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Modifiable determinants of iron deficiency and anemia among primigravidae and multigravidae in western Kenya: a secondary analysis of the Mama SASHA cohort study on vitamin A

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Abstract

Modifiable determinants of iron deficiency and anemia among primigravidae and multigravidae in western Kenya: a secondary analysis of the Mama SASHA cohort study on vitamin A
By Alysse Kowalski

Background: Maternal anemia during pregnancy is an important public health problem.

Objective: This study aimed to identify determinants of iron deficiency (ID) and anemia among pregnant women in western Kenya.

Methods: Between March-April 2011, 505 pregnant women attending their first antenatal care visit from eight health facilities were enrolled in the Mama SASHA cohort study on vitamin A. Standardized questionnaires were used to collect data on household characteristics, food security and dietary diversity, uptake of health services, and participation in existing agriculture, water, sanitation and nutrition programs. Weight and mid-upper arm circumference were measured and capillary samples were drawn for purposes of determining hemoglobin (Hb), vitamin A, iron, and inflammation status. Vitamin A and iron status indicators were adjusted for inflammation using the correction factor approach described by Thurnham. Primary outcomes of interest were anemia defined as Hb <11.0 g/dL and ID defined as plasma ferritin <12 mg/L. Multivariable logistic regression was used to identify modifiable determinants of ID and anemia overall and by parity.

Results: The prevalence of ID was 22.6% and was significantly higher among primigravidae compared to multigravidae (29.4% vs. 19.7%, p=0.02). The prevalence of anemia was 31.5% and did not differ by parity. In multigravidae, the receipt of iron supplements in the pregnancy prior to the current one (POR 0.49 (0.27, 0.87)) and receipt of WASH interventions (POR 0.53 (0.29, 0.97) were each associated with reduced odds of ID. Stillbirth/miscarriage in the pregnancy prior to the current one and a birth interval of less than one year however were both associated with increased odds of ID (POR 5.54 (2.06, 14.87), and POR 4.25 (1.68, 10.74), respectively). Among primigravidae none the determinants considered were associated with ID or anemia. The only modifiable factor associated with anemia was early convalescence infection (POR: 5.23 (1.91, 14.29). No dietary or household food security indicators were associated with either ID or anemia in multiparas or primiparas.

Conclusion: Our results indicate the etiology of ID may differ by gravidity, although further research is needed to identify determinants associated with ID among primigravidae. Due to these etiological differences, different prevention and control strategies for primigravidae and multigravidae may be warranted. Furthermore we found evidence to support a lasting impact of WASH support programs, like LifeStraw in protecting against ID.

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Chapter 1 Literature Review

Anemia comes from the Greek word *anaimia*, which directly translates to "without blood." Anemia is the state of clinically detectable, low hemoglobin concentration. At the individual level, the consequences of anemia include fatigue and impaired cognitive development. At a societal level, the consequences of anemia have implications for economic growth and development. Pregnant women and young children are at increased risk for anemia due to increased demands by the body during periods of rapid growth. Therefore, global estimates on the prevalence of anemia tend to focus on these populations.

The 2013 Lancet series on Maternal and Child Nutrition estimated the global prevalence of anemia was 38.2% (34.3, 42.0) among pregnant women and 42.7% (38.7, 46.9) among children <5.(R. E. Black et al.) This estimate has remained unchanged over the past five years.(R. E. Black et al.) There are regional disparities in the prevalence of anemia; the greatest burden is seen in Africa and Southeast Asia. It has been estimated that 50% of pregnant women and 40% of non-pregnant women in Africa are anemic.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) The prevalence of anemia among pregnant women in East Africa is estimated to be 35.6% (31.4, 39.9); 17.1% (15.0, 19.1) of which is due to iron deficiency.(Black RE) In Kenya, 1999 estimates of anemia among pregnant women were 55.1% (48.1–61.9) and 69% (66.5–71.4) among preschool-age children.

The etiology of anemia is multifactorial, although iron deficiency (ID) is estimated to be the cause of 50% of the global burden of anemia.(World Health Organization; WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) While global estimates for the prevalence of ID do not exist, it is thought to be the most common nutritional disorder in the world. The consequences of anemia are the same regardless of its origin. While iron deficiency anemia (IDA) is the most severe form of ID, less severe ID still has consequences on birth outcomes and cognitive development.

This literature review provides an overview of ID and anemia in pregnancy, describing the different causes of anemia and ID, the progression of ID to IDA, and the consequences poor iron status in general and during pregnancy. It provides a background on iron metabolism and the stages of ID, as well as describing the clinical indicators for diagnosis. Lastly, this section describes potential interventions to treat and prevent anemia and ID. Where possible, specific information is provided about ID and anemia in Kenya and other malaria-endemic areas, as the dataset used in analysis is from a malaria endemic region of western Kenya.

The literature for this section was drawn from Medline, Web of Science, Popline, and Google Scholar. The search was restricted to 2003-January 2014 with the exception of landmark articles and included peer reviewed articles, published textbooks, and conference proceedings. All sources were published in English and search terms focused on determinants of iron status and anemia in pregnancy, consequences of ID and anemia, and methods for measuring and estimating iron status in regions with high levels of infection.

Types and Causes of Anemia

Anemia is defined by having a low hemoglobin concentration. Hemoglobin is found in red blood cells and binds oxygen during respiration, however when the hemoglobin concentration is low, the body's ability to bind adequate amounts of oxygen s impaired. This results in tissue hypoxia.(*Nutrition and Health in Developing Countries*)

Anemia is multifactorial; therefore an anemia diagnosis is non-specific and does not identify the underlying cause of the anemia. Anemias can be categorized by underlying cause. Three broad categories of anemias include: genetic anemias, nutritional anemias, and anemias from infection. The mechanism by which hemoglobin concentrations are reduced, differs across these categories. Hemoglobin concentrations can be reduced by blood loss, decreased erythrocyte production, and increased erythrocyte destruction. The different categories of anemia are described below.

Genetic anemias arise from inherited blood disorders that affect hemoglobin synthesis. Inherited blood disorders are common in sub-Saharan Africa; in western Kenya, it has been shown that two out of three pre-school children have one or more inherited blood disorders.(Suchdev et al.) Blood disorders are thought to confer a survival advantage against death from *Plasmodium falciparum* malaria, although the mechanisms are not understood and likely involve a host of factors. It is thought that genes have been naturally selected over time, resulting in high population frequencies of inherited blood disorders in malaria-endemic areas.(Weatherall)

Inherited blood disorders that affect hemoglobin synthesis. Broadly speaking, hemoglobinopathies refer to structural abnormalities in the globin proteins, while thalassemias refer to the underproduction of globin proteins. Hemoglobinopathies and thalassemias can exist simultaneously as well as independently. In western Kenya, children with α^{+}-thalassaemia had hemoglobin concentrations 4–6 g/L lower than children without blood disorders.(Suchdev et al.)

Nutritional anemias can result from deficiencies in iron, vitamin A, folate, and vitamin B_{12}. Nutrient deficiencies affect erythrocyte production, although the different deficiencies impact erythrocytes differently. Folate and vitamin B_{12} deficiencies effect nucleic acid synthesis resulting in macrocytic red blood cells; ID effects erythropoiesis, resulting in the production of microcytic red blood cells; and vitamin A deficiency interferes with iron metabolism.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*)

Nutritional anemias are often driven by diet quality. The quality of a women's diet is affected by social factors including poverty, household food security, and household food allocation.(Khan and Bhutta) Food preferences and cultural food taboos, particularly during pregnancy are also contributing factors to inadequate consumption.(Shannon K et al.; Begum S and B) ID is the most common cause of anemia worldwide. Nutritional ID can result from low dietary intake or absorption. Dietary iron exists in two forms, heme and non-heme iron. Foods rich in heme iron include organ meat, oysters, and egg yolks. Non-heme iron can be found in dark green leafy vegetables and iron fortified cereals.

Anemias of infection can be caused by malaria, intestinal parasites, and HIV. Parasitic infections such as hookworm, trichuriasis, amebiasis, and schistosomiasis can result in blood loss which contributes to ID and anemia.(*Nutrition and Health in Developing Countries;* WHO *Iron*

Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers) Malaria infection results in hemolysis, in which red blood cells are destroyed. In Tanzania 60% of severe anemia cases in infants were due to malaria, while 30% were due to ID.(Menendez et al.) Concurrent ID and malaria will exacerbate anemia.(Menendez et al.)

Anemia in pregnancy. The recommended daily intake of iron is 15 mg/day for non-pregnant women ages 10-45 years; in pregnancy the recommended intake increases to 45 mg/day.(*Nutrition and Health in Developing Countries*) In pregnancy the blood volume expands, requiring the production of additional hemoglobin to maintain the same hemoglobin concentration.(*Preventing and Controlling Anaemia through Primary Health Care: A Guide for Health Administrators and Programme Managers*) Substantial amounts of iron are also deposited in the placenta and fetus during pregnancy.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) Women need an additional 700-850 mg of body iron over the course of a pregnancy to maintain adequate iron status.(Milman "Prepartum Anaemia: Prevention and Treatment"; WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) While some iron is retained through stopping menstruation, many pregnant women have inadequate iron stores and few change their dietary practices enough to not require supplementation.(Umbreit; Milman "Prepartum Anaemia: Prevention and Treatment")

Following childbirth, the iron deposited in fetal and supportive tissues is lost from the mother. Blood loss during childbirth can result in ID or IDA for the mother.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) Iron is expressed through the breast milk, however lactational amenorrhea more than compensates for the small amounts of iron a mother loses from breastfeeding.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*)

There are also broader, more distal social factors that contribute to a women's increased risk of ID and anemia during pregnancy. High parity and short interpregnancy intervals deplete the tissues of essential nutrients and increase the risk of ID and anemia for the mother and child.(Ahmed, Hossain and Sanin) Globally, IDA contributes to 447,000 maternal deaths that equate to 12.9 million disability-adjusted life years.(Bhutta et al.)

Because an anemia diagnosis is non-specific, understanding its etiology is important when considering an individual treatment plan or public health intervention at the population level. At the population level, the etiology of anemia is greatly influenced by the location of the population of interest, as both genetic anemias and anemias of infection are more common in subtropical and tropical climates.

Iron Metabolism

Iron is essential for metabolic processes concerned with oxygen transport, oxidative metabolism, and cellular growth. Most iron is used by hemoglobin, but myoglobin, cytochromes, and enzymes all require iron to function.(*Nutrition and Health in Developing Countries*)

Iron metabolism begins with dietary iron intake. Dietary iron exists in heme and non-heme forms as mentioned earlier. Heme iron is more bioavailable than non-heme iron. In meat, 15-35% of

heme iron is bioavailable compared to non-heme sources where absorption ranges from 2% to 20%.(Monsen) Iron absorption is inhibited by phytates and lignins, found in high concentrations in coffee and tea; whole grains; and dark green leafy vegetables. Calcium chelates iron and is found in dairy products. (Umbreit) Non-heme iron absorption is facilitated by meat and ascorbic acid.

After being consumed, iron is absorbed through the lumenal membrane of the duodenum.(Umbreit) From that point forward, iron is transported by a carrier protein, as free-floating iron is toxic to the body. Iron is transported in the plasma by the transferrin protein.(Huebers and Finch) Transferrin binds with transferrin receptor protein expressed on the surface of cells. Transferrin receptor protein is expressed in proportion to the cell's need for iron (e.g. the greater the tissue's demand for iron, the more transferrin receptor protein it will express).(*Nutrition and Health in Developing Countries*) Transferrin binds with transferrin receptor where iron is taken up by the cell.

Transferrin transports iron to the bone marrow where erythropoiesis, or red blood cell production takes place. Red blood cells are packed with hemoglobin. They are anaerobic and lack organelles to create additional space for hemoglobin within the cell. Each hemoglobin molecule is made up of four protein subunits, each composed of protein and a heme group. Heme is an organometallic complex with a centrally located iron, surrounded by a porphyrin ring. Each heme can hydrogen bond with one oxygen molecule. Following production, erythrocytes are released into the bloodstream.

Red blood cells circulate in the blood stream for 120 days at which point they are recycled by the spleen. Macrophages in the red pulp of the spleen phagocytize the red blood cells, breaking them down into iron and bilirubin. Bilirubin is transported to the liver where it is excreted in bile. Iron is bound to transferrin and transported to the spleen or liver where it is either stored or transported to the bone marrow for red cell production. Iron is bound to the apoferritin protein for storage and stored as ferritin. The majority of iron is sequestered and reused.(*Nutrition and Health in Developing Countries*) Small amounts are lost through desquamation and excreted through the stool.(*Nutrition and Health in Developing Countries*)

ID reduces the number of hemoglobin molecules, which in turn reduces the body's oxygen carrying capacity. This reduced carrying capacity is detected by the kidneys, which then send erythropoietin to the bone marrow to stimulate red cell production. Erythropoietin signals for increased red cell production, however because there is not ample hemoglobin, the quality of the red blood cells is reduced. The cells become smaller and microcytic and hypochromic.

Stages of Iron Deficiency

IDA results from long-term negative iron balance that progressively diminishes iron stores, iron transport, and iron-dependent metabolic functions. Iron deficiency progresses through three stages: depletion of storage iron, iron deficient erythropoiesis, and IDA.(*Nutritional Anemias*)

Storage iron depletion is the earliest sign of ID. There is no functional consequence of storage iron depletion, however, the body lacks iron reserves to meet any future physiological or pathological requirements.(*Nutritional Anemias*) Following storage iron depletion, the body

progresses into iron deficient erythropoiesis. In iron deficient erythropoiesis the rate of iron delivery to the bone marrow is inadequate for red blood cell production. Anemia is not detectable at this point. At the point at which anemia is detectable, oxygen delivery has been impaired as well as significant tissue ID which results in functional consequences.(*Nutritional Anemias*) Reduced oxygen capacity results in fatigue and can result in tachycardia and tachypnea during physical exertion. Other physical manifestations include hepatosplenomegaly; kolnychlia or concave spoon nails; and cheilosis, or cracking in the corners of the mouth. The body's craving for iron can also result in pica behaviors, such as the consumption of ice or soil. Geophagy has been documented in Kenya.(Geissler; Geissler, Prince, et al.; Geissler, Shulman, et al.)

Diagnostic Indicators

The WHO classifies the public health significance of anemia in an area by its prevalence. The WHO classification system can ben seen in Table 1.(WHO *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*)

Table 1. Public health significance of anemia prevalence(WHO *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*)

Significance level	Prevalence
Normal	4.9 or lower
Mild	5-19.9
Moderate	20-39.9
Severe	≥40

Hemoglobin concentration is the indicator used for diagnosing anemia. The diagnostic cutoffs for anemia are dependent on sex, age, and physiologic state. The cutoff is lower for women than men due to the loss of iron through menstruation. The cutoff is lower for children and pregnant women due to the body's increased iron demands to support growth. In pregnant women, anemia is defined as having a hemoglobin concentration <11 g/dL and severe anemia is Hb <7 g/dL (see Table 2).(WHO *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*)

Table 2. Classification of Anemia Status in Pregnancy(WHO *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*)

Anemia Status	Hemoglobin Concentration
Mild	100-109 g/L
Moderate	70-99 g/L
Severe	<70 g/L

Anemia can be diagnosed in field settings by using a Hemocue hemoglobinometer (Hemocue).(Cohen and Seidl-Friedman) The Hemocue uses spectroscopy to measure hemoglobin concentration in a drop of blood collected from a capillary blood sample. Elevation above sea level is known to increase hemoglobin concentrations, therefore adjusting hemoglobin concentrations for the affect of elevation is standard.(WHO *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*) At higher altitudes the lower partial pressure of oxygen, reduces oxygen's binding capacity. The body responds by producing additional hemoglobin to compensate. Smoking is also known to increase hemoglobin

concentrations.(WHO *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*)

Hematocrit is used as another indicator for anemia. Hematocrit is the percent of the total volume of a blood sample represented by packed red blood cells. Normal hematocrit is approximately 40% in women and 45% in men. Hematocrit measurement requires a centrifuge and is often inappropriate in field settings.

Mean corpuscular volume allows for the determination of whether red blood cells are micro- or macrocytic. This can be useful identifying the cause of anemia; IDA results in hypochromic, microcytic red blood cells, while folate and B_{12} deficiencies are macrocytic in nature.

Plasma ferritin concentration estimates the size of iron stores. In an adult, 1 μg/L in plasma ferritin indicates the presence of approximately 8 mg of storage iron.(Bothwell) Plasma ferritin is quantified using an enzyme linked immunosorbent assay (ELISA).(Erhardt et al.) Ferritin concentrations <12 μg/L indicate depleted iron stores.(WHO *Assessing the Iron Status of Populations : Including Literature Reviews : Report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6–8 April 2004*)

Ferritin is an acute phase proteins, and its levels increase in the presence of infection and inflammation. The acute phase protein response can elevate ferritin levels, making them appear to be in normal range, when in fact they are low. Thus, in areas with low levels of inflammation, low serum ferritin concentrations are highly specific for ID, but its use in areas with high levels of infection and inflammation is problematic.(*Nutritional Anemias*)

Total iron binding capacity (TIBC) is a measure of the free, unbound plasma transferrin concentration. Transferrin levels rise in response to iron storage depletion before there is evidence of inadequate supply of iron to tissues. TIBC is also affected by inflammation.

Serum transferrin saturation with iron is normally 30-35%. The percent serum transferrin saturation decreases as the iron supply decreases. Serum transferrin saturation levels less than 15% are insufficient to maintain normal levels of iron synthesis. Low transferrin saturation in combination with high TIBC is indicative of ID. However, transferrin saturation can be reduced by other causes as well. Both serum iron and TIBC are measured through chromogenic techniques.

Erythrocyte protoporphyrin concentration increases in ID. Once the iron supply is insufficient for hemoglobin synthesis, the formation of heme from iron and protoporphyrin is also impaired. Excess protoporphyrin accumulates in the red blood cells where it can be measured as free erythrocyte protoporphryin. Elevated erythrocyte protoporphyrin concentration is not specific to ID and be elevated due to other causes.

Soluble transferrin receptor (sTfR) concentration increases in the presence of ID and iron deficient erythropoiesis. Transferrin receptor is a protein expressed on the cell surface to bind transferrin. Cells express transferrin receptor in proportion to their need for iron. Soluble

transferrin receptor is present in the plasma in direct proportion to transferrin receptor expressed on cells. As iron depletion occurs, cells begin expressing additional transferrin receptor, and the soluble transferrin receptor concentration increases as well. Soluble transferrin receptor is not sensitive to inflammation.(Beguin) It is quantified using ELISA methods.(Erhardt et al.)

There is no gold standard indicator for assessing ID. The indicators described above each measure different time points in the progression from iron sufficiency to IDA. Serum ferritin is the closest thing to a gold standard, however its sensitivity to infection and inflammation make it less than ideal in settings where these are rampant. Different methods have been proposed for estimating iron status in areas with high levels of inflammation and infection including multiple indicator approaches that diagnose ID on the basis of having more than one abnormal indicator of iron status and correction factor approaches that aim to adjust ferritin values for the presence of infection.

In the multiple indicator approach, indicators (typically three) are considered in combination.(*Nutritional Anemias*) If a minimum number of indicators are abnormal (typically two or more in the three indicator approach), ID status is assigned.(*Nutritional Anemias*) The multiple indicator approach for diagnosing IDA considers the detection of anemia in association with a combination of laboratory tests indicating ID, specifically a measure indicating low iron stores and suboptimal iron delivery (iron deficient erythropoiesis). Considering more than one indicator has been shown to improve the accuracy of measuring IDA. In the ferritin model, serum ferritin, transferrin saturation, and erythrocyte protoporphryin are considered as indicators of iron status. Two or more abnormal indicators are said to indicate impaired iron status.(*Nutritional Anemias*) The ferritin model was used in NHANES II and III.(*Nutritional Anemias*) Other combinations include hemoglobin, serum transferrin receptor, and serum ferritin.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*)

In the correction factor approaches, serum ferritin is adjusted for the presence of inflammation as measured by the acute phase proteins, C-reactive protein (CRP) and α-1-acid glycoprotein (AGP). CRP rises quickly in response to infection and/or inflammation, but also subsides quickly. AGP is slower to respond, but remains elevated longer and is a better indicator of chronic, sub-clinical infection.(WHO *Assessing the Iron Status of Populations : Including Literature Reviews : Report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6–8 April 2004*) In the Thurnham method a correction factor is developed from the ratio of the geometric mean of the healthy group to the geometric mean of the inflammation group.(Thurnham et al.) In the regression method a correction factor is developed from linear regression models using CRP and AGP as predictors of ferritin.

Consequences of Anemia and Iron Deficiency

Because iron is used throughout the body, the consequences of ID and anemia are numerous. There are consequences of ID that occur before anemia can be detected due to tissue ID. Tissue ID adversely affects muscle's ability to use energy sources. The most severe consequences of ID are reduced work capacity and impaired cognitive development in children and adolescents. For these reasons, at the population level, ID has strong socioeconomic consequences.(Grantham-

McGregor and Ani) Some individuals do not experience symptoms of ID, however it has been shown that oxygen consumption is reduced.(WHO *Assessing the Iron Status of Populations : Including Literature Reviews : Report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6–8 April 2004*) Other consequences of ID include impaired immune function, DNA replication and repair, and hormone production and regulation.*(WHO Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers)* The body's absorption of heavy metals also increases.(*Nutrition and Health in Developing Countries;* WHO *Assessing the Iron Status of Populations : Including Literature Reviews : Report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6–8 April 2004*)

Because not enough oxygen is getting into the body in anemia, the heart is forced to work harder, resulting in tachycardia, which could result in chest pain and tachypnea. This results in other symptoms including: fatigue, weakness, dizziness, headache, and restless legs. (Milman "Anemia--Still a Major Health Problem in Many Parts of the World!") Associations have been shown between anemia and emotional stability and depression.(Milman "Anemia--Still a Major Health Problem in Many Parts of the World!") Other physical symptoms include glossitis, stomatitis, and koilonychia. The consequences of ID previously described increase in severity as iron status worsens, specifically work productivity and slowed child development.(Stoltzfus) Mild-to-moderate anemia may increase susceptibility to infectious disease.(Stoltzfus) Extremely severe anemia can result in death.(Stoltzfus)

Pregnant women and children are at high risk for developing ID and anemia and there are unique consequences of IDA in these groups. Mothers and neonates are at an increased risk of mortality in the perinatal period (22 weeks gestation to seven days after birth).(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*). As high as 40% of maternal perinatal deaths have been linked to anemia.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) IDA in the first two trimesters increases the incidence of pre-term labor and low-weight births.(Scholl et al.) During delivery, mothers are at an increased risk of hemorrhage and sepsis.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) Blood loss during delivery can further aggravate anemia into postpartum.

Anemic mothers are 30% less likely to have favorable birth outcomes.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) Children are at an increased risk of prematurity, low birth weight, and perinatal mortality.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) Low birth weight is the result of impaired intrauterine growth and development and has been associated with chronic disease later in life including coronary heart disease, hypertension, and type 2 diabetes.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*)

Full term infants are normally born with adequate iron stores, but infants born to anemic mothers can be born with less than half of the normal iron reserves and will require more iron than what

is supplied by breast milk.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) Studies have shown that infants and children born to iron deficient mothers have poorer cognitive development and a lower IQ than infants and children born to iron-replete mothers.(Milman "Anemia--Still a Major Health Problem in Many Parts of the World!") In infants and preschool children, IDA results in decreased motor activity, attention, and social interaction.(Pollitt) The consequences of IDA in infancy are thought to be largely irreversible.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*)

Prevention and Control Strategies

It is important to identify the modifiable determinants of ID and anemia to develop effective treatment strategies at the individual level, or public health prevention and control programs at the population level. This also requires understanding who is at risk so high risk individuals can be targeted, and tailoring interventions so they are culturally appropriate. Strategies for the prevention and control of nutritional ID include fortification and improving dietary diversity. Strategies for reducing IDA from infection include water, sanitation, and hygiene (WASH) interventions, and insecticide treated bed nets (ITN). Supplementation is a key strategy for preventing ID in pregnancy.

Fortification has been shown to be effective in improving nutritional status, however rigorous monitoring programs need to be put in place to ensure subpopulations (such as men) do not experience iron overload. Fortification typically targets staple grains, however this may not be practical in agrarian societies such as western Kenya where the majority of households grow and mill their own grains. Fortification strategies aim to improve iron stores, however supplementation would likely still be necessary during pregnancy to meet the body's demands. Improving dietary diversity is a food-based approach to improving iron status. In the context of iron, this typically means improving meat consumption. Costs and effects of dietary diversity interventions are not known at this time.(WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*)

Other strategies to prevent and control anemias from infection include WASH interventions. These interventions are thought to reduce the risk of parasites that contribute to anemia. Additionally malaria prevention programs, such as ITN that decrease the prevalence of malaria, thereby reduce the risk of ID from hemolysis of red blood cells.

In pregnancy, iron supplementation is a key strategy to preventing ID. Supplements come in tablet or syrup form, typically made with ferrous sulfate. Side effects from iron supplements include: constipation, stomach pain, heartburn, nausea, vomiting, and diarrhea. Iron supplements can also make the stool turn black.(Umbreit) The side effects from supplements are a barrier to adherence. Reproductive health programs that support appropriate and adequate birth spacing could contribute to improved iron status.

Anemia in Kenya

In Kenya, a 1999 survey estimated the prevalence of IDA among pregnant women to be 55.1%. The maternal mortality rate in Kenya has increased over the past two demographic and health surveys (DHS) from 414 deaths per 100,000 live births in 2003 to 488 maternal deaths per

100,000 live births in 2008-09.(Division of Nutrition) Neonatal deaths accounted for approximately 60 percent of <5 mortality.(Division of Nutrition)

The 2008-2009 DHS reported on iron supplementation use during pregnancy. The proportion of women taking any iron supplements during pregnancy increased from 41% in 2003 to 60% in 2008-09, however most women take supplements for less than 60 days.(Kenya National Bureau of Statistics and ICF Macro) In Western province, over half (54%) of women reported taking iron tablets or supplements for less than 60 days during the pregnancy of their most recent birth and 36% report not taking any iron supplements at all.(Kenya National Bureau of Statistics and ICF Macro) Nationally only 2.5% of women took supplements for ≥90 days, in Western province this was only 1%.(Kenya National Bureau of Statistics and ICF Macro)

Iron supplementation is often administered concurrently with folic acid supplementation. The recommended dose is 60mg iron and 400µg folic acid daily from the first month of pregnancy for 6 months. Iron folic acid (IFA) supplementation has been shown to reduce maternal anemia, maternal mortality, and LBW.(Haider et al.) Previously, high dose (200 mg) iron supplements were used in Kenya. These supplements were associated with side effects such as constipation and gastrointestinal effects including nausea, vomiting and diarrhea. The Ministry of Health (MOH) has recently introduced enteric coated and combined formulations (60mg Fe and 400µg FA).(Division of Nutrition) The lower dose should help with side effects and ideally improve compliance.

Targeted IFA supplementation to pregnant mothers is the primary IDA prevention strategy used by the MOH in Kenya.(Division of Nutrition) IFA supplementation is a routine service provided with antenatal care.(Division of Nutrition) Primary challenges to accessing IFA supplementation include low and delayed receipt of antenatal care by pregnant women, low provider knowledge, and IFA supplement stock outs.(Division of Nutrition) Less than half of all pregnant women receive the recommended four or more antenatal care visits.(Kenya National Bureau of Statistics and ICF Macro) Approximately 13% of women in rural areas receive antenatal care within the first four months of pregnancy.(Kenya National Bureau of Statistics and ICF Macro) It has been shown that most providers have limited knowledge of which routine services should be administered.(Division of Nutrition) A number of health workers insist on screening women for anemia first and only prescribing IFA supplements to women with anemia.(Division of Nutrition) Prior to switching to a combined IFA supplement, data from the Kenya Service Provision Assessment in 2012 showed only 41% of health facilities had iron tablets and that 74% had folic acid supplements.(Division of Nutrition) ITN usage in Kenya has increased among pregnant women. In 2003, only 4% of pregnant women slept under an ITN compared with 49% in 2008-09.(Kenya National Bureau of Statistics and ICF Macro)

In 2012, the Ministry of Health Division of Nutrition released, *Accelerating Reduction of IDA Among Pregnant Women in Kenya*, a six year, ~$12 million plan of action for IDA prevention and control during pregnancy.(Division of Nutrition) The plan has policy, service delivery, behavior change communication, supply chain management, and monitoring components to strengthen IFA supplementation and reduce IDA.(Division of Nutrition) The plan also acknowledges the importance of multi-sector collaboration with other divisions including divisions of Reproductive Health and Malaria and Environmental Health.(Division of Nutrition)

While previous research into the determinants of anemia in western Kenya has been conducted, this analysis will explore the contributions of different determinants that have not been considered previously. These include indicators of maternal and household diet, household food security status, WASH, and obstetric history among multigravidae. This analysis will also explore differences in determinants by gravidity. Lastly, this analysis aims to identify modifiable determinants of ID and/or anemia to inform future anemia prevention and control strategies.

Chapter 2: Manuscript

Modifiable determinants of iron deficiency and anemia among primigravidae and multigravidae in western Kenya: a secondary analysis of the Mama SASHA cohort study on vitamin A

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Abstract

Background: Maternal anemia during pregnancy is an important public health problem.

Objective: This study aimed to identify determinants of iron deficiency (ID) and anemia among pregnant women in western Kenya.

Methods: Between March-April 2011, 505 pregnant women attending their first antenatal care visit from eight health facilities were enrolled in the Mama SASHA cohort study on vitamin A. Standardized questionnaires were used to collect data on household characteristics, food security and dietary diversity, uptake of health services, and participation in existing agriculture, water, sanitation and nutrition programs. Weight and mid-upper arm circumference were measured and capillary samples were drawn for purposes of determining hemoglobin (Hb), vitamin A, iron, and inflammation status. Vitamin A and iron status indicators were adjusted for inflammation using the correction factor approach described by Thurnham. Primary outcomes of interest were anemia defined as Hb <11.0 g/dL and ID defined as plasma ferritin <12 mg/L. Multivariable logistic regression was used to identify modifiable determinants of ID and anemia overall and by parity.

Results: The prevalence of ID was 22.6% and was significantly higher among primigravidae compared to multigravidae (29.4% vs. 19.7%, p=0.02). The prevalence of anemia was 31.5% and did not differ by parity. In multigravidae, the receipt of iron supplements in the pregnancy prior to the current one (POR 0.49 (0.27, 0.87)) and receipt of WASH interventions (POR 0.53 (0.29, 0.97) were each associated with reduced odds of ID. Stillbirth/miscarriage in the pregnancy prior to the current one and a birth interval of less than one year however were both associated with increased odds of ID (POR 5.54 (2.06, 14.87), and POR 4.25 (1.68, 10.74), respectively). Among primigravidae none the determinants considered were associated with ID or anemia. The only modifiable factor associated with anemia was early convalescence infection (POR: 5.23 (1.91, 14.29). No dietary or household food security indicators were associated with either ID or anemia in multiparas or primiparas.

Conclusion: Our results indicate the etiology of ID may differ by gravidity, although further research is needed to identify determinants associated with ID among primigravidae. Due to these etiological differences, different prevention and control strategies for primigravidae and multigravidae may be warranted. Furthermore we found evidence to support a lasting impact of WASH support programs, like LifeStraw in protecting against ID.

Introduction

The consequences of anemia during pregnancy for the mother and child are well established. Anemia in late pregnancy increases the risk of maternal mortality.(Stoltzfus RJ) There is strong causal evidence for a link between maternal iron deficiency anemia (IDA) and low birth weight and increased perinatal mortality.(Allen; Christian; Rasmussen and Stoltzfus) In young children, the prevalence of IDA peaks around 18 months of age and then falls as iron requirements decline and iron intake is increased through complementary foods.(Robert E. Black et al.) Anemia has consequences on cognitive development. For every 10 g/L decrease in hemoglobin concentration, a child's IQ decreases by 1.73 IQ points 73 (95% CI 1·04–2·41).(Stoltzfus RJ) These consequences are thought to be largely irreversible.

While the etiology of anemia can be multifactorial, it is generally believed that approximately half of all cases of anemia are due to iron deficiency (ID). A 2002 WHO review assumed 60% of anemia was due to iron deficiency in non-malaria areas and 50% in malaria areas.(Tanuja Rastogi) However, this rule of thumb may not be accurate in areas with high levels of infection and inflammation. This was reflected in the 2013 Lancet estimates of the global prevalence of anemia where the prevalence of anemia in east Africa was estimated to be 34%, only 17% of which was estimated to be due to iron deficiency.(R. E. Black et al.) Due to this multifactorial nature, understanding the local etiology and determinants of anemia are important to the development of local anemia prevention and control programs.

Where anemia is in fact due to ID, early detection and treatment in pregnancy is important, as the increased iron demands of pregnancy can cause ID to progress to IDA. ID progresses to IDA through three stages: depletion of iron stores, iron deficient erythropoiesis, and anemia, resulting in a reduction in hemoglobin concentration due to a restricted supply of iron to the bone marrow for erythropoiesis.(Gibson) A major cause of IDA is low consumption of iron rich foods.(Bhargava, Bouis and Scrimshaw)

The first aim of this analysis was to estimate the prevalence of anemia and iron deficiency in a cohort of pregnant women in western Kenya. The second aim was to identify modifiable determinants of ID and/or anemia and explore any differences in determinants by gravidity.

Methods

Overview

Sweet potato Action for Security and Health in Africa (SASHA) is a five-year, multi-partner project led by the International Potato Center to improve the food security and livelihoods of poor families in sub-Saharan Africa by maximizing the potential of the orange-fleshed sweet potato (OFSP). Mama SASHA was a proof-of-concept project (PoCP) that tested the effect of an integrated intervention that coupled homestead OFSP production with enhanced health services and nutrition education on the vitamin A and health statuses of pregnant and lactating women and their children. Almost 5000 women in the intervention sites received vouchers for OFSP and nutrition education through antenatal care, postnatal care, and community-based mother support groups. Women in the control sites received usual care. Mama SASHA took place from 2011-

2014 with baseline and endline surveys occurring in March-April 2011 and March-April 2014, respectively.

Mama SASHA was embedded within the USAID AIDS, Population and Health Integrated Assistance Program (APHIA II/APHIAplus) and was implemented in eight health facilities in Bungoma and Busia counties in western Kenya. Health facilities were purposively selected based on size-related variables (i.e. number of service providers, antenatal clinic attendance numbers, and population served), coverage by APHIA II/APHIAplus community health workers (CHW), and location criteria (e.g. selected health facilities were approximately 30-50 km apart from each other).

The Cohort Study on Vitamin A (COVA) was a 28-month longitudinal cohort study nested within the larger Mama SASHA PoCP. COVA is a sub-study of Mama SASHA that follows a cohort of 500 women from mid-pregnancy through 9 months postpartum. While the aim of COVA was to rigorously assess the impact of Mama SASHA on maternal and infant vitamin A and health statuses, this is a secondary analysis examining the modifiable determinants of iron status among pregnant women using the COVA enrollment data.

Study Population

The enrollment of 505 pregnant women into COVA took place between November 2012 and March 2013. Prior to being enrolled, women were referred to the study by ANC nurses from the 8 participating health facilities (4 intervention, 4 control) screened for eligibility, and consented. In order to be eligible, pregnant women had to be: attending their first ANC visit; 17-40 years of age; 10-24 weeks gestation determined by maternal self-report of last menstrual period (or if unavailable, by nurse palpation); intending to breastfeed; and intending to reside in the catchment area until child was 10 months of age. Women were ineligible if they: did not meet the eligibility criteria; had previous involvement with Mama SASHA in an earlier pregnancy; did not reside in the catchment area for the health facility visited.

Data Collection and Procedures

Questionnaire Data

Data were collected using a standardized questionnaire administered by trained research assistants in English, Kiswahili, or Luyha, depending on the respondent's preference. The questionnaire was developed in English, translated into Kiswahili and Luhya, piloted and revised prior to the start of the study. While the enrolllment questionnaire contained a number of modules, modules relevant to this analysis include: 1) household characteristics; 2) household food security and dietary diversity; 3) maternal diet; 4) uptake of health services; and 5) project participation and participation in other programs.

Household food security was assessed using the FANTA Household Food Insecurity Access Scale (HFIAS).(Coates, Swindale and Bilinsky) The HFIAS has been validated in different developing country contexts and provides an accurate measure of food insecurity in the 30 days preceding the survey when adapted and translated to the local setting.(Coates, Webb and Houser; Frongillo and Nanama; Knueppel, Demment and Kaiser; Webb, Coates and Houser) The HFIAS has been validated for use in the Rift Valley and Coast provinces of Kenya (Webb Girard, unpublished). Household dietary diversity and consumption of iron-rich food and maternal diet

were assessed according to the FAO Guidelines for Measuring Household and Individual Dietary Diversity.(Kennedy, Ballard and Dop; Swindale and Bilinsky) Levels of household dietary diversity were defined by household consumption of foods from different food groups in the previous 24 hours as reported by the mother. Consumption of 3 or fewer different food groups was classified as low, 4-5 food groups, moderate, and >6 as high dietary diversity.

Collection of Biochemical and Anthropometric Data

Mid-upper arm circumference (MUAC) was measured using a stretchable, non-flexible tape at the mid-point of the non-dominant upper arm, between the acromion process and the tip of the olecranon. Under-nutrition was defined as MUAC <22 cm.(Ferro-Luzzia and James)

Biochemical indicators were assessed from a capillary blood sample. Hemoglobin (Hb) concentration was quantified in the field using the Hemocue hemoglobinometer model HB201^{+}. Hemoglobinometers were calibrated each morning according to protocol. Hb was adjusted for altitude. Pregnant women with Hb concentrations < 11.0 g/dL were considered anemic.(WHO *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*)

Approximately 500 µL of capillary blood was collected into a microtainer with EDTA for plasma separation (Becton Dickinson). The blood was then centrifuged at 1500 × g for 5 min at 27^{o}C and the plasma was transferred into cryovials (Thermo Fisher Scientific). The plasma samples were stored at -20^{0}C using solar powered freezers the health facilities and then periodically collected and transferred to the Kenya Medical Research Institute/CDC Malaria Laboratory in Kisumu, Kenya for long-term storage. Samples were shipped to Germany for analysis.

Samples were analyzed using a 5-protein ELISA method that simultaneously quantified retinol binding protein (RBP), plasma ferritin, soluble transferrin receptor, C-reactive protein (CRP), and α-1-acid glycoprotein (AGP).(Erhardt et al.) Abnormal biochemical indicator values were as follows: RBP, <1.17µmol/L, plasma ferritin, <12 µg/L; soluble transferrin receptor, >8.3 mg/L; CRP, >5 mg/L; and AGP, >1.0 g/L.

Ethics

Ethical approval for the study was obtained from the Kenya Medical Research Institute (KEMRI) Ethical Review Committee and Emory University Institutional Review Board. Pregnant women were informed on the purpose and procedures of the study, risk and constraints due to participation, strict confidentiality of the personal data, and the ability to refuse any or all portions without having to justify the refusal or losing access to health or project services. All participants provided written informed consent prior to data collection.

Statistical Analysis

Plasma ferritin and RBP are affected by subclinical inflammation. Therefore plasma ferritin and RBP were adjusted for inflammation using the correction factor approach described by Thurnham.(Thurnham et al.) In this approach the population was divided into categories by stage of infection: reference (no infection), CRP < 5ug/L; AGP < 1 g/L; incubation, CRP ≥ 5ug/L; AGP < 1 g/L; early convalescence, CRP ≥ 5ug/L; AGP ≥ 1 g/L; late convalescence, CRP < 5ug/L; AGP ≥ 1 g/L. Category-specific correction factors were then estimated from the ratio of

the geometric mean of the reference group to the geometric mean of the stage of infection. Plasma ferritin and RBP values were then adjusted by multiplying the individual values by the appropriate category-specific correction factor.(Grant et al.; Thurnham et al.) An alternative approach to adjusting for inflammation uses linear regression to develop correction factors. While we considered modifying the approach described by Engle-Stone et al. we felt it was inappropriate for this population, as correlation analyses by stage of infection did not show linear relationships between plasma ferritin and CRP or AGP or RBP and CRP or AGP.(Engle-Stone et al.)

Descriptive statistics were calculated for all variables. Measures of central tendency were examined for continuous variables. Continuous variables were assessed for normality by examination of skewness and kurtosis values and visual inspection of the histograms. Variable that did not adhere to a normal distribution were log transformed prior to further analysis. Normally distributed variables are reported as mean ± standard deviation. Log transformed variables are reported as geometric mean ± standard deviation. Frequency distributions were calculated for categorical variables and presented as frequencies and percentages.

Dichotomous variables were created for the outcomes of interest. Iron deficiency was defined as adjusted ferritin <12 mg/L. Anemia was defined as Hb <11.0 g/dL. Indicators of maternal nutritional status, inflammation status, diet, household food security status, household diet, and water, sanitation, and hygiene (WASH) were considered as potential modifiable determinants. Maternal age, gestational age, marital status, maternal education level, asset score, and district were considered as non-modifiable determinants. Separate bivariate analyses were carried out between ID and anemia and potential determinants. Continuous variables were compared using student's t-tests. Categorical variables were compared using chi-square tests.

Multivariate logistic regression was used to identify determinants associated with ID or anemia status. Variables were modeled as continuous variables where possible. Dummy variables were used for categorical variables with more than two levels. Due to the biological differences between primiparity and multiparity and the observed significant difference in prevalence of ID by parity, multivariable logistic regression models were stratified on parity where the sample size allowed.

Confounding was considered present if there was a >10% change between crude and adjusted prevalence odds ratio (POR) estimates. Models were adjusted for maternal age, gestational age, district, marital status, maternal education level, parity, household asset score, and MUAC. District was used as a proxy for health facilities as the sample sizes for the different health facilities were too small. Models were assessed for multicollinearity, defined as a condition index >30 and two or more variance decomposition proportions >0.5. No models exhibited multicollinearity. All statistical analyses were carried out in SAS 9.3 (SAS Institute, Cary, NC). Statistical significance was defined as $P < 0.05$.

Results

Sociodemographic characteristics of the 505 pregnant women are presented in Table 1. Women were an average of 24.59 ± 5.53 years of age. Thirty percent of the women had completed less

than a primary education (61.2%) and the majority (86.3%) were in monogamous relationships. Approximately 5% of women were from female-headed households. Forty percent of women did not work, while 35.4% worked in agriculture. Less than 4% of the population was HIV positive. Sixty-six percent of women reported receiving programmatic WASH assistance. Of these women, 98% had received support from LifeStraw®, a water filter produced by Vestergaard.

The average gestational age of first antenatal care visit was 20.44 ± 5.13 weeks. Thirty percent of women were primigravidae (n=153). Among the multigravidae, 52% reported receiving iron supplements in their most recent previous pregnancy; 6% of women had a stillbirth/miscarriage in their most recent previous pregnancy; and 7% had an interpregnancy interval of less than one year.

Anthropometric, biochemical, and diet indicators are presented in Table 2. Mean maternal MUAC was 26.03 ± 3.02 and 11% were malnourished, based on a cutoff of <22 cm. Practically all (99.6%) women reported consuming vitamin A rich food at least once in the previous week. Maternal self-reported consumption of iron rich foods at least once in the previous week was high (82.8%), however consumption of organ meat was low (10.3%). The prevalence of low, moderate, and high dietary diversity were 7.7%, 46.5%, and 45.7%, respectively. Food groups consumed by >50% of households with low food dietary diversity included cereals and dark green leafy vegetables. In moderate dietary diversity, consumption from the same food groups as households from low dietary diversity was observed as well as the addition of consumption from milk and oil groups. In high dietary diversity, the addition of consumption from vitamin A rich fruit and legumes and nut groups was observed. Twenty-three percent of households reported severe food insecurity. Maternal and household diet patterns and household food security distributions did not differ by ID or anemia status.

The prevalence of ID was 22.6% and was significantly higher among primigravidae compared to multigravidae (29.4% vs. 19.7%, p=0.02). The prevalence of anemia was 31.5% and did not differ by parity. Three women (0.59%) had severe anemia (Hb <7.0 g/dL), all of which were multigravidae. Among those with anemia, 37.7% had concurrent ID while 27% had concurrent vitamin A deficiency. The prevalence of vitamin A deficiency was 22%.

Biochemical indicators of ID and anemia by stage of infection are shown in Table 3. Seventy-six percent of the population was classified as having no subclinical infection (reference subgroup, CRP < 5ug/L; AGP < 1 g/L). The prevalence of ID was 22% in the reference subgroup compared to 22.6% estimated in the total population. The prevalence of anemia was 23.2% in the reference subgroup compared to 31.5% estimated in the total population.

Overall, no measures of maternal diet, household diet, household food security, or household WASH were associated with the odds of ID or anemia in crude or adjusted models (Table 4). Maternal MUAC was associated with ID; for every centimeter increase in MUAC, the odds of ID decreased 10% (POR 0.90 (0.83, 0.97)). Pregnant women in early convalescence (CRP ≥ 5ug/L; AGP ≥ 1 g/L) were 5.23 (1.91, 14.29) times more likely to be anemic compared to those in the reference group. However, inflammation was not associated with ID. The sample size did not allow for analysis of stages of infection by parity. The remaining findings are presented by parity.

Among multigravidae, receipt of iron supplements in a previous pregnancy was protective against ID (POR 0.49 (0.27, 0.87)). An interpregnancy interval of less than 1 year and experiencing a miscarriage/stillbirth in the previous pregnancy were each associated with significantly increased odds of ID 4.25 (2.06, 14.87); and 5.54 (2.06, 14.87), respectively. No associations were observed between these obstetric history indicators and anemia.
No meaningful and significant associations were observed between determinants of maternal and household diet or household food security. Among multigravidae, those who received WASH support had a 47% (POR 0.53 (0.29, 0.97)) reduction in the odds of being iron deficient compared those who did not receive WASH support, controlling for maternal age, gestational age, MUAC, maternal education level, parity, marital status, household wealth, and district. A similar but nonsignificant association was observed between WASH support and ID among primigravidae. Similar, but nonsignificant associations were observed between WASH support and anemia among both multigravidae and primigravidae. Non-modifiable determinants maternal age and gestational age were associated with ID and both ID and anemia in crude models, but these associations were nonsignificant in adjusted models.

Among primigravidae, we identified no significant associations between modifiable factors such as maternal nutritional status, diet, food security, or WASH and ID or anemia. Asset score and district of residence were significantly associated with anemia in crude models, but not in adjusted models. No other indicators were significantly associated with anemia or ID among primigravidae.

Discussion

In the Mama SASHA cohort study on vitamin A, situated in a malaria-endemic area of western Kenya, anemia is a moderate public health problem. The observed prevalence of mild–moderate anemia (31%) is consistent with the Lancet Maternal and Child Nutrition series estimate for the East Africa region and prevalence from western Kenya reported by Alusala et al.(Alusala et al.; Robert E. Black et al.) Iron deficiency, defined as adjusted plasma ferritin <12 μg/L was observed in 23% of the pregnant women. Our most salient findings were differences in the modifiable determinants of ID and anemia between primigravidae and multigravidae. In fact, among primigravidae, none of the determinants we analyzed were significantly associated with either ID or anemia. This lack of significant associations may be the result of a small sample size as only 153 participants were primigravidae. Among multigravidae, we saw significant associations between indicators of maternal obstetric history and ID that persisted after adjusting for confounding factors including maternal age, gestational age, district, marital status, maternal education level, parity, household wealth, and MUAC. In multigravidae, previous participation in a WASH program was also protective against ID but not anemia.

A significantly greater proportion of primigravidae were iron deficient compared to multigravidae. A greater proportion of primigravidae were anemic as well, although this was not statistically significant. Increased anemia prevalence in primigravidae has been reported previously.(Alusala et al.; Huddle, Gibson and Cullinan; Ndyomugyenyi et al.; Shulman et al.) It has been shown that in malaria endemic areas, *P. falciparum* infection in pregnancy is greatest among primigravidae and parasitemia concentrations decrease with increasing

gravidity.(Greenwood et al.; McGregor and Smith; McGregor; Steketee et al.) This difference is attributed to an altered immune response to malaria.(Huddle, Gibson and Cullinan) The prevalence of malaria peaks at 13-16 weeks gestation with hemolysis and subsequent anemia from hemolysis occurring in the second trimester, around the time this study took place.(Brabin)

Shulman et al. reported observing a higher prevalence of severe anemia among primigravidae and grand multigravidae. (Shulman et al.) In our study, severe anemia was only observed among multigravidae. Multigravidae are thought to be at increased risk for anemia due to the cumulative demands on iron stores from successive pregnancies.

There has been speculation that a short interpregnancy interval is associated with maternal IDA, but this has not been shown definitively. In a 2007 meta-analysis aimed at examining the effects of short interpregnancy intervals on maternal and child nutritional outcomes, three of the four studies examining the association between short interpregnancy interval and maternal anemia did not find a significant association, however there were methodological limitations in the included studies.(Dewey and Cohen) A 2012 review concluded their were too few quality studies to assess the impact of short interpregnancy intervals and maternal nutritional status, however short interpregnancy intervals were associated with pre-term birth, low birth weight, stillbirth, and early neonatal death in high and moderate income countries.(Wendt)

Adverse pregnancy outcomes in a previous pregnancy have been shown to be a risk factor for developing similar adverse pregnancy outcome or perinatal mortality in the pregnancy immediately following the pregnancy with the adverse outcome.(Greenwood; Singh, Goli and Parsuraman) Our results show increased odds of iron deficiency in the current pregnancy among multigravidae experiencing a previous stillbirth or miscarriage in the pregnancy immediately preceding the current pregnancy compared to multigravidae who did not.

Iron supplements have been shown to be effective in improving hemoglobin status during pregnancy and reduce the risk of low birth weight.(Haider et al.; Imdad and Bhutta) Using demographic and health survey data, Dibley and colleagues showed a reduction in risk of death in children under five if the mother reported consuming any antenatal iron-folic acid supplements.(Dibley et al.) This finding was striking in the absence of IFA compliance data, which is known to be low. We observed receipt of iron supplements in the pregnancy immediately preceding the current pregnancy reduced the odds of maternal ID in the current pregnancy.

One of the unique aspects of this study was its ability to examine associations between exposure to WASH interventions and ID and anemia. Previous participation in a WASH program was protective against ID after controlling for maternal age, gestational age, district, marital status, maternal education level, parity, household wealth, and MUAC. Ninety-eight percent of pregnant women reporting previous WASH program participation, participated in LifeStraw. LifeStraw produces water filters for both individual and household point of use water filtration. As a cross sectional assessment of association, the mechanism by which LifeStraw could improve iron status is unknown. While the LifeStraw Personal pipe reduced diarrheal disease in a randomized control trial, inconsistent usage of the personal pipe by the end of the five-month intervention questioned reporting bias in reporting diarrheal episodes.(Boisson et al.) It is possible that usage

differs between the personal pipe and family filtration system. It is unknown if there was an educational component to the program that could have changed household WASH practices.

This study is not without limitations. First, this study was cross-sectional, so it is not possible to evaluate the effects of the modifiable determinants considered on ID or anemia risk. Second, the small sample size among primigravidae in particular did not permit multivariable models examining the association between stages of infection and ID and anemia stratified on parity and likely weakened power to detect other potentially important associations. Third, this study lacked data on specific causes of inflammation and hemoglobinopathies. In particular, malaria, hookworm, and sickle cell trait have been named as determinants of hemoglobin concentration and anemia among pregnant women in other studies in Kenya.(Alusala et al.; Shulman et al.) Infection, malaria infection in particular, may alter concentrations of iron indicators, independent of iron status.(Engle-Stone et al.) Use of the inflammation markers CRP and AGP allowed us to correct for the effect of inflammation and infection, to the extent that their effect on iron status indicators is mediated through the acute-phase response.(Engle-Stone et al.)

Engle-Stone et al. observed that while different iron status indicators identified the same high-risk groups, different determinants were implicated depending on the iron indicator used.(Engle-Stone et al.) This has important implications for informing the design of anemia prevention and control programs that often operate with limited resources. Therefore further research is needed to determine the best methods for assessing iron status in populations where inflammation and infection are prevalent.

Our results show an increased prevalence of ID and anemia in primigravidae consistent with other findings. While we lack data on specific infectious agents and genetic characteristics, we observed unique associations between ID and a women's obstetric history from the pregnancy immediately preceding her current pregnancy and previous participation in a WASH program. In conclusion, the determinants of ID and anemia may differ by gravidity, making the case for additional research on determinants of ID and anemia and for subsequent differential prevention and control strategies for primigravids and multigravids.

Chapter 3 Public Health Implications

Iron deficiency anemia is known to increase the risk of adverse pregnancy outcomes for the mother and neonate. Anemia, however is multifactorial, and in addition to severe iron deficiency, anemia can be caused by other nutritional deficiencies of vitamin A, folate, and B12, as well as, genetic hemoglobinopathies, and infection, particularly malaria and intestinal parasites. While it is widely held that 50% of anemia is due to iron deficiency when only hemoglobin measurements are available, our results and the results of others call this assumption into question in areas with high levels of inflammation and infection.(Engle-Stone et al.; WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) However, distinguishing anemia due to iron deficiency from anemia of other causes is challenging, and to date, there is no "gold standard" indicator or method for doing so in areas with high levels of infection and inflammation.

If 50% of anemia is in fact not due to iron deficiency, this will have important implications for anemia prevention and control programs, which commonly use iron supplementation as the primary, and often only, strategy for reducing the burden of anemia during pregnancy. Taking this assertion one step further, our results show that the determinants of ID and anemia may differ between primigravidae and multigravidae, which could imply differential treatment strategies are needed by gravidity.

Our results suggest the determinants of ID and anemia differ between primigravids and multigravids. It is well documented that malaria infection is more common among primigravidae.(Alusala et al.; Huddle, Gibson and Cullinan; Ndyomugyenyi et al.; Shulman et al.) In multigravidae, our results suggest that the obstetric history of a women's previous pregnancy is associated with her iron status in her current pregnancy. This association could be explained through a variety of pathways including hemoglobinopathies playing a role in adverse birth outcomes or depleted iron stores from short interpregnancy intervals. Furthermore, the protective association between ID and participation in the WASH program suggest intestinal parasites might play a role in iron status among multigravidae.

Currently, iron and folic acid supplementation are universally recommended during pregnancy. (WHO *Iron Deficiency Anaemia Assessment, Prevention and Control: A Guide for Programme Managers*) However, these treatments are likely to be ineffective if iron deficiency is not the determinant, or sole determinant of anemia. Therefore alternative strategies or combinations of strategies may be needed to reduce the anemia burden. In instances where malaria is thought to be a contributing factor, intermittent preventative treatment in pregnancy (IPTp) may be an effective strategy to reduce maternal anemia. Controlled efficacy trials show IPTp improves birth weight and hemoglobin levels, but its impact on the burden of anemia and malaria are not well documented.(Parise et al. 1998; Shulman et al. 1999) It is not known if gravidity was considered in these interventions. A community-based study in the Gambia saw improvements in hemoglobin concentrations among primigravids in the chemoprophylaxis intervention group, but not among multigravids in the intervention group or among the placebo control group.(Greenwood et al.)

The results of combination interventions are inconsistent. IPTp combined with iron supplementation did not show additional benefits of IPT on hemoglobin levels compared to iron supplementation alone.(Van eijck 2007) In an IPTp + antihelminth intervention, the intervention was efficacious in clearing parasite infections and improving hemoglobin levels compared to an IFA supplementation only comparison group.(Ouedraogo et al.) The authors speculated IFA compliance may have contributed to this finding.(Ouedraogo et al.) However, these inconsistencies may be due to different etiologies of anemia within the study populations.

In Kenya, IFA supplementation is the primary strategy used for the prevention of IDA by the MOH in Kenya. While important, IFA may not result in reductions in anemia incidence, if iron deficiency is not the primary determinant of anemia and inefficiently use limited resources. The MOH Division of Nutrition's six year plan for reducing IDA in pregnancy cites the importance of multisector collaborations with the divisions of reproductive health and malaria and environmental health, but clear strategies are not outlined in this document.(Division of Nutrition)

Measurement of IDA is also challenging in areas with high levels of infection and inflammation, which can have downstream effects of misguiding anemia treatment and control strategies. While methods for measuring hemoglobin concentration exist, there is no "gold standard" indicator or method for assessing iron deficiency. Given anemia is caused by factors other than ID, simply using hemoglobin as a basis for an iron supplementation program may be misguided. Plasma ferritin is an indicator of iron stores when inflammation and infection are low. However, ferritin is an acute phase protein whose concentration will spike with infection or inflammation. One method for adjusting ferritin concentrations for the effect of inflammation was described by Thurnham.(Thurnham et al.) In this method cutoffs are used for CRP and AGP to divide the population into subgroups by stage of infection and different correction factors for each stage of infection are determined based on a ratio of the reference group to each subgroup.(Thurnham et al.) However because this approach relies on cutoff values, an inherent amount of information is lost from the study population. Soluble transferrin receptor is not affected by the acute phase response, however there is concern that it may be affected by malaria.(Williams et al.) Therefore further research is needed to identify indicators or methods for assessing ID during pregnancy in areas with high levels of infection and inflammation.

Hemoglobinopathies appear to be prevalent in western Kenya, yet they are not considered in anemia prevention and control strategies. Sickle cell trait has been shown to be protective against malaria, however the sickle cell trait itself results in hemolysis and frequently severe anemia.(Aidoo et al.; Friedman; Pasvol)

The broader implications from our findings are that anemia prevention and control strategies may need to be tailored by gravidity and the local context. Further methods research on iron status indicators is needed as well as more extensive evidence on gravidity-specific anemia treatment strategies. The prevalence of maternal anemia has remained constant over the past five years, and further research is needed to reduce the burden of maternal anemia.

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