ANAEMIA AND MICRONUTRIENTS DEFICIENCY AMONG PREGNANT WOMEN AND ADOLSECENT GIRLS IN EASTERN SUDAN

By

Ishraga Izzeldin Abdelrahim

(B.Sc., M.Sc. Biochemistry)
(U of K)

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Supervisor

Professor Mustafa Idris Elbashir
MBBS, MD, PhD
Professor of Biochemistry
(U of K)

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Co-supervisor
Dr. Ishag Adam Ahmed

MBBS, MD, PhD
Associate professor of Obstetrics & Gynaecology
DEDICATION

To
My Mother, Father, Sisters and Brother

My Husband and Kids

The soul of Dr. Ahmed Tamam
ACKNOWLEDGEMENTS

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## Abbreviations

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<tr>
<td>Hb</td>
<td>hemoglobin</td>
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<tr>
<td>IPI</td>
<td>interpregnancy interval</td>
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<td>LBW</td>
<td>low birth weight</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>5'-Methyl THF</td>
<td>5'-methyltetrahydrofolate</td>
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<td>THF</td>
<td>tetrahydrofolate</td>
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<td>MS</td>
<td>methionine synthase</td>
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<td>NTDs</td>
<td>neural tube defects</td>
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<tr>
<td>MTHFR</td>
<td>methylene tetrahydrofolate reductase</td>
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<tr>
<td>FAAS</td>
<td>flame atomic absorption spectrophotometry.</td>
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<tr>
<td>LOD</td>
<td>limit of detection</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<td>SRM</td>
<td>standard reference material</td>
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<tr>
<td>OD</td>
<td>optical density</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<td>OR</td>
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ABSTRACT

Background: Anaemia has been reported to be associated with poor maternal and perinatal outcomes. In Sudan, no data exist about the type of anaemia, and its maternal and perinatal effect.

Objectives: We conducted this study in eastern Sudan to investigate the prevalence and types of anaemia among pregnant women and adolescent schoolgirls of eastern Sudan.

Methodology and Results: We determined the levels of haemoglobin (Hb), ferritin, folate and vitamin B₁₂ and some minerals (zinc and copper) in sera of pregnant women and compared them with nonpregnant women as well as adolescent schoolgirls. Among two hundred and seventy nine pregnant Sudanese women were enrolled in the study, anaemia (Hb < 11 g/dl) and iron deficiency (ferritin< 15 µg/l) were prevalent in 80.3% and 14.3% of the study sample, respectively. Of the total sample, 11.1% had iron-deficiency anaemia. Serum folate (< 6.6 ng/ ml) and vitamin B₁₂ (< 150 pg/ ml) deficiency was reported in 57.7% and 1%, respectively, and none of the women had both folate and vitamin B₁₂ deficiencies. Regression analysis showed that ferritin, serum folate and vitamin B₁₂ levels were not significantly associated with anaemia.

In addition, a cross-sectional study was conducted among adolescent schoolgirls where one hundred eighty one (96.8%) out of 187 were shown to have anaemia (Hb <12 gm/dl); 21% had mild anaemia (Hb: 11.0-11.9 gm/dl), 66.8% had moderate anaemia (Hb: 8.0-10.9 gm/dl), and 12.1 % had severe anaemia (Hb < 8gm/dl), respectively. Iron deficiency (serum ferritin < 12µg/l), iron deficiency anaemia (Hb <12gm/dl and serum ferritin < 12µg/l) and folate deficiency (serum folate < 3 ng/ml) were prevalent in 17.6%, 16.5% and 69% of these girls, respectively. Eight percent and 7% of these girls had zinc (< 70 µg/dl) and copper deficiency (< 80 µg/dl), respectively. 26 (14%) girls had ≥ two micronutrients deficiencies. Serum ferritin and zinc were significantly lower in patients with
severe anaemia. Hb levels were significantly positively correlated with zinc levels ($r=0.161$, $P=0.03$) and with copper levels ($r=0.151$, $P=0.03$).

Furthermore, a case-control study (38-40 parturient women in each arm of the study) was conducted to investigate levels of maternal serum folate and ferritin in relation to short interpregnancy interval (short IPI) (< 18 months), preterm delivery (< 37 weeks) and low birth weight (LBW) (< 2500gm). There were no significant differences in levels of serum folate and ferritin in parturient women with; short IPI, preterm delivery, LBW and controls. There were significantly lower levels of folate and ferritin in women who had short IPI and preterm delivery than in the controls. In univariate analyses, there was no significant association between serum folate, ferritin, preterm delivery and LBW. Univariate and multivariate analyses showed significant association between low serum folate level (<2.5 ng/ml) and short IPI and preterm delivery (OR = 3.5; 95% CI =1.1-10.6; $P = 0.02$) and significant association between low serum folate levels and short IPI (OR = 1.9; 95% CI =1.0-3.6; $P = 0.03$). We also compared serum folate, zinc and copper levels among well matched two groups of pregnant Sudanese women (41 in each arm); with short IPI (< 18 months) and control group with referral IPI (18-30 months) at the mean (SD) gestational age of 10.1 (2.0) weeks. The mean (SD) serum zinc concentration was significantly lower, 86.8 (35.3) versus 90.7(22.5) µg/dl; $P = 0.02$, serum copper was significantly higher, 251.2 (81.0) versus 216.0 (71.7) µg/dl, $P= 0.04$ in women with short IPI. There was no significant difference in the levels of serum folate between the two study groups. There was significant positive correlation between haemoglobin and folate levels ($r= 0.218$, $P= 0.04$). There was no correlation between haemoglobin, IPI, serum zinc and copper levels.

Conclusion: The main findings of this study were; high prevalence of anaemia and folate deficiency, and relatively low prevalence of iron deficiency anaemia and very low prevalence of Vitamin $B_{12}$ deficiency
ال المستخلص

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187 Ljd7%96.8)180LD بـ Kيردك كـğ 8ـ كـحـتـه ـهـبـث فـرـى. (p= 0.03 ,r=0.151) ـ سـبـعـنـبـكـدـغـدـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذـذ~~~
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- ملاحظة: 
**الخلاصة:** 
هناك نقص في الحديد في الدم مع نقص في الحديد في الدم، بالإضافة إلى انخفاض في الحديد في الدم مع نقص في الحديد في الدم، حيث أن النسبة بين الداء والسفديات مع انخفاض في الحديد في الدم، بالإضافة إلى انخفاض في الحديد في الدم، حيث أن النسبة بين الداء والسفديات مع انخفاض في الحديد في الدم، بالإضافة إلى انخفاض في الحديد في الدم، حيث أن النسبة بين الداء والسفديات مع انخفاض في الحديد في الدم.
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Elhassan M. Elhassan1, Abdelrahim II, Ali NI, Elbashir MI and Adam I. Serum Folate, Zinc, Copper and Short Interpregnancy Interval, submitted

CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW
CHAPTER ONE

1. Introduction and Literature Review

1.1 Anaemia and pregnancy

Anaemia is a widespread public health problem associated with an increased risk of morbidity and mortality, especially in pregnant women and young children. It is a disease with multiple causes, both nutritional (vitamin and mineral deficiencies) and non-nutritional (infection) that frequently co-occur. It is assumed that one of the most common contributing factors is iron deficiency, and anaemia resulting from iron deficiency is considered to be one of the top ten contributors to the global burden of disease. Global prevalence of anaemia in pregnant and non-pregnant women is 41.8% and 30.2% respectively, and Africa shows the highest prevalence [1].

Measurement of haemoglobin (Hb) is essential for the diagnosis of nutritional anaemia and is one of the most common, easiest and least expensive methods. Unfortunately the Hb measurement is not very sensitive and specific for iron deficiency. Thus, to determine if iron deficiency is responsible for anaemia, it is usually necessary to include other indicators for example ferritin. Ferritin is currently considered the most important indicator of the iron status as even in the first stage of iron deficiency, its concentration decreases. Therefore it is the most sensitive indicator. However, it is important to note that ferritin is increased by many factors, including infection and inflammation, thus a high value does not necessarily indicate a good iron status [2].

Following WHO and international guidelines anaemia defines as Hb less than 12gm/dl, mild anaemia means Hb concentration 11.0-11.9 gm/dl,
moderate anaemia means Hb concentration 8.0-10.9 gm/dl and severe anaemia if Hb concentration is less than 8gm/dl. Iron deficiency means serum ferritin concentration less than 12 µg/l, iron deficiency anaemia (IDA) means haemoglobin concentration less than 12 gm/dl and serum ferritin less than 12 µg/l. Serum folate and vitamin B₁₂ deficiencies were defined as levels less than 6.6 ng/ ml and < 150 pg/ ml, respectively. Its adverse health consequences affect people with varied degrees of affluence and from all age groups, particularly women of childbearing age and children. Anaemia can lead to poor mental and educational performances [3].

Anaemia in pregnancy is a global problem and is associated with increased maternal morbidity and mortality [4]. It is also regarded as a major risk factor for an unfavorable outcome of pregnancy. It has been associated with premature labour and low birth weight [LBW] [5], and maternal and perinatal mortality [6, 7, 8]. Severe anaemia has consistently been associated with maternal mortality [9].

In 1987, the United Nations Children's Fund and representatives from 45 countries launched the Safe Motherhood Initiative (SMI) for reducing maternal mortality worldwide by 50% by 2000 [10]. One of the major goals of the Initiative was to eradicate anaemia during pregnancy. In developing countries, the cause of anaemia during pregnancy is multi-factorial and includes nutritional deficiencies of iron, folate, and vitamin B₁₂, parasitic diseases, such as malaria and hookworm and Short interpregnancy interval [IPI] [11, 12]. The relative contribution of each of these factors to anaemia during pregnancy varies greatly by geographical location. Iron deficiency remains the major cause of anaemia and is the most widespread single nutrient deficiency in the world. It is estimated that 75% of anaemia is due to
iron deficiency, followed by folate and vitamin B_{12} deficiencies [10]. Iron deficiency anaemia during pregnancy is prevalent because additional iron is needed to supply the mother’s expanding blood volume (±20% increase) and to support the needs of the growing fetus and placenta [13]. Identifying the magnitude of anaemia and its determinants in pregnant women is essential for evidence-based intervention modalities, particularly in developing countries where awareness is lacking and resources are scarce.

Based on these associations and the high prevalence of iron deficiency anaemia, supplementation programs were expected to reduce poor outcome of pregnancy, but the results were often disappointing [9]. This was often attributed to program implementation weaknesses, but more profound anaemia, accounting for most of the anaemia-related increased risk of maternal death, is likely to have complex, multiple causes [14]. A study from Malawi showed that 60% of iron-deficient women had other deficiencies as well, and many had signs of inflammation [15]. Similarly, a study from Tanzania identified iron deficiency, malaria, hookworms, and other infections as major causes of anaemia [16].

Another anaemia vulnerable group is adolescent girls who are undergoing rapid growth periods. Adolescence, like pregnancy, increases iron requirements in girls to accommodate the demands of growth and iron losses due to the onset of menstruation. Adam et al., 2009 recently observed that, adolescent pregnancy is a huge problem in Sudan, and adolescent pregnant women and elder schoolgirls were at higher risk for anaemia [17].
1.2 Folate

Folate, a member of the B-vitamin family, is a polyglutamate compound. Folic acid is a hematopoietic water-soluble vitamin. After absorption, free folic acid is reduced to tetrahydrofolate by the enzyme dihydrofolate reductase and circulated in plasma as N5-methyl derivative of tetrahydrofolate. The biologically most potent forms of tetrahydrofolates are found primarily as polyglutamate derivatives. Folic acid is stored as a polyglutamate derivative of tetrahydrofolate in the liver. Folate is one carbon carrier that facilitates interconversion of methenyl, formyl, formimino, methylene and methyl groups. Various one carbon tetrahydrofolate derivatives are used in biosynthetic reactions. They are required, for example, in the synthesis of purines and pyrimidines, heterocyclic bases required for synthesis of RNA and DNA [18], in repair of DNA and in gene expression through the process of DNA methylation [19]. Deficiency of folic acid itself or deficiency of vitamin B₁₂ which leads to functional folate deficiency, affects rapidly dividing cells because of their large requirement for thymidine for DNA synthesis [20]. Thus macrocytic anaemia associated with megaloblastic changes in the bone marrow is characteristic of folate deficiency. In addition, folate is a coenzyme for the enzymatic conversion of many amino acids and in vitamin metabolism, figure 1.
Figure 1. Folate-dependent homocysteine metabolic pathway.

5'-Methyl THF ≡ 5'-methyltetrahydrofolate
THF ≡ tetrahydrofolate
MS ≡ methionine synthase

Folate is needed for normal embryonic development and growth, and deficiency has been associated with the development of neural tube defects and low birth weight. Maternal folate deficiency remains a frequent and mostly unrecognized disorder, and is associated with recurrent miscarriage, placental abruption and intrauterine growth restriction [21].

A low intake of micronutrients and of vitamins such as folate [22, 23] and low circulating concentrations of folate are associated with an increased risk of adverse birth outcomes [21] and spontaneous abortion [24]. Ronnenberg
et al., 2002 also found that folate deficiency tended to be more common among women with spontaneous abortion than in those with live births [25], and George et al., 2002 found that there was a trend towards a protective effect among women with high folate levels [26]. Evidence consistently shows that a low folate level at time of conception is associated with neural tube defects (NTDs) and that a daily supplement of 0.4 mg of folic acid confers protection against NTDs. The mechanisms by which low folate could cause spontaneous abortion, and the mechanisms of the protective effect of folic acid on NTDs remain unknown [27]. Some studies also suggest that folic acid may protect against other types of birth defects [28, 29, 30]. Folate deficiency has been tentatively associated with abruption placentae and preeclampsia [31], and early vascular effects related to folate deficiency might also increase the risk of spontaneous abortion. Low folate increases the incidence of NTDs, and fetuses affected with NTDs are more commonly aborted spontaneously [32]. Adequate folate status promotes fetal growth. This is supported by the recent report of an analysis of more than five million birth records in California that showed small but significant reductions in the rates of low-birth weight and very-low-birth weight infants and preterm delivery after folic acid fortification [33]. Many studies support that there is an association between low folate intake and increased risk of infant low birth weight and of preterm delivery [34, 35, 36, 37, 38, 39, 40]. Biological plausibility for this association centers on the theory that elevated total homocysteine due to poor folate status along with the presence of the mutation in methylene tetrahydrofolate reductase (MTHFR) gene where cytosine at position 677 is substituted by thymine (C677T) leads to decidual vasculopathy, which can result in preterm delivery [35].
Maternal folate concentration decreases from the fifth month of pregnancy, and continues to do so during the first post-partum months, irrespective of lactation [41, 42]. By the sixth post-partum month, 20% of mothers remain deficient of folate [43]. Smits and Essed, (2001) concluded that commencement of a further pregnancy before complete folate restoration has taken place will result in a higher risk of maternal deficiency [42]. Concentrations of other micronutrients such as zinc and vitamins A and B6 also fall during pregnancy but they return to normal within a few weeks following delivery and/or do not affect the outcome of pregnancy [44, 45]. Thus folate deficiency appears to be the most important nutritional factor associated with the higher risk of poor pregnancy outcome after a short interpregnancy interval (IPI) [42]. It has been also found that a two-fold increase in the risk of neural tube defect was observed for pregnancies conceived within six months of a previous live birth [46]. The effect of short IPI on the risk of smallness for gestational age also has been documented [47].

Adolescents have increased requirements for folate during puberty. Thoradeniya et al (2006) found that both folic acid and iron deficiency are important in the aetiology of nutritional anaemia in adolescent schoolgirls and women of childbearing age, with iron being the leading factor [48]. Ahmed (2000) suggested that iron deficiency could be the major factor in the Bangladeshi population [49]. A similar situation was observed in India [50]. However, Ronnenberg et al., (2000) showed a higher contribution of folic acid and \( B_{12} \) deficiencies than of iron deficiency among non-pregnant women of childbearing age in China [51].
The relationship between IPI and perinatal health is receiving increasing attention. Appropriate pregnancy spacing has been recommended to achieve better perinatal outcomes. Thus, birth spacing is an important consideration when planning a family. Adam et al., (2008) observed that, short interval between conceptions is related to adverse outcomes such as preterm labour and LBW [8]. The increased risk of adverse pregnancy outcomes related to short IPI has been attributed to a number of mechanisms including maternal nutritional depletion [52] and hormonal imbalance [53]. It has been hypothesized that maternal folate depletion may play a major role in pregnancy outcome [42]. Furthermore, recently it has been reported that the excess risk of fetal growth restriction that is associated with short IPI was higher in women who did not use folic acid before pregnancy [54].

Thus folate status during pregnancy and pregnancy following a short IPI is correlated to complications of pregnancy and is an important parameter affecting both maternal morbidity and mortality and pregnancy outcomes [46, 42, 32].

1.3 Vitamin B\textsubscript{12}

B\textsubscript{12} (cobalamin) is the other hematopoietic water-soluble vitamin. It consists of cobalt in the centre of a corrin ring. In foods B\textsubscript{12} usually occurs bound to protein in the methyl or 5'-deoxyadenosyl forms. B\textsubscript{12} is released from the protein by acid hydrolysis in the stomach or trypsin digestion in the intestine. It then must combine with intrinsic factor, a protein secreted by the stomach, which carries it to the ileum for absorption. Methy cobalamin is required along with N5-methyltetrahydrofolate for the conversion of homocysteine to methionine. The 5'-deoxyadenosyl derivative is required for
the methylmalonyl-CoA mutase reaction, which is a key step in the catabolism of some branched-chain amino acids [18].

Megaloblastic anaemia associated with B₁₂ deficiency is thought to reflect the effect of B₁₂ on folate metabolism. The B₁₂-depenant homocysteine to methionine conversion appears to be the only pathway by which N5-methyltetrahydrofolate returns to the tetrahydrofolate pool, figure 1. Thus in B₁₂ deficiency there is a buildup of N5-methyltetrahydrofolate and a deficiency of the tetrahydrofolate derivatives needed for purine and deoxythymidinol monophosphate biosynthesis. Essentially all of the folate becomes trapped as the N5-methy derivative [18].

Homocysteine is a sulphur-containing amino acid that is a demethylated derivative of methionine. Homocysteine is metabolized via two main pathways: remethylation to methionine or transulphuration to cystathionine and then to cysteine. A defect in either leads to an accumulation of circulating homocysteine [55]. The remethylation of homocysteine into the amino acid methionine is blocked by a lack of folate and vitamin B₁₂ which results in hyperhomocysteinaemia [56], figure 1. It is recognized that hyperhomocysteinaemia produces thrombogenesis, vasodilation and endothelial damage, and is associated with cardiovascular and cerebrovascular disease, as well as recurrent miscarriage, placental abruption, pre-eclampsia, intrauterine growth restriction and perinatal death [57]. The causes of these complications of pregnancy may be traced to the first gestational weeks [58].
1.4 Minerals

Minerals are required as cofactors of enzymatic reactions, as structural components of enzymes and mitochondrial cytochromes, and as active electron and proton carriers in the ATP-generating respiratory chain. Copper is involved in the function of several cuproenzymes that are essential for life. Ceruloplasmin, which contains copper catalyses the conversion of ferric ions to the ferrous form, favoring absorption of iron from the gastro-intestinal tract. It also plays a role in the mobilization of iron to plasma from tissue stores [59].

Zinc is an essential part of more than 100 enzymes, some of which are involved in energy metabolism. It is also a cofactor for the synthesis of a number of enzymes, DNA and RNA [60].

Zinc is important in adolescence because of its role in growth and sexual maturation and it is known that serum zinc levels decline in response to the rapid growth and hormonal changes that occur during adolescence [61].

During pregnancy, inadequate stores or intake of minerals can have adverse effects on the mother, such as anaemia, hypertension, complication of labour and even death. Furthermore, the fetus can be affected, resulting in stillbirth, pre-term delivery, intrauterine growth retardation, congenital malformations, reduced immunocompetence and abnormal organ development [62, 60].

The essential nature of micronutrients has been recognized through the identification of clinical conditions associated with severe deficiencies of particular vitamins or minerals, and through subsequent animal experiments. While the importance in pregnancy of a few micronutrient deficiencies, such as iodine, has been long recognized, the role of many others is only recently
becoming appreciated [60]. It is also important to understand the significance of how deficiencies of other micronutrients may cause anaemia in communities where concurrent deficiency of several micronutrients is common. However, a few studies have characterized the relation between anaemia and deficiency in trace elements. Trace elements such as selenium, zinc and copper are essential nutrients with regulatory, immunologic, and antioxidant functions resulting from their actions as essential components or cofactors of enzymes throughout metabolism [63, 64]. Nishiyama et al (1999) found that the combination of iron and zinc therapy significantly increased haemoglobin levels in pregnant women [65]. Serum zinc concentrations decreased in contrast with significant increases in copper, as shown before [66, 67]. Ma et al., (2004) reported that zinc deficiency is common in Chinese pregnant women, showing high frequencies of marginal zinc deficiency in both anaemic and non-anaemic groups. They also found that serum copper concentrations are in inverse relationship with maternal haemoglobin concentrations. They also reported low frequencies of marginal copper deficiency in both groups [68]. Buamah et al., (1984) observed that low serum copper concentrations in pregnant women during midgestation were associated with an increased risk for anencephaly [69].

Upadhyaya et al., (2004) reported that serum zinc and iron levels were significantly reduced during pregnancy and despite routine supplementation of iron and folate pregnant women had significantly low haemoglobin and iron levels as compared to age matched controls [70].

Jameson (1976) was the first to comprehensively evaluate maternal zinc status and pregnancy outcome. He reported that low serum zinc concentration is associated with congenital anomalies and preterm delivery.
in the first and third trimester respectively [71]. Caulfield et al., 1998 also related zinc deficiency to complications of pregnancy and delivery, such as pre-eclampsia, premature rupture of membranes, and pre-term delivery, and with fetal growth retardation and congenital abnormalities [72]. Several studies have shown an association between maternal plasma zinc levels and birth weight [73]. Maternal morbidities such as pregnancy induced hypertension, prolonged labour and pre- and post-term deliveries may be increased in zinc deficient pregnant women [74]. Other minerals, like selenium and copper deficiencies may be associated with adverse outcomes of pregnancy and reduced fetal growth [75]. Jiang et al., (2005) investigated the prevalence of deficiency for vitamins B_{12}, folate among others, and zinc, iron, and copper during early pregnancy. They documented that the prevalence of multiple micronutrient deficiencies was common among women in the 1st trimester of pregnancy in rural Nepal and that simultaneous deficiencies for two or more micronutrients affected more than 80% of the pregnant women [76].

Copper deficiency during embryonic and fetal development can result in numerous gross structural and biochemical abnormalities [59].

Adolescence is defined by the WHO as the period between childhood and adulthood, spanning from 10 to 19 years of age. During adolescence, physiological requirements for iron and zinc peak at the time of the pubertal growth spurt, which in girls generally occurs between 10 and 15 years. Several other physiological processes that accompany puberty in females have a major impact on their requirements for iron and zinc, including sexual maturation, onset of menarche, and increased erythropoiesis [77, 78]. Even when the growth spurt has ceased, adolescents may require additional
iron and zinc to replenish body iron stores and depleted tissue zinc pools as a result of these increased demands [57, 79]. Unfortunately, many adolescents fail to meet these high physiological requirements for iron and zinc during puberty. The quality of their diets is often poor. This has been attributed to poor food selection patterns, and low energy intakes arising from concerns about body weight, and possibly from a sedentary lifestyle [61].

Baker et al (2001) showed that poor micronutrient intake and status increase the risk of small-for-gestational age births in pregnant adolescents [80].

1.5 Anaemia and pregnancy in Sudan
Sudan is the biggest African country, with around 40 millions populations. Anaemia is a big burden during pregnancy. Recently Adam et al., have shown that pregnant women of eastern Sudan are more susceptible to anaemia irrespective to their age or parity and even cases of severe anaemia have been reported [81, 82, 83]. Pica and infections were the risk factors for anaemia during pregnancy in that part of the country [82, 84]. Anaemia has been reported to be associated with poor maternal and perinatal outcomes [85]. In spite of this burden and impact of anaemia during pregnancy in Sudan, no data exist about the type of anaemia, its maternal and perinatal effect in Sudan. Thus, studies investigating these parameters are vital and may be of great interest, so as to provide health planners and caregivers with fundamental guidelines for the implementation of preventive measures. Identifying the magnitude of anaemia and its determinants in women is essential for evidence-based intervention modalities, particularly in developing countries where awareness is lacking and resources are scarce. Thus, we conducted this study as a continuation of an on-going research [86]
and to strengthen collaboration between the Department of Obstetrics and Gynecology and the Department of Biochemistry [87].

**GENERAL OBJECTIVES:**
The general aim of this study was to investigate the prevalence and types of anaemia among pregnant women and adolescent schoolgirls of eastern Sudan.

**SPECIFIC OBJECTIVES:**
1. To determine the levels of Hb, ferritin, folate and vitamin B$_{12}$ in sera of pregnant women.
2. To compare the levels of Hb, ferritin, folate and vitamin B$_{12}$ in sera of pregnant women with nonpregnant women.
3. To measure serum levels of Hb, ferritin, folate, copper and zinc amongst adolescent schoolgirls.
4. To investigate the prevalence of anaemia, iron, zinc, copper and folate, deficiencies amongst adolescent schoolgirls.
5. To examine the relationship of these micronutrients with Hb levels.
6. To study the role of maternal serum ferritin and folate in relation to short IPI (< 18 months), preterm delivery (< 37 weeks) and LBW (< 2500gm).
7. To investigate the role of maternal serum folate, zinc and copper levels in relation to short IPI.
CHAPTER TWO
MATERIALS AND METHODS
CHAPTER TWO
Materials and methods

2.1 Study design:
A cross-sectional study was carried out to investigate the prevalence and types of anaemia among pregnant women of eastern Sudan. In addition a cross-sectional study was conducted to investigate the prevalence of anaemia, iron, folate, zinc and copper deficiencies amongst adolescent schoolgirls in New Halfa, eastern Sudan and to examine the relationship of these micronutrients with Hb levels. Furthermore a case-control study was conducted to investigate levels of maternal serum folate, ferritin, zinc and copper in relation to short IPI (< 18 months), preterm delivery (< 37 weeks) and LBW (< 2500gm).

2.2 Study centres:
Gadarif Teaching Hospital, Gadarif, eastern Sudan
Wad Medani Teaching Hospital, Geizera, Sudan
Khartoum Teaching Hospital, Khartoum, Sudan
Alhara Aloula Girls’ high school in New Halfa, eastern Sudan

2.3 Research ethics:
Informed verbal consent was obtained from all patients and controls to participate in the study.

The study was approved by the Faculty Research Board, Faculty of Medicine, University of Khartoum.
2.4 Study population:

2.4.1 Cases:

A. Two hundred seventy nine women with a singleton baby were included in the study. Those who have antepartum haemorrhage were excluded.

B. A total number of one hundred eighty seven adolescent schoolgirls aged 14–18 years were enrolled in the study. Those with history of any systemic illness such as hepatic disorders, renal disease, thyroid disease, arthritis, blood disorders or diabetes mellitus were excluded.

C. One hundred ninety six women with a singleton baby participated in the study. Primiparae, those with intrauterine fetal death and antepartum haemorrhage, hypertension and diabetes mellitus were excluded. Groups of case-control stratum were performed; each group consisted of 38-40 women. Cases were those who had given birth to low birth weight, preterm delivery, those with short IPI (<18 months) (with its subgroups). Consecutive patients with term delivery, those who delivered baby with weight $\geq$ 2500 gm and referral group for IPI (18-30 months) acted as controls. Controls for preterm deliveries were matched for gestational age during pregnancy and were followed-up till delivery so as to confirm their term gestational age.

IPI was defined as the time between the woman’s previous delivery, miscarriage and the first day of the last menstrual period for the index pregnancy. The date of the last normal menstrual period was used to determine gestational age. However, when the discrepancy between gestational age determined in this way and gestational age calculated from ultrasound scanning was greater than 2 weeks, the ultrasound estimate was preferred.
Short IPI was defined as eighteen or less months duration. Then short IPI categories were adopted as follows; 0-5, 6-11 and 12-17 completed months. Maternal age was defined as completed years at time of delivery. Parity was defined as the number of previous births after completing 28 weeks of gestation, including stillbirths. LBW was defined as birth weight less than 2.5 kg. Preterm delivery was defined as birth before completed 37 weeks gestational age.

2.5 Sample collection:

2.5.1 Haematology
Blood films for malaria were collected.
Blood samples for measurement of maternal and adolescents haemoglobin concentration were taken.

Venous blood samples (5ml) were collected from each woman and allowed to clot in plain tubes, and sera were stored at -20°C until analyzed in the lab in Khartoum for measurement of serum ferritin, folate, and B₁₂ levels and copper and Zinc concentrations.

2.5.2 Histopathology:
Full thickness placental blocks of around 2-3 cm were taken from the placentae, kept in neutral buffer formalin for histo-pathology and placental malaria examinations.

2.6 Data collection:
Well- structured questionnaires were used to collect socio-demographic characteristics.
2.7 Malaria detection:
Blood films for malaria were prepared, Giemsa-stained and the number of asexual *P. falciparum* parasites per 200 white blood cells were counted and double-checked blindly by an expert microscopist.

Placental malaria infection was based on the pathological classification of Bulmer et al (1993) [88], which was classified as: uninfected (no parasites or pigment); active (parasites in intervillous spaces); active-chronic (parasites in maternal erythrocytes and pigment in fibrin or cells within fibrin and/or chorionic villous syncytiotrophoblast or stroma); past-chronic (no parasites and pigment confined to fibrin or cells within fibrin).

2.8 Haemoglobin measurement:
Hb concentration was estimated by HemoCue haemoglobinometer (HemoCue AB, Angelhom, Sweden).

2.9 Ferritin measurement:
Ferritin was measured by radioimmunoassay and activity was measured by $\gamma$ counter.

2.9.1 Reagents
1. $^{125}$I-ferritin to which 10 ml incubation buffer was added
2. Ferritin standards: 5, 20, 50, 100, 200 and 500 µg/l
3. Ferritin antibody to which 11 ml of distilled water was added before use.
4. Incubation solution
5. Precipitating reagent (P.R.)
2.9.2 Experimental procedure

1. T included only 100 µl of $^{125}$I-ferritin
2. NSB was prepared by adding 100 µl of $^{125}$I-ferritin to 200 µl incubation solution
3. $S_0$ was prepared by mixing 100 µl each of incubation solution, $^{125}$I-ferritin and ferritin antibody
4. Standards were prepared as follow:
   100 µl each of ferritin standard, $^{125}$I-ferritin and ferritin antibody
5. Samples were prepared by adding 100 µl each of $^{125}$I-ferritin and ferritin antibody to 100 µl of sample.
6. Then the five sets were mixed thoroughly and incubated at 37°C for one hour.
7. 500 of precipitating reagent were added followed by thorough mixing and a second incubation at room temperature for 15 minutes.
8. Then the sets except T were centrifuged at 3000 x g for 15 minutes.
9. The supernatant was discarded.
10. Ferritin concentration was measured using a $\gamma$ counter.

2.10 Measurement of folate

IMMULITE folate was determined by competitive, liquid-phase, ligand-labeled, protein binding chemiluminescent assay using IMMULITE and IMMULITE 1000 systems, and SIEMENS (5210 Pacific Concourse Drive Los Angeles, Ca 90045-6900 USA kits for folate and $B_{12}$).

Sensitivity of the assay was 0.8 ng/ml and the calibration range was 1-24 ng/ml.
2.10.1 Principle of the assay

IMMULITE folic acid is a boil, competitive, liquid-phase, ligand-labeled, protein binding chemiluminescent assay with in situ immobilization, and with an anti-ligand detection system.

The solid phase, a polystyrene bead enclosed with a murine monoclonal antibody specific for folic acid binding protein.

After the sample preparation procedure, the patient sample, ligand-labeled folic acid analog and folic acid binding protein are simultaneously introduced into the test unit, and incubated for approximately 30 minutes at 37°C with intermittent agitation.

During this time, folic acid in the sample competes with the ligand-labeled folic acid analog for a limited amount of folic acid binding protein, and the folic acid binding protein is captured by the antibody on the bead.

Unbound analog is then removed by a centrifugal wash

2.10.2 Materials needed

1. Folic acid test units: each barcode-labeled unit contains one bead coated with murine monoclonal anti-folic acid binding protein antibody.
2. Folic acid reagent wedges:
   Wedge A containing folic acid binding protein with preservative.
   Wedge B containing alkaline phosphatase (bovine calf intestine) conjugated to anti-ligand in buffer, with preservative.
3. Folic acid adjustors: Two vials (high and low) of lyophilized folic acid in a human protein-based matrix, with preservative.
Each vial was reconstituted with 3.0ml deionized water, mixed by gentle swiriling until full dissolution.
The adjustors were boiled like patient samples.
4. ligand-labeled folate: 5.0 ml of ligand-labeled folate in buffered human protein-based solution, with preservative.
5. Borate-KCN buffer solution: 125ml of borate-KCN buffer solution, with preservative
6. Dithiothreitol solution: 3ml of dithiothreitol solution

2.10.3 Kit components
2. Chemiluminescent substrate
3. Probe wash module
4. Probe cleaning kit
5. Sample cup holders
6. Sample cups
7. Sample cup caps
8. Tri-level, multi-constituent control

2.10.4 Other Materials
1. A covered boiling waterbath
2. An ambient waterbath

2.10.5 Experimental procedure
2.10.5.1 Preparation of working solution
Was prepared on a daily basis by mixing 1000 µl borate-KCN buffer solution, 20 µl ligand-labeled folate and 20 µl dithiothreitol.
2.10.5.2 Sample Pretreatment

1. 200 µl of each adjustor, control, or patient serum were pipetted into a test tube.
2. 1000 µl of the working solution were added to all tubes.
3. Test tubes were vortex mixed.
4. All tubes are capped and placed in a covered, boiling waterbath (100 °C) for 15-20 minutes.
5. Tubes were removed from the waterbath and cooled in an ambient waterbath for 5 minutes.
6. At least 350 µl of the treated sample were pipetted to the sample cups.
7. Sample cups, test units and reagent wedges A and B were loaded onto the system and the assay was run.

2.11 Measurement of B_{12}:

B12 was determined by solid-phase, competitive chemiluminescent enzyme immunoassay using IMMULITE and IMMULITE 1000 systems, and SIEMENS (5210 Pacific Concourse Drive Los Angeles, Ca 90045-6900 USA kits for folate and B_{12} ).

Sensitivity of the assay was 125 pg/ml and the calibration curve range was 150-1200pg/ml.

2.11.1 Principle of the assay

IMMULITE /IMMULITE 1000 B_{12} is a solid-phase, competitive chemiluminescent enzyme immunoassay.

A preliminary heat denaturation step: B_{12} in the patient sample is released from carrier proteins by incubation at 100°C in the presence of dithiothreitol and potassium cyanide.
Treated sample and hog intrinsic factor are simultaneously introduced into an IMMULITE / IMMULITE 1000 test units containing a polystyrene bead coated with a B12 analog.
Test units are incubated for approximately 30 minutes at 37°C with intermittent agitation.
B12 competes with the B12 analog on the solid phase for a limited number of B12 binding sites on the purified intrinsic factor.
Alkaline phosphatase-labeled anti-hog intrinsic factor is introduced for another 30 minute cycle.
The unbound enzyme conjugate is removed by a centrifugal wash.

2.11.2 Materials needed

1. Vitamin B12 test units: each barcode-labeled unit contains one bead coated with B12 analog.
2. Vitamin B12 reagent wedges:
   Wedge A containing B12 binding protein (purified hog intrinsic factor) with preservative.
   Wedge B containing alkaline phosphatase (bovine calf intestine) conjugated to murine monoclonal anti-hog intrinsic factor antibody in buffer, with preservative.
3. Vitamin B12 adjustors: Two vials (high and low) of lyophilized B12 in a human protein-based matrix, with preservative.
   Each vial was reconstituted with 4.0ml deionized water, mixed by gentle swirling until full dissolution.
   The adjustors were subjected to the same heat denaturation step as patient samples.
4. Borate-KCN buffer solution with preservative
5. Dithiothreitol solution
2.11.3 Kit components

2. Chemiluminescent substrate
3. Probe wash module
4. Probe cleaning kit
5. Sample cups
6. Sample cup caps
7. Tri-level, multi-constituent control

2.11.4 Other Materials

1. A covered boiling waterbath
2. An ambient waterbath

2.11.5 Experimental procedure

2.11.5.1 Preparation of working solution

Was prepared on a daily basis by mixing 1000 µl borate-KCN buffer solution and 20 µl of dithiothreitol.

2.11.5.2 Sample Pretreatment and measurement

1. 200 µl of each adjustor, control, or patient serum were pipetted into a test tube.
2. 1000 µl of the working solution were added to all tubes
3. Test tubes were vortex mixed.
4. All tubes are capped and placed in a covered, boiling waterbath (100 °C) for 15-20 minutes.
5. Tubes were removed from the waterbath and cooled in an ambient waterbath for 5 minutes.
6. At least 350 µl of the treated sample were pipetted to the sample cups.
7. Sample cups, test units and reagent wedges A and B were loaded onto the system and the assay was run.

2.12 Measurement of copper and zinc:

2.12.1 Apparatus
Copper and zinc concentrations were measured using flame atomic absorption spectrophotometry (FAAS). Measurements were performed on a Phoenix-986 atomic absorption spectrophotometer (Biotech Engineering Management Co. Ltd. (UK)).

Instrumental parameters were as follows:
Hollow cathode lamp for copper (3.0 mA, 324.7 nm),
Bandpass 0.2 nm,
Fuel-acetylene (pressure 0.07 MPa, flow rate 2.0 l/min),
Oxidant-air (pressure 0.2 MPa, flow rate l/min)

2.12.2 Experimental procedure
The samples were diluted (1:5) with deionized water.
Standard solutions (prepared in deionized water) were run in the range of 0.1-0.5 mg/l for copper and 0.05-0.2 mg/l for zinc, with detection limit of 0.05 mg/l for copper and 0.02 mg/l for zinc.

The calibration graph was plotted using the concentration method, limit of detection (LOD) was estimated according to the following equation (Wetz & Sperling, 1999): LOD=3SD/a, where SD stands for standard deviation for 20 measurements of the blank and "a" stands for the slope of the calibration line.
2.12.3 Quality assurance and quality control
The method of analysis was verified by comparison with the certified values of standard reference material (SRM). For that purpose IAEA-A-13 (freeze dried bovine blood) was used. 0.5030 g of IAEA-A-13 was dissolved in 10 ml of concentrated nitric acid and put in a hot plate until the acid was evaporated to near dryness; finally the volume was completed to 25 ml with deionized water.

A comparison of the certified reference values for the standard reference material (IAEA-A-13) with practical values verified the accuracy of the method.

<table>
<thead>
<tr>
<th>Element</th>
<th>Practical value</th>
<th>Reference value</th>
<th>RSD (%)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>4.22 ± 0.007</td>
<td>4.3</td>
<td>0.15</td>
<td>98.14</td>
</tr>
<tr>
<td>Zinc</td>
<td>14.23 ± 0.1</td>
<td>13</td>
<td>0.7</td>
<td>109.5</td>
</tr>
</tbody>
</table>

All samples were run in duplicates and the mean value of optical density (OD) was calculated.

2.13 Statistics
Data was entered in computer using SPSS for Windows and double checked before analyses.

2.13.1 Pregnant women and types of anaemia
Values were calculated and expressed as mean (SD) and proportions, which were compared by Chi square test. Logistic regression model was constructed where anaemia as the dependent variable and baseline characteristics (age, parity, education, antenatal care) serum ferritin, serum
folate and serum vitamin B<sub>12</sub> as possible influencing factors. Odd ratio, 95% confidence interval were calculated. P < 0.05 was regarded as significant.

2.13.2 Adolescent schoolgirls and types of anaemia
Data were summarized as mean (SD) when normally and median (25–75 percentile) when not normally distributed. To investigate the relationship between the indicators of micronutrient status and the degree of severity of anaemia, mild anaemia, moderate and severe anaemia, Krushal-Wallis (in case if the data were not normally distributed) or ANOVA (in normally distributed data) tests were used to analyze the difference of these micronutrients between the three grades of anaemia. Pearson’s correlation test was used to assess the association of Hb with other micronutrients.

2.13.3 Anaemia and adverse pregnancy outcomes
Values were calculated and expressed as mean (SD) and proportions, which were compared by Chi square test. Folate and ferritin data were found to be not normally distributed data hence; Mann-Whitney U test and Kruskal – Wallis H were used to analyze two groups or more. Univariate analyses were performed initially and variables with significant results (P < 0.05) were analyzed by multivariate analyses too. LBW, preterm labour and short IPI as dependent variables and ferritin, folate and maternal socio-demographic characteristics as independent variables. P < 0.05 was regarded as significant.
CHAPTER THREE

RESULTS
CHAPTER THREE

Results

A cross-sectional study aimed to investigate the prevalence and types of anaemia among pregnant women of eastern Sudan. Data from two hundred seventy nine out of 300 women who completed study are presented.

Their mean (SD) age and parity was 25.9 (6.4 years and 1.7 (2.4), respectively. Two hundred and twenty four women (80.3%), 40 (14.3%) and 31(11.1%) of 279 women had anaemia, iron deficiency and iron deficiency anaemia, respectively. Only three women had severe anaemia. The mean (SD) haemoglobin (Hb) and ferritin values were 9.8 (1.1) g/dl and 64.5(59.0) µg/l respectively (Table 1).

The mean (SD) serum folate and vitamin B12 levels were 6.5(4.5) ng/ ml and 216 (90.1) pg/ ml, respectively. Folate deficiency was prevalent in 161(57.7%) women. Vitamin B12 deficiency was prevalent in 3 (1%). None of the women had both folate and vitamin B12 deficiencies (Table 2). The percentages of women with folate deficiency, vitamin B12 and with both folate and vitamin B12 deficiencies were not significantly different in the anaemic group than in the non-anaemic group (Table 2).

Table 3 shows the results of logistic regression, where serum ferritin, folate and vitamin B12 were not significantly associated with anaemia in these women.

Maternal and placental blood films were positive in six cases and the blood films for malaria were positive in two maternal, placental and cord setting.
Six (2%), 6 (2%) and 82 (28.0%) of the placentae showed active, active-chronic, past-chronic infection on histopathology examination respectively, while 199 (68.0%) of them showed no infection.

A cross-sectional study was also conducted to investigate the prevalence of anaemia, iron, folate, zinc and copper deficiencies among adolescent schoolgirls and to examine the relationship of these micronutrients with Hb levels.

During the study period, 187 adolescent schoolgirls had completed data. Their age ranged 11-18 with mean (SD) of 13.9 (1.3) years. One girl was excluded because she was found to have *P. falciparum* malaria.

Hb level range was 4.1-17.0 with mean (SD) 9.8(1.4) g/dl. The mean (SD) or median (25–75 Percentile) values of the anthropometric measurements and these elements are shown in Table 4.

Following WHO and international guidelines (1990), zinc and copper deficiencies were defined as levels less than 75 µg/dl and 80 µg /dl, respectively.

One hundred eighty one (96.8%) out of these 187 adolescent schoolgirls had anaemia. 21% had mild anaemia, 66.8.1% and 12.1 % of them had moderate anaemia and severe anaemia respectively. 33 (17.6%) and 31 (16.5%) girls had iron deficiency and iron deficiency anaemia, respectively. Folate deficiency was prevalent in 129 girls (69%).

8% and 7% of these girls had zinc and cooper deficiency respectively. 26 (14%) girls had ≥ two micronutrients deficiencies.
Table 5 reflects mean or median concentrations of serum ferritin, folate, zinc and copper in the three grade of anaemia. Serum ferritin and zinc levels were significantly lower in patients with severe anaemia.

Among adolescent schoolgirls, Hb levels were significantly positively correlated with zinc levels ($r=0.161$, $P=0.03$) and with copper levels ($r=0.151$, $P=0.03$) (Figures 2 and 3).

Hb, ferritin and folate were not significantly correlated (Figure 4, 5 and 6). Also there were no significant correlations between ferritin, folate, zinc and copper (Figures 7, 8, 9, 10 and 11).

During the study period there were 26150 deliveries in these three hospitals. Table 6 compares cases and controls regarding age, parity, weight and Hb. Cases were those who had given birth to low birth weight, preterm delivery, those with short IPI (with its subgroups). Consecutive patients with term delivery, those who delivered baby weight $\geq 2500$ gm and referral group for IPI acted as controls. Controls for preterm deliveries were matched for gestational age during pregnancy and were followed-up till delivery so as to confirm their term gestational age. There were no significant differences between cases and control in their basic characteristics.

Table 7 shows folate and ferritin concentrations according to the IPI. There was no significant difference in serum folate and ferritin in subgroups of short and referral group of IPI.

There were no significant differences in serum ferritin and folate levels in those women with preterm delivery and LBW in comparison to control. However, serum ferritin and folate levels were significantly lower in women
with preterm delivery and short IPI in comparison with women who had preterm delivery and referral IPI (Table 8).

Table 9 displays univariate and multivariate analyses that show significant associations between low serum folate levels and preterm delivery (OR = 3.5; 95% CI =1.1-10.6; P = 0.02) and short IPI (OR =1.9; 95% CI =1.0-3.6; P = 0.03).

Table 10 reflects basic characteristics between women with short IPI (<18 months) (cases) and those with referral IPI (18-30 months) (controls). The two groups (41 in each arm) were well matched and there were no significant differences between cases and controls in their basic characteristics.

Table 11 compares serum levels of folate, zinc and copper between women with short IPI and those with referral IPI.

The mean (SD) serum zinc concentration was significantly lower, 86.8(35.3) µg/dl in women with short IPI versus 90.7(22.5) µg/dl in women with referral IPI; P = 0.02, serum copper was significantly higher, 251.2 (81.0) µg/dl in women with short IPI compared to 216.0 (71.7) µg/dl, P= 0.04 in women with referral IPI. There was no significant difference in the levels of serum folate between the two study groups.

There was significant positive correlation between haemoglobin and folate levels (r= 0.218, P= 0.04), figure 12. There were no correlations between haemoglobin, IPI, serum zinc and copper levels, figures 13, 14, and 15. Furthermore, IPI, folate, zinc and copper were not significantly correlated (Figures 16- 21).
Table 1. Classification of Sudanese pregnant women (n = 279) by groups for anaemia and iron deficiency *

<table>
<thead>
<tr>
<th>Hematological status</th>
<th>N (%)</th>
<th>Haemoglobin, g/dl</th>
<th>Serum ferritin, µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>279</td>
<td>9.8(1.1)</td>
<td>64.5(59.0)</td>
</tr>
<tr>
<td>Normal</td>
<td>55(19.7)</td>
<td>11.6(0.7)</td>
<td>80.8(55.0)</td>
</tr>
<tr>
<td>Anaemia only</td>
<td>224(80.3)</td>
<td>9.4(0.8)</td>
<td>63.4(59.8)</td>
</tr>
<tr>
<td>Iron deficiency only</td>
<td>40 (14.3)</td>
<td>9.8(1.1)</td>
<td>8.3(3.6)</td>
</tr>
<tr>
<td>Iron-deficiency anaemia</td>
<td>31(11.1)</td>
<td>9.3 (0.8)</td>
<td>8.5 (3.5)</td>
</tr>
</tbody>
</table>

* Data are shown as mean (SD) and n (%) as appropriate.
<table>
<thead>
<tr>
<th>Hematological status</th>
<th>Total (n=279)</th>
<th>Anaemic (n=224)</th>
<th>Non anaemic (n=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate deficiency only</td>
<td>161 (57.7)</td>
<td>135 (60.2)</td>
<td>26 (47.2)</td>
</tr>
<tr>
<td>Vitamin B\textsubscript{12} deficiency only</td>
<td>3 (1)</td>
<td>2 (0.8)</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>Both folate and vitamin B\textsubscript{12} deficiency</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>None</td>
<td>115 (41.2)</td>
<td>87 (38.8)</td>
<td>28 (50.9)</td>
</tr>
</tbody>
</table>

* No significant differences in all these values between anaemic and non anaemic groups
Table 3. Factors associated with anaemia among pregnant women of eastern Sudan using logistic regression analysis.

<table>
<thead>
<tr>
<th>The variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>1.0</td>
<td>0.9-1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Parity</td>
<td>0.8</td>
<td>0.6-1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Lack of antenatal care</td>
<td>2.3</td>
<td>0.7-6.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>0.9</td>
<td>0.9-1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Serum folate</td>
<td>0.9</td>
<td>0.8-1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Serum Vit B&lt;sub&gt;12&lt;/sub&gt;</td>
<td></td>
<td>0.9-1.0</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Anthropometric and biochemical measures of the adolescent schoolgirls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>25–75 Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>43.7(9.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>154.1(6.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>18.3(3.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemoglobin, g/dl</td>
<td>9.8(1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. ferritin, µg/l</td>
<td>45.9</td>
<td>19.5-97.5</td>
<td></td>
</tr>
<tr>
<td>S. folate, ng/ml</td>
<td>2.2</td>
<td>1.5-3.7</td>
<td></td>
</tr>
<tr>
<td>S. zinc, µg/ml</td>
<td>115.0(22.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. copper, µg/ml</td>
<td>125.1(33.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Mean (SD) or median (25–75 percentiles) concentrations of selected micronutrients by grade of anaemia among adolescent schoolgirls

<table>
<thead>
<tr>
<th>Biochemistry</th>
<th>Mild anaemia (n= 38)</th>
<th>Moderate anaemia (n= 121)</th>
<th>Severe anaemia (n= 22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. ferritin, µg/l</td>
<td>48.8(24.6-124.6)</td>
<td>49.5(22.4-110-3)</td>
<td>16.6(4.5-56.5)</td>
<td>0.003</td>
</tr>
<tr>
<td>S. folate, ng/ml</td>
<td>2.2(1.6-4.0)</td>
<td>2.0(1.4-3.2)</td>
<td>2.1(1.7-3.5)</td>
<td>0.6</td>
</tr>
<tr>
<td>S. zinc, µg/ml</td>
<td>121.5(22.6)</td>
<td>113.9(21.0)</td>
<td>106.0(20.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>S. copper, µg/ml</td>
<td>125.7(35.9)</td>
<td>125.6(32.9)</td>
<td>114(31.2)</td>
<td>0.4</td>
</tr>
</tbody>
</table>
### Table 6. Comparison of the basic characteristics of the cases and controls women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preterm delivery</th>
<th>Controls</th>
<th>P</th>
<th>Low birth weight</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>26.4 (5.7)</td>
<td>28.1 (2.5)</td>
<td>0.08</td>
<td>28.8 (19.5)</td>
<td>28.8 (6.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Parity</td>
<td>2.5 (2.6)</td>
<td>2.5 (2.6)</td>
<td>0.1</td>
<td>2.2 (1.9)</td>
<td>3.0 (2.3)</td>
<td>0.6</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.6 (11.9)</td>
<td>66.4 (12.4)</td>
<td>0.2</td>
<td>67.2 (8.5)</td>
<td>69.1 (14.7)</td>
<td>0.3</td>
</tr>
<tr>
<td>Haemoglobin, g/dl</td>
<td>9.8 (0.6)</td>
<td>9.9 (0.9)</td>
<td>0.4</td>
<td>10.4 (0.8)</td>
<td>10.5 (1.2)</td>
<td>0.9</td>
</tr>
<tr>
<td>Variable</td>
<td>Folate ng/ml</td>
<td>Ferritin µg/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 6 months</td>
<td>5.4 (1.6-9.5)</td>
<td>58 (26-85)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-11 months</td>
<td>4.8 (2.8-7.1)</td>
<td>48 (24.5-84.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-17 months</td>
<td>4.8 (2.6-8.1)</td>
<td>45 (24-130)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-30 months</td>
<td>5.5 (3.2-9.2)</td>
<td>36 (23-82)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>Folate, ng/ ml</td>
<td>Ferritin µg/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------------</td>
<td>---------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm labour</td>
<td>4.6 (2.6-8.2)</td>
<td>59 (21-124)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.9 (2.2-6.0)</td>
<td>33.0 (15-98)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.6</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm labour and short IPI</td>
<td>2.6 (0.9-4.6)</td>
<td>48 (15.7-78.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preterm labour and referral IPI</td>
<td>5.1 (3.1-9.4)</td>
<td>103 (26-227)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.008</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight</td>
<td>6.0 (3.3-8.9)</td>
<td>55 (22.0-83.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.3 (3.2-8.7)</td>
<td>45.5 (25.0-101.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.8</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight and short IPI</td>
<td>6.5 (2.2-8.9)</td>
<td>58 (26-83)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low birth weight and referral IPI</td>
<td>5.5 (3.3-8.6)</td>
<td>34 (20-83)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 9. OR and 95% CI of serum ferritin, folate, interpregnancy interval and pregnancy outcome

<table>
<thead>
<tr>
<th>Variable</th>
<th>Folate</th>
<th></th>
<th></th>
<th>Ferritin</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>Preterm labour</td>
<td>0.9</td>
<td>0.8-1.0</td>
<td>0.2</td>
<td>1.0</td>
<td>0.9-1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Preterm labour and short IPI</td>
<td>3.5</td>
<td>1.1-10.6</td>
<td>0.02*</td>
<td>0.9</td>
<td>0.9-1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>0.9</td>
<td>0.9-1.0</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9-1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Low birth weight and short IPI</td>
<td>0.9</td>
<td>0.8-1.1</td>
<td>0.6</td>
<td>0.9</td>
<td>0.9-1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Short IPI</td>
<td>1.9</td>
<td>1.0-3.6</td>
<td>0.03*</td>
<td>0.9</td>
<td>0.9-1.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* adjusted for confounding factors
Table 10. The basic characteristics of the women with short interpregnancy interval (cases) and those with referral interpregnancy interval (controls)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women with short IPI (n=41)</th>
<th>Women with referral IPI (n=41)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>26.1(7.7)</td>
<td>27.6 (5.4)</td>
<td>0.2</td>
</tr>
<tr>
<td>Parity</td>
<td>2.8 (1.5)</td>
<td>2.9 (1.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>10.6(1.6)</td>
<td>10.0(2.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>61.0(8.8)</td>
<td>59.6 (12.4)</td>
<td>0.1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>151.8(32.2)</td>
<td>155.1(17.4)</td>
<td>0.2</td>
</tr>
<tr>
<td>Body mass index</td>
<td>23.8(5.0)</td>
<td>23.9(4.5)</td>
<td>0.6</td>
</tr>
<tr>
<td>Mid upper arm circumference, cm</td>
<td>28.5(3.6)</td>
<td>27.2(3.2)</td>
<td>0.5</td>
</tr>
<tr>
<td>Haemoglobin, g/dl</td>
<td>10.3 (1.0)</td>
<td>10.6(1.0)</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Table 11. Serum folate, zinc and copper levels in women with short interpregnancy interval (cases) and those with referral interpregnancy interval (controls)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women with short interpregnancy interval (n=41)</th>
<th>Women with referral interpregnancy interval (n=41)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-folate, ng/dl</td>
<td>5.3 (4.5)</td>
<td>5.3 (4.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>S. zinc, µg/ml</td>
<td>86.8 (35.3)</td>
<td>90.7 (22.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>S. copper, µg/ml</td>
<td>251.2 (81.0)</td>
<td>216.0 (71.7)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Figure 2. Correlation between serum zinc and Hb among adolescent schoolgirls ($r=0.161$, $P=0.03$)
Figure 3. Correlation between serum copper and Hb among adolescent schoolgirls (\(r=0.151, P=0.03\))
Figure 4. Correlation between Ferritin and Hb among adolescent schoolgirls ($r=0.051$, $P=0.8$)
Figure 5. Correlation between folate and Hb among adolescent schoolgirls ($r=0.038$, $P=0.6$)
Figure 6. Correlation between serum folate and ferritin among adolescent schoolgirls ($r = 0.059, P = 0.4$)
Figure 7. Correlation between serum zinc and ferritin among adolescent schoolgirls ($r = 0.137$, $P = 0.1$)
Figure 8. Correlation between serum zinc and folate among adolescent schoolgirls ($r=0.084$, $P=0.2$)
Figure 9. Correlation between serum copper and ferritin among adolescent schoolgirls ($r = 0.027$, $P = 0.7$)
Figure 10. Correlation between serum copper and folate among adolescent schoolgirls ($r = 0.090$, $P = 0.2$)
Figure 11. Correlation between serum copper and zinc among adolescent schoolgirls ($r=0.12$, $P=0.8$)
Figure 12. Correlation between folate and Hb among pregnant women with short and referral IPI
(r=0.218, P=0.04)
Figure 13. Correlation between interpregnancy interval and Hb (r = 0.124, P = 0.2)
Figure 14. Correlation between serum zinc and Hb among pregnant women with short and referral IPI (r= 0.076, P= 0.4)
Figure 15. Correlation between serum copper and Hb among pregnant women with short and referral IPI (r = 0.012, P = 0.9)
Figure 16. Correlation between serum zinc and interpregnancy interval ($r = 0.035$, $P = 0.7$)
Figure 17. Correlation between serum copper and interpregnancy interval ($r=0.177$, $P=0.1$)
Figure 18. Correlation between serum folate and interpregnancy interval ($r = 0.005$, $P = 0.9$)
Figure 19. Correlation between serum zinc and folate among pregnant women with short and referral IPI ($r = 0.186$, $P = 0.9$)
Figure 20. Correlation between serum copper and folate among pregnant women with short and referral IPI ($r = 0.142$, $P = 0.2$)
Figure 21. Correlation between serum copper and zinc among pregnant women with short and referral IPI ($r=0.2$, $P=0.07$)
CHAPTER FOUR

DISCUSSION
CHAPTER FOUR

Discussion

The main findings of the current study are; high prevalence of anaemia and folate deficiency, and relatively low prevalence of iron deficiency anaemia and very low prevalence of Vitamin B₁₂ deficiency.

Although the prevalence of anaemia in the pregnant women in this study was higher (80.3%) than previously reported in the neighboring area in eastern Sudan [79], it was low compared with those reported in pregnant women in Malawi (where 84.8% of the subjects had moderate anaemia) [90] and in Tanzania (where the prevalence of severe anaemia was 7%) [91].

It should be noted that although ferritin deficiency was present in 14.3 % of the women this number could be an underestimation, since other factors found to be associated with anaemia in pregnancy include haemolysis and markers of infection, such as C-reactive protein and urine nitrite [92] which were not measured in the present study. It is documented that ferritin is an acute-phase protein and its level rises in cases of infection, thus leading to underestimation of iron deficiency [93]. Furthermore, the haemolysis associated with malarial infection leads to the release of free iron and thus to an apparent increase in serum ferritin, which, in the presence of infection, behaves as an acute-phase protein. Serum concentrations of ferritin are therefore poor indicators of iron deficiency in areas where malaria is common, since, in such areas, the serum concentrations of ferritin in the iron deficient are elevated by malaria infection.
The low prevalence of vitamin B12 deficiency could be explained by high consumption of animal products by these women. Interestingly even if the animal products were not consumed, vitamin B12 level was found to be relatively high in the neighboring Ethiopia [94]. The main explanation for that was the habit of consuming fermented food. Gadarif is on Ethiopian border and people are sharing same habits including diet. Consumption of fermented food products may have increased vitamin B12 levels in the diet and thus reduced the risk of vitamin B12 deficiency anaemia. Several vitamin B12-producing microorganisms have been isolated during the fermentation of food [95, 96]. Certainly, based on the Ethiopian data these microorganisms are also a folate-producing microorganism. So it is not surprising that fermented food also contributed some folate, although plasma folate may not reflect true folate status in malarial endemic areas such as Gadarif, where levels may be elevated through erythrocyte hemolysis induced by malaria. In Ethiopia however, fermented products were reported to be the staple food for more than 10 million Ethiopians [94] and further investigation into the presence of vitamin B12 analogs in these women consuming fermented food and in the food itself is warranted.

One of the limitations in our study was that, folate status was determined using serum samples as opposed to determining the red blood cell concentration of folate which would have provided a better indication of tissue folate status. Folate concentration is much higher inside the erythrocytes than in sera. During hemolysis, intra-erythrocytic folate leaks into the plasma and folate may rise temporarily and mask a folate deficiency.

In the current study no significant association was observed between anaemia and serum ferritin, folate and vitamins B12 levels. In Tanzania, it
has been reported that anaemia was significantly associated with serum ferritin but not serum folate and vitamin B\textsubscript{12}. However these two studies should be compared cautiously. Firstly, because in the Tanzanian study the haemoglobin cutoff point used was 9 gm/dl and we used 11 gm/dl. Other nutrients, such as vitamin A and vitamin B\textsubscript{6} that are associated with anaemia, were not measured. Subjects were also not tested for hookworm or other parasitic diseases besides malaria a potential cause of anaemia due to intestinal blood loss. These results might have been changed if these were investigated too.

The prevalence of anaemia, iron, folate, copper and zinc deficiencies and their interactions in the etiology of anaemia was investigated among adolescent school girls in eastern Sudan. The current study provides evidence that adolescent school girls of eastern Sudan have a high prevalence of anaemia, iron deficiency anaemia, folate and other deficiencies. The prevalence of anaemia in this setting is higher than the prevalence of anaemia that recently reported among adolescent girls in Nigeria, Bangladesh, Vietnam, Sri Lanka and Indonesia [97, 98, 99, 100, 101], and even higher than the prevalence of anaemia that was reported among pregnant women in the study area itself [89]. This is not surprising as adolescents health in Sudan is not yet fit in any of the health programme like the antenatal care for the pregnant women.

In this study iron deficiency alone may not explain anaemia in these adolescent girls as 69% of them had folate deficiency. We have recently reported that 57.7% of pregnant women in eastern Sudan had folate deficiency [102]. The accurate diagnosis of folate deficiency is difficult to establish at a population level because no test can reliably be used to reflect
actual metabolic levels of folic acid [103]. However, the contribution of folic acid deficiency to anaemia in this study population could not be ascertained, because no peripheral blood cell morphology examinations were carried out. Though folates are present in various foods of animal and vegetable origin, dietary intake may be low due to inadequate storage or excessive cooking [104].

The current study showed that, 8% of these girls had zinc deficiency. However, serum zinc is not a reliable indicator of body zinc stores [105], It has proven useful at the population level [106]. Although zinc intake and other food constituents were not quantified in our study, high-phytate in the foods could in part explain our results, as phytates reduce zinc absorption [107]. Zinc deficiency is commonly overlooked although it is a widespread public health problem with nearly half of the world’s population at risk of insufficient intake [108]. It causes reduced growth rate, abnormal neurobehavioural development and impaired resistance to infections [109].

Our estimate that simultaneous deficiencies for ≥ 2 micronutrients affected 14% of girls and there was positive correlations between Hb and zinc and Hb and copper, suggesting potential metabolic interactions possibly derived in part from a shared dietary deficit of good food sources. Coexisting nutritional deficiencies could reduce the potential benefit of a single nutrient supplement in improving nutrition status and morbidity [110, 111]. The coexistence of multiple micronutrient deficiencies with iron deficiency may increase the risk of anaemia and limit haematological response [112].

In the contrary the competition between these elements should be born in mind, like the competition between bivalent iron and zinc for mucosal
uptake in the gut may result in one interfering with the absorption of the other [113, 114]. Previously it has been reported that improvement in Hb concentration due to iron-folic acid treatment, was lower among pregnant Nepalese women receiving supplemental zinc compared with those not receiving zinc [115, 116]. Furthermore, a recent trial among Indonesian infants has also shown that combined iron and zinc supplementation is less efficacious than iron supplementation alone in improving iron status [117]. Old in vitro and in vivo studies have shown that a mutual inhibition exists at the site of intestinal transport [118]. Folic acid supplements have been shown to increase zinc excretion in men with mild zinc deficiency [119].

Copper deficiency states are relatively rare in man but have been associated with premature infants [120], patients on total parental nutrition [121], and severely malnourished children [122]. Hypochromic anaemia can be responsive to copper supplementation [123].

Thus, there was high prevalence of anaemia and other elements deficiencies, interventions are required to prevent and control anaemia in this setting and further research is needed.

Concerning the investigation of levels of folate, ferritin and IPI, the main findings of the current study were, no significant differences in folate and ferritin levels between groups (and subgroups) of short IPI and the referral group, and ferritin and folate levels were not different between those with LBW, preterm delivery and controls. Folate and ferritin levels were significantly lower in those women who had preterm delivery and short IPI. Thus the study confirmed the hypothesis of the role of folate depletion in women with short IPI and preterm delivery [42], but it failed to show any
difference in folate level in subgroups of short IPI in comparison with the referral group. Recently van Eijsden et al., (2008) reported that, women with short IPI who did use folate were at higher risk of fetal growth restriction in those women [54]. In the current study folate levels were not significantly lower in those women who delivered LBW babies. This is in contrast to the previous reports where low folate levels were found to be predictors for low birth weight babies [124]. One of explanations for this finding is; primiparae were excluded from the study so as to investigate IPI too. We recently found that primiparae were the vulnerable group of women for low birth weight in Sudan [125].

High levels of ferritin in women with preterm delivery and short IPI in this study should be interpreted cautiously since other factors found to be associated with ferritin levels in pregnancy include haemolysis and markers of infection, such as C-reactive protein and urine nitrite [92] were not measured in the present study. It is documented that ferritin is an acute-phase protein and its level rises in cases of infection [93]. However, it has been observed that high ferritin levels were predictors for preterm delivery [126].

One limitation of this study was that consumption of fermented food products as it was claimed before and that might have increased folate levels in the diet and serum too. Furthermore, serum folate and ferritin were measured during delivery; these might have been totally different picture if these measurements were made in early pregnancy. Thus, further research is needed to explore this issue in more depth.
The main findings of the current study were: no significant differences in folate levels between groups. While serum zinc was significantly lower, serum copper was significantly higher in women with short IPI. Thus the study did not confirm the hypothesis of the role of folate depletion in women with short IPI [42]. Previously it was reported that low folate levels were found to be predictors for low birth weight babies [125]. Furthermore, we recently observed that women with short IPI and preterm delivery had significantly lower folate levels [18]. The important point desired to be mentioned here is the significantly positive correlation between folate and haemoglobin levels in the current study. One limitation of this study was that food intake was not quantified.

Low zinc levels in women with short IPI in this study should be interpreted cautiously since copper was higher in these women. In neighboring Ethiopia, 74 % of pregnant women had low plasma zinc and it was the major positive predictors of haemoglobin [95]. Recently high prevalence of zinc deficiency (64.6%) was reported amongst Indian pregnant women possibly due to the low dietary intake of zinc [129]. In China zinc deficiency was found to be 51.05%, the prevalence of zinc deficiency higher in anaemic than in non-anaemic women and copper and haemoglobin levels were found inversely correlated [69]. Zinc is necessary for normal growth in pregnancy. Its deficiency increases the risk of intrauterine growth restriction, prematurity, pre-eclampsia and some other complications [130, 131, 132]. Thus; further research is needed to explore this issue in more depth possibly through longitudinal studies.


**Conclusion**

Anaemia is a big burden during pregnancy, and pregnant women of eastern Sudan are more susceptible to anaemia irrespective to their age or parity. In addition, anaemia among adolescent girls has negative consequences on growth, school performance, morbidity and reproductive performance.

We reported types and causes of anaemia in Sudanese pregnant women. The main findings of this study are; high prevalence of anaemia and folate deficiency, and relatively low prevalence of iron deficiency anaemia and very low prevalence of Vitamin B\textsubscript{12} deficiency.

The low prevalence of vitamin B\textsubscript{12} deficiency could be explained mainly by high consumption of fermented food, which contains several vitamin B\textsubscript{12}-producing microorganisms [97].

None of the women had both folate and vitamin B\textsubscript{12} deficiencies, and the percentages of women with folate deficiency, vitamin B\textsubscript{12} and with both folate and vitamin B\textsubscript{12} deficiencies were not significantly different in the anaemic group than in the non-anaemic group.

We also concluded that serum ferritin, folate and vitamin B\textsubscript{12} levels were not significantly associated with anaemia.

Serum ferritin and zinc were significantly lower in patients with severe anaemia. Hb levels were significantly positively correlated with zinc levels and with copper levels. Thus, interventions are required to prevent and control anaemia in this group.
Folate and ferritin levels were significantly lower in those women who had preterm delivery and short IPI. Thus the study confirmed the hypothesis of the role of folate depletion in women with short IPI and preterm delivery, but it failed to show any difference in folate level in subgroups of short IPI in comparison with the referral group.

The increased risk of adverse pregnancy outcomes related to short IPI has been attributed to maternal nutritional depletion. No significant differences in folate levels between groups. This study also showed a significantly positive correlation between folate and haemoglobin level.

The main findings of this study were: serum zinc was significantly lower and that of copper was significantly higher in women with short IPI.
Recommendations

It is of interest to investigate the levels of micronutrients in early pregnancy to evaluate the role of these micronutrients in pregnancy and pregnancy outcome. Thus, further research is needed.

To introduce health care programme for adolescents in Sudan to assess prevalence and types of anaemia and deficiency of micronutrients and to initiate supplementation programmes.
References


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