopenia is not also un common, affecting 8-10% of pregnancies but often times it is physiologic and worsens as pregnancy advances but rarely drops below critical level and resolves after delivery (10,11).

Tetra hydro folic acid (THFA) is the biologically active form of folic acid, important for the synthesis of amino acids, purine and thymidine. Unlike microcytic-hypochromic features in case of iron deficiency anemia, folate and/or vitamin B12 deficiency anemia typically consists of macrocytosis and hyper segmented polymorpho nuclear leucocytes (PMNs) and is gradually progressing. Less commonly, neutropenia (rarely <1000/mm3) and thrombocytopenia (rarely <50,000/mm3)will also occur during folate/Vit B12 deficiency (12,13).

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Although higher proportion of megaloblastic anemia (MA) are due to dietary deficiency, reduced absorption and/or derangement in the metabolism of these vitamins are also responsible for the development of MA. In addition to bone marrow and peripheral blood morphology features, reduced serum level of these vitamins indicates the presence of deficiency. In the absence of laboratory evidences, initial treatment with folate/Vit B12, especially for severe anemia is recommended (14).

One case report described sudden onset Vit B12 deficiency during pregnancy detected at 22 weeks of gestational age leading to pancytopenia at 30 weeks and treated with Vit B12. Hematologic profile improved and delivered live baby at term with no complications (15).In another case report, a young pregnant mother presented with pancytopenia and was found to have megaloblastic, hyper-cellular changes in a subsequent bone marrow biopsy. This presentation was determined to be secondary to toxic effects after heavy use of nitrous oxide, suggesting the potential contribution of toxic chemical exposure affecting the hematopoietic path way leading to reduced out puts of these cell lines (16).

In the past few years, TASH, a central referral teaching Hospital, located in Addis Ababa, the capital of Ethiopia has been receiving more cases of severe anemia during pregnancy from Adama and Assala Hospitals, located 100-200 kms east of the capital. All the cases referred have similar clinical presentations; new onset nasal and/or gum bleeding, generalized body weakness and palpitation during the current pregnancy. In addition of being from similar locality and having similar signs and symptoms, the cases hematologic profile consistently showed anemia, low platelets and reduced white cell counts. The peripheral morphology and bone marrow examination revealed megaloblastic anemia (17).

The residence area of these index cases is a rift valley region of eastern Shoa and the vicinity and is known for endemic infectious diseases like malaria and other water borne diseases. The water is known to be contaminated with excess florin affecting children and adult's teeth and bone development as well as strength. It is also an area where most of the countries manufacturing industries, commercial establishments and large scale modern farming like flower farms are located.

The purpose of this study is to compare the socioeconomic status, regular nutrition, and potential environmental exposure of cases (pregnant mothers with bi/pan-cytopenia and megaloblastic bone marrow change) and healthy pregnant controls residing in eastern Shoa and Arsi zones of Oromia region and had antenatal care (ANC) and/or delivery in Adama or Assala hospital or referred from these hospitals to TASH for better care due to the hematologic disorder. Identifying potential cause/s will help to plan future largescale studies to specifically know the cause of this fatal disorder and propose preventive strategies.

## PATIENTS AND METHODS

The study was conducted in Adama and Assala Hospitals, located in east Shoa and Arsi zone of Oromia region, 100-200 kms east of Addis Ababa, the capital of Ethiopia.

A case control study design was utilized to identify risk factors that could be associated with MA during pregnancy. Cases were pregnant mothers diagnosed to have bi or pancytopenia with Hgb<10.5gm/dl (hematocrit (HCT)<33%), WBC <4000/mm3, platelet <00,0000/mm3) whose peripheral morphology and bone marrow examination revealed MA and no medical or other reason identified for the disorder. Controls, on the other hand, were healthy pregnant mothers with normal hematological profile.

Sample size of 52 cases and 155 controls was calculated based on the following assumptions: A 95% level of confidence, a power of 80% to detect real association of exposure variable and un-control to control ratio1:3. Statcalc for unmatched case – control study was used assuming 40% exposure in case and 20% in controls.

When a case was identified during ANC or in the delivery/emergency rooms of both hospitals or in TASH (for cases referred from either of the two hospitals), structured data collection tool was administered by trained nurse and subsequent three controls, who visited to either hospitals (Adama or Assala) where the cases were identified were selected by simple random sampling and their data was captured. All cases were treated with 5 mg folic acid tablet to be taken every day and vitamin B12 injection by the investigators. Besides, blood products were transfused as indicated. Data were analyzed using STATA version 14 statistical package.

The study was approved by AAU, College of Health Sciences, Adama and Assala Hospital Medical College institution review board (IRBs) and written consent was secured from study participants before data was collected.

## RESULT

In this study, a total of 50 cases and 156 controls participated. All cases and controls were from east Shoa and Arsi zone of Oromia regional state of Ethiopia, located 100-200 kms east of the capital Addis Ababa. As shown in Table 1, the mean (±SD) age of cases and controls was 25.8 (±5.2) and 25.1 (±4.5), respectively.

Most of the cases were Muslims, while majority of the controls were Christians and belong to Oromo ethnic group. More than 50 % of cases were farmers as opposed to 10% among the control group.

**Table 1:** Socio-demographic characteristics of megaloblastic anemia study participants, East Shoa Zone of Oromia region, Ethiopia.

| **Socio-Demographic variables** | **Cases n (%)** | **Controls n (%)** | **p-value** |
|---|---|---|---|
| Age: mean | 25.8(5.2) | 25.1(4.5) | 0.340 |
| Religion | | | |
| Christian | 12(24.5) | 100(64.9) | <0.001 |
| Muslim | 37(75.5) | 54(35.1) | |
| Ethnicity | | | |
| Oromo | 46(92.0) | 100(64.1) | <0.001 |
| Others | 4(8.0) | 56(35.9) | |
| Marital status | | | |
| Married | 49(98.0) | 153(99.3) | 0.431 |
| Unmarried | 1(2.0) | 1(0.7) | |
| Occupation | | | |
| Farmer | 28(56.0) | 16(10.3) | <0.001 |
| Housewife | 17(34.0) | 117(75.5) | |
| Others* | 5(10.0) | 16(10.3) | |

* Government employee, Privet employee, Self employed, Merchant and student

The mean platelet count, white blood cell (WBC) count and hematocrit of cases and controls was found to be 30,497.6 and 194,270.6; 3,251 and 11,671; 15.4% and 36.7%, respectively (Table 2).

All cases had undergone peripheral smear and bone marrow examination by the standard method and revealed megaloblastic bone marrow changes.

**Table 2:** Hematologic profile of megaloblastic anemia study participants, East Shoa Zone of Oromia region, Ethiopia.

| **Counts** | **Cases** | | **Controls** | | **P-value** |
|---|---|---|---|---|---|
| | **n** | **Mean (SD)** | **n** | **Mean (SD)** | |
| Lowest WBC | 50 | 3251 (±2637.2) | 154 | 11671.1 (±6076.9) | <0.001 |
| Lowest HCT | 50 | 15.4 (±4.4) | 154 | 36.7 (±7.9) | <0.001 |
| Lowest Platelets | 50 | 30497.6 (±21894.2) | 153 | 194270.6 (±66644.1) | <0.001 |

Tables 3 and 4 show the details of study participants' diet. Almost all of them have starch staple diet. Cases on average have starch staple diet for 4.5 days per week and the control group 2.3 days per week. Eighty-four percent of cases and 96.8% of controls consume legumes/nuts on average 3.5and 2 days per week respectively. Seventy-two percent of cases and 92.3 % of controls consume dark green leafy vegetable on average 2.6 and 1.5 days per week respectively. Fifty-six percent of the cases and 80.8% of the controls consume Vitamin A rich fruits and vegetables 2.1 and 1.7 days per week respectively.

Nearly thirty-seven percent of cases and 50.6 % of controls eat organ meat on average 1.5 and 1.2 days in a week respectively; and 40% of cases and 60% of controls eat flesh meat and fish on average 1.6 and 1.2 days per week.

Fifty-four percent of cases and 83.3 % of controls consume egg on average 1.4 and 1.3 days per week. Sixty-six percent of cases and 87.8 % of controls consume milk and milk products on average 2.5 and 1.4 days per week respectively. Seventy-four percent of cases and 79.5 % of controls eat oil and fat containing foods on average 3.8 and 1.5 days per week respectively.

Fifty-four percent of cases and 90.4% of controls use spices, condiments, beverages on average 3.6 and 1.9 days per week respectively. Nearly all of the cases and controls eat breakfast and dinner and 72 % of cases and 96.8 % of controls eat lunch.

**Table 3:** Food composition of megaloblastic anemia study participants, East Shoa Zone of Oromia region, Ethiopia.

| Food items | Cases n (%) | Controls n (%) | P-value | COR (95% CI) | AOR (95% CI) |
|---|---|---|---|---|---|
| Starch Staple | | | | | |
| Yes | 50(100) | 152(97.4) | 0.574 | | |
| No | 0(0) | 4(2.6) | | | |
| Legumes/nuts | | | | | |
| Yes | 42(84.0) | 151(96.8) | <0.001 | 0.17(0.05,0.56) | 0.4(0.09, 1.82) |
| No | 8(16.0) | 5(3.2) | | | |
| Dark green leafy vegetables | | | | | |
| Yes | 36(72.0) | 144(92.3) | <0.001 | 0.21(0.09, 0.50) | 1.10(0.26, 4.56) |
| No | 14(28.0) | 12(7.7) | | | |
| Vitamin A Rich fruits & Vegs | | | | | |
| Yes | 28(56.0) | 126(80.8) | <0.001 | 0.30(0.15, 0.60) | 0.29(0.10, 0.80) |
| No | 22(44.0) | 30(19.2) | | | |
| Other fruits & Vegetables | | | | | |
| Yes | 38(76.0) | 143(91.7) | 0.003 | 0.29(0.12, 0.68) | 1.33(0.37, 4.73) |
| No | 12(24.0) | 13(8.3) | | | |
| Organ meat | | | | | |
| Yes | 18(36.7) | 79(50.6) | 0.089 | | |
| No | 31(63.3) | 77(49.4) | | | |
| Flesh meet and fish | | | | | |
| Yes | 20(40.0) | 94(60.6) | 0.011 | 0.43(0.23, 0.83) | 1.14(0.48, 2.75) |
| No | 30(60.0) | 61(39.4) | | | |
| Eggs | | | | | |
| Yes | 27(54.0) | 130(83.3) | <0.001 | 0.23(0.12, 0.47) | 1.01(0.36, 2.85) |
| No | 23(46.0) | 26(16.7) | | | |
| Milk & Milk products | | | | | |
| Yes | 33(66.0) | 137(87.8) | <0.001 | 0.27(0.13, 0.57) | 0.26(0.10, 0.69) |
| No | 17(34.0) | 19(12.2) | | | |
| Oil and fats | | | | | |
| Yes | 37(74.0) | 124(79.5) | 0.414 | | |
| No | 13(26.0) | 32(20.5) | | | |
| Sweets | | | | | |
| Yes | 24(48.0) | 79(50.6) | 0.745 | | |
| No | 26(52.0) | 77(49.4) | | | |
| Spices, condiments, beverages | | | | | |
| Yes | 27(54.0) | 141(90.4) | <0.001 | 0.12(0.06, 0.27) | 0.19(0.07, 0.51) |
| No | 23(46.0) | 15(9.6) | | | |

**Table 4:** One week mean feeding frequencies for selected food items in megaloblastic anemia study participants, East Shoa Zone of Oromia region, Ethiopia

| Food items | Mean frequency/week<br>Cases<br>Mean (SD) | Control<br>Mean (SD) | P-value |
|---|---|---|---|
| Starch Staple | | | |
| Yes | 4.5(2.3) | 2.3(0.6) | <0.001 |
| No | | | |
| Legumes/nuts | | | |
| Yes | 3.5(2.3) | 2.0(0.5) | <0.001 |
| No | | | |
| Dark green leafy vegetables | | | |
| Yes | 2.6(1.8) | 1.5(0.5) | <0.001 |
| No | | | |
| Vitamin A rich fruits and vegs | | | |
| Yes | 2.1(1.4) | 1.7(0.6) | 0.029 |
| No | | | |
| Other fruits & vegetables | | | |
| Yes | 2.4(1.8) | 1.6(0.5) | <0.001 |
| No | | | |
| Organ meat | | | |
| Yes | 1.5(1.2) | 1.2(0.4) | 0.039 |
| No | | | |
| Flesh meet and fish | | | |
| Yes | 1.6(1.2) | 1.2(0.4) | 0.012 |
| No | | | |
| Eggs | | | |
| Yes | 1.4(1.2) | 1.3(0.4) | 0.264 |
| No | | | |
| Milk and Milk products | | | |
| Yes | 2.5(2.0) | 1.4(0.5) | <0.001 |
| No | | | |
| Oil and fats | | | |
| Yes | 3.8(2.5) | 1.5(0.6) | <0.001 |
| No | | | |
| Sweets | | | |
| Yes | 2.9(2.3) | 1.5(0.6) | <0.001 |
| No | | | |
| Spices, condiments, beverages | | | |
| Yes | 3.6(2.5) | 1.9(0.5) | <0.001 |
| No | | | |

As shown on table 5, majority (88%) of cases and nearly all (98.7%) of controls wear shoes always. Fifty-six percent of cases reported contact with fertilizers as opposed to 14.7% of the controls (AOR 7.3, 95% CI 3.6-15, P<0.05). Out of the 50 cases, 34 (68%) reported contact with animals/manure while only 27 (17.3%) controls reported similar contact. More than half (51%) of cases lived with livestock or animal manure as oppose to only 14.1% of the controls though the odds ratio did not show statistically significant difference. Forty percent of cases reported contact with pesticides and weedicides but only one tenth of controls reported such exposures (AOR 6.2, 95% CI 2.8-13.5) making this exposure statistically significant.

Only 1.3% of controls reported to have swam, waded or soaked in river, pool or lake, while 16% of cases reported such practice. Out of the 50 cases, 10 (20%) were working or living close to flower farms and none of the control groups live or work around flower farms. Seven (14%) cases used herbs but none of the controls used herbs for any reason in the past year.

**Table 5:** Risk factors and potential exposure of megaloblastic anemia study participants, East Shoa Zone of Oromia region, Ethiopia.

| Environmental exposure | Case n (%) | Control n (%) | P-value | AOR (95%CI) |
|---|---|---|---|---|
| Frequency of Shoe use | | | | |
| Always | 44(88.0) | 154(98.7) | 0.002 | |
| Sometimes | 5(10.0) | 1(0.6) | | |
| Never | 1(2.0) | 1(0.6) | | |
| Any contact with Fertilizer | | | | |
| Yes | 28(56.0) | 23(14.7) | <0.001 | |
| No | 22(44.0) | 133(85.3) | | 3.89(1.37, 11.03) |
| Contact with animals/manure | | | | |
| Yes | 34(68.0) | 27(17.3) | <0.001 | |
| No | 16(32.0) | 129(82.7) | | |
| Lived with livestock or animal manure | | | | |
| Yes | 25(51.0) | 22(14.1) | <0.001 | |
| No | 24(49.0) | 134(85.9) | | |
| Any contact with pesticide or weedicide | | | | |
| Yes | 20(40.0) | 15(9.7) | <0.001 | 3.27(1.01, 10.62) |
| No | 30(60.0) | 140(90.3) | | |
| Swim, wade or soak in river, pool or lake | | | | |
| Yes | 8(16.0) | 2(1.3) | <0.001 | |
| No | 42(84.0) | 154(98.7) | | |
| Work in or live close to flower farming | | | | |
| Yes | 10(20.0) | 0(0.0) | <0.001 | |
| No | 40(80.0) | 156(100) | | |
| Work in or live close to a factory | | | | |
| Yes | 13(26.0) | 0(0.0) | <0.001 | |
| No | 37(74.0) | 156(100) | | |
| Use of herbs for any reason | | | | |
| Yes | 7(14.0) | 0(0.0) | <0.001 | |
| No | 43(86.0) | 156(100) | | |

Sixty-eight percent of cases has current history of bleeding from one or more sites as compared to 10.3% of the controls. One fifth of cases had past history of bleeding from one or more sites, while only 1.2% of controls had such a history. Four of the 50 cases with bi/pancytopenia had been previously diagnosed and treated for anaemia and only less than one percent of the control groups reported treatment for anaemia in the past. Six percent of cases with bi/pancytopenia reported family history of bleeding disorder as opposed to only less than one percent of those without bi/pancytopenia.

Nearly a quarter of the cases had a history of past malarial attack but only 0.6% of the control group reported a prior history of malarial attack.

The Mean GA of cases and controls at the time of delivery was 35(+/-5.4) and 38(+/-3.2) weeks, $P<0.05$. The mean gravidity amongst the cases was nearly 3 and for the control group 2. There were two maternal deaths from cases, two had postpartum hemorrhage (PPH) but none from control group. Nearly a quarter (25%) of pregnancies with bi/pancytopenia were complicated by still birth as opposed to 4% of such incidents amongst the control group (Table 6).

**Table 6:** Reproductive history of megaloblastic anemia study participants, East Shoa Zone of Oromia region, Ethiopia.

| Reproductive history | Case n (%) | Control n (%) | P-value |
|---|---|---|---|
| Gravidity: mean(SD) | 2.9(±2.1) | 2.1(±1.3) | 0.002 |
| Gestational Age: mean (SD) | 35.2(±5.4) | 37.8(±3.2) | <0.001 |
| Maternal Outcome | | | |
| Alive | 46(92.0) | 151(100) | 0.003 |
| Maternal death | 2(4.0) | 0(0) | |
| PPH and live | 2(4.0) | 0(0) | |
| Perinatal Outcome | | | |
| Live birth | 36(75.0) | 144(96.0) | <0.001 |
| Still birth | 12(25.0) | 6(4.0) | |

## DISCUSSION

Although there are no well-studied environmental exposures associated with MA to date, in this study we have tried to explore potential environmental chemical exposures that may predispose to MA affecting multiple cell lines in the bone marrow. This case control study revealed pregnant mothers from east Shoa and Arsi zones of Oromia region,who were diagnosed to have MA (bi/pan cytopenia) were more likely to have contact with fertilizers and pesticides, to live near flower farming, to have contact with live stocks and animal manure, live close to factory and use herbal medication prior to the onset of the current disease than the controls. Adjusted Odds Ratio showed significant association of the disease with fertilizer and pesticide exposure although the study did not identify specific chemicals.

Because of the similarity in the clinical presentation and the geographic proximity of residential area of index cases reported in the case series and pregnant mothers affected by the disease in in this case control study, and as the stated residential area of the cases is a rapidly industrializing and urbanizing region of the country, exposure to similar toxic chemicals in a setup where industrial waste management is underdeveloped is highly likely (17).

Folic acid deficiency is the most common cause of MA accounting for 95% of MA during pregnancy, especially in low-income countries. Compared to iron deficiency anemia, MA is associated with poor maternal and perinatal outcome (18,19). The average GA at the time of delivery for cases in this study was lower than controls (35weeks vs 38 weeks) indicating the disease may be a predisposing factor for preterm labor, preterm delivery and all the complications related with prematurity. Similar to our cases, MA during pregnancy begins most often in either the third trimester or shortly after delivery (20).

Unlike nutritional deficiency MA that tends to be gradual in onset, the presentation of our patients was acute and complicated by bleeding (8, 15, 16).Our cases red blood cell indices, peripheral morphology and bone marrow examination revealed classic features of MA.

The cases and controls are comparable in most sociodemographic variables including age but most of our cases were farmers compared to the controls. Though in this study difference in nutritional pattern and frequency of eating certain food items between cases and controls was observed, there was no much difference in folic acid and vitamin B12 rich diet suggesting that derangement in metabolism of folic acid/Vit B12 may also be a cause for MA as anything that interferes with absorption, transport and chemical reaction at cellular level may also manifest with the same clinical and laboratory feature of dietary deficiency MA (16). Cobalamin and folate arenecessary for the production of tetrahydrofolate, and therefore DNA synthesis and hence are vital for rapid proliferation of cells as in the case of growing fetus (21).

Symptoms and signs of anemia appear when the drop in hemoglobin reaches the critical level and impairs tissue oxygenation, leading to compensatory tachycardia. Our cases had all clinical features of severe anemia and bleeding from different parts of the body before blood product was transfused. Adequate number of functionally competent platelets are required to achieve optimal hemostasis in the body. As the severity of MA worsens, neutropenia and thrombocytopenia develop, leading to fever and bleeding. Circulating platelets are often functionally compromised, although megakaryocytes appear normal (22,23).

The difference in the meanlowest hematocrit, platelet and white cellcount is significant between cases and controls in this study. The lowest mean platelet level in cases was 30,000/mm3, which is below the lowest critical level required to achieve hemostasis. Disorders in folic acid/Vit B12 metabolism affect cellular proliferation at different stage of cell division. Mild to moderate derangements manifest with red cell abnormality and birth defects. When the derangement becomes sever, it affects all cell lines during hematopoiesis (21). The finding of pancytopenia/ bicytopenia in our cases compared to controls suggest the derangement in folic acid/vit B12 metabolism could be significant to affect multiple cell lines.

The rate of PPH in cases was 4% and there were two maternal deaths (case fatality rate of 4%) but no PPH and mortality was recorded in controls. The deaths were cases with severe anemia and thrombocytopenia, who went to spontaneous labor and referred from Adama Hospital to TASH and delivered up on arrival before anemia and low platelet was corrected. One out of four deliveries in cases were stillbirth, compared to 4% in controls suggesting that the disorder also contributes to poor perinatal outcome.

In conclusion, although this study could not specifically identify the cause/s of the cytopenias in the study subjects, it has identified some environmental exposures as risk factors and potential causes of megaloblastic anemia.

***Recommendation:*** Based on the preliminary data of risk factors identified in this study, future large-scale community based study may unravel the cause/s of severe megaloblastic anemia particularly affecting pregnant mothers in east shoa and Arsi zones of Oromia region of Ethiopia.

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