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Associations between iron status and psychosocial wellbeing among pregnant women in Cape Coast, Ghana: a longitudinal study

Ruth A Pobee,[1, 2] Jacob Setorglo,[3] Moses Klevor,[4] Laura E. Murray-Kolb[1,5*]

[1]Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA USA;

[2]Department of Emergency Medicine, University of Illinois at Chicago

[3]Department of Medical Biochemistry, University of Cape Coast, Ghana;

[4] Department of Clinical Nutrition and Dietetics, University of Cape Coast, Ghana

[5]Department of Nutrition Science, Purdue University, West Lafayette, IN, USA

***Corresponding Author**: Laura E. Murray-Kolb, Department of Nutrition Science, Purdue University, West Lafayette, IN 47907, Tel: 765-496-3570

Short Title: Iron and psychosocial wellbeing in pregnancy

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Authorship

R.A.P., designed research; conducted research; supervised field work, analyzed data, wrote initial draft of manuscript. J.S., and M.K., conducted research and supervised field work, while L.E.M.-K. designed research, obtained funding for research, and participated in manuscript development. All authors read and approved the final manuscript.

Ethical approval

Ethical approval was obtained from the Ghana Health Service Ethical Review Committee, University of Cape Coast Institutional Review Board, Cape Coast Teaching Hospital Ethical Review Committee and The Pennsylvania State University Institutional Review Board.

Abstract:

Objective: To determine the associations among iron status, depressive/anxiety symptoms, and quality of life (QoL) throughout pregnancy.

Design: This longitudinal study recruited participants in their 1st trimester (< 13 weeks; n=116) and followed in their 2nd (n=71) and 3rd (n=71) trimesters. Sociodemographic, food security, anxiety, depressive symptoms, and QoL questions were collected. Hemoglobin (Hb), ferritin (Ft), and transferrin saturation (TSAT) were determined. Women were categorized as iron improvers or non-improvers based on changes in iron status. Associations were assessed using difference-in-difference analyses.

Setting: Cape Coast, Ghana between October 2017 to September 2018.

Participants: Pregnant women, 18-38 years.

Results: Improvement in Ft levels from the 1st to 2nd trimester were associated with reduced depressive symptoms (-2.96 vs -0.58, p=0.028), and higher overall QoL (13.99 vs 1.92, p=0.006) particularly role physical (23.32 vs -2.55, p=0.025) and role emotional (27.50 vs 10.06, p=0.025) subscales. Improvement in Hb levels during the same period were linked to less anxiety, particularly fear factor (-2.62 vs -0.51, p=0.020); and worsened physical health aspect of QoL (-21.80 vs -3.75, p=0.005). Improvement in TSAT levels from 2nd to 3rd trimester were associated with increased total anxiety (1.56 vs -0.64, p=0.030) and panic factor (0.45 vs -0.26, p=0.004) and decreased total QoL (-1.08 vs 7.94, p=0.017), specifically role physical (-10.98 vs 11.93, p=0.018).

Conclusion: Increases in iron status from first to second trimester were related to improvements in psychosocial wellbeing, implying potential benefit of iron supplementation on affect in early pregnancy. Larger studies are needed to confirm these findings.

Key words: Pregnancy, iron deficiency, anxiety, depressive symptoms, quality of life.

Introduction

Iron deficiency and poor psychosocial health may have a devastating impact on pregnancy and its outcomes. During pregnancy, mothers go through many physiological, nutritional, and emotional changes and adjustments [(1)]. Iron is one important nutrient whose requirement increases during pregnancy, mainly due to the rapid fetal growth and cell division, expansion of plasma volume, growth of the fetal placental unit and the fulfillment of the maternal requirements [(2)]. Maternal iron deficiency (ID) is a risk factor for poor maternal and child outcomes particularly preterm delivery, low birth weight, and poor child cognitive and motor development [(3)]. Maternal anemia has been associated with maternal and infant mortality, mood changes, emotional disturbances and psychological distress during pregnancy [(4)]. Additionally, psychosocial disturbances including depression, anxiety, and low quality of life (QoL) may be exacerbated during pregnancy due to the various hormonal changes (estrogen and progesterone) that occur [(5)]. Depression and anxiety are associated with a woman's social and biological function and can lead to self-harm and suicide [(6)]. Psychosocial outcomes have broad implications for maternal and child mental and physical health, with long-lasting negative social and economic impacts [(7)].

The mechanism by which iron is related to psychosocial wellbeing is not well understood, however it has been proposed to be linked to the known requirement of iron for neurotransmitter production and function [(8)]. Iron is required for the synthesis of serotonin, norepinephrine and dopamine [(9)]. Iron is also required for the degradation of these neurotransmitters via its role in monoamine oxidase [(10)]. Insufficient iron will, therefore, limit the production, degradation and clearance of these neurotransmitters which may consequently lead to poor psychosocial outcomes. Another proposed mechanism is associated with chronic or severe psychological stress exposure which activates the hypothalamic-pituitary-adrenocortical (HPA) axis, increasing proinflammatory cytokines, thus disrupting iron absorption and utilization, which eventually may lead to iron deficiency [(11)].

Existing literature suggests that ID individuals have higher depressive and anxiety scores but lower quality of life scores than iron sufficient individuals [(12–16)]. Several intervention studies indicate that treatment with iron can improve these outcomes [(17,18)] while others do not support

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these findings [19,20]. Differences in findings may be due to the heterogeneity of the studies (different questionnaires to assess outcomes of interest, different iron biomarkers to determine iron status, and different interventions used as treatment). Additionally, most of the studies done to date, even though longitudinal in nature, were conducted in high-income countries and were among postpartum women but not pregnant women [12,17]. The existing literature on the relation between iron status and psychosocial outcomes during pregnancy is sparse. Despite the devastating consequences of both psychosocial disorders and iron deficiency on pregnancy and its outcomes, very little is known about the relation between iron deficiency and psychosocial wellbeing during pregnancy, especially in developing countries. Longitudinal studies in low-income countries are needed to determine if this relation persists.

Therefore, this study aimed to determine the associations between iron status and psychosocial wellbeing (including depressive symptoms, anxiety symptoms, and quality of life) in Ghanaian women during pregnancy. The hypothesis postulated that improvement in iron status from the 1st to 2nd, 1st to 3rd, or 2nd to 3rd trimester would correlate with better psychosocial outcomes within the respective trimesters.

Methods

Study design

A longitudinal study was carried out from October 2017 to September 2018. Women were recruited at <13 weeks gestation, when they reported for their first antenatal visit in clinics and hospitals which form the major health centers in the Cape Coast and Elmina Metropolis. Multistage sampling was employed to select healthcare facilities. Initial data on antenatal attendance throughout the year were gathered from the regional Ministry of Health office to identify suitable health facilities. Nine facilities were purposefully selected based on previous antenatal attendances that aligned with our research requirements. Simple random sampling was subsequently applied to select seven health facilities for the study, which comprised Moree Clinic, Cape Coast Teaching Hospital, Cape Coast Metropolitan Hospital, Ewim Polyclinic, University of Cape Coast Hospital, Elmina Urban Health, and Abura Dunkwa District Hospital.

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Screening and recruitment

At their first antenatal visit, prospective participants were informed about the study by the nurses on duty, and interested pregnant women were directed to the research team. A screening form was used to determine eligibility, which included attendance at any of the seven selected prenatal clinics in Central Region of Ghana; aged between 18-38 years at enrolment; <13 weeks gestation at enrolment (determined by last menstrual period or ultrasound); expecting a singleton pregnancy with no known congenital anomalies; and no known history of diabetes mellitus or hypertension. Participants were excluded if they did not attend any of the seven selected prenatal hospitals/clinic, were under 18 or above 38 years at enrolment, had gestational age exceeding 13 weeks, were carrying multiple pregnancies, had congenital anomalies or had a history of hypertension or diabetes at enrolment. Eligible and interested participants gave written informed consent. Data on sociodemographic characteristics (age, socioeconomic status (SES), income level, years of schooling, parity, marital status, food insecurity (FIS)), psychosocial wellbeing (depression, anxiety, and QoL), anthropometry, blood pressure, and collection of blood samples occurred immediately, unless the woman requested to come back at a later date. Four trained field assistants helped with data collection at each of the three time points. After the first visit was completed, each participant was provided with a date for her second trimester visit. The same participants were then followed into their second (13-27 weeks) and third trimesters (28-36 weeks). At the end of the first two visits, each participant received a bar of soap plus money to cover transportation cost, as incentives, and at the end of the third visit, each participant received a baby onesie in addition.

Data collection procedures

Participants were asked basic sociodemographic information such as age, marital status, parity, and food insecurity which were recorded on a tablet. A food frequency questionnaire was used to evaluate the consumption of iron rich foods (red meat), vegetables, and fruit (orange, pineapples, mangoes, pawpaw) during the week prior to data collection [(21)]. Information on health status such as diabetes, hypertension, and malaria were asked. Food insecurity was assessed using an 8-item version of the US Household Food Security Survey Module [(22)]. Weight and height measurements were collected at each trimester visit using a Seca scale and portable stadiometer, respectively.

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Sample population

Out of the initial 119 participants recruited in the first trimester with complete data, 46 pregnant women withdrew from the study due to reasons such as miscarriage, spouse refusal, and being unreachable by phone. Seventy-three pregnant women were tracked into their second trimester. By the third trimester, 72 pregnant women remained in the study, with 15 dropping out between the second and third trimesters due to reasons such as relocation, delivery, or refusal to continue participation. Fourteen pregnant women who were absent during the second trimester visit returned for the third trimester visit. However, the number of participants with complete socio-demographic data used for analyses were 116, 71, and 71, while 111, 68, and 65 participants provided a blood sample for the first, second, and third trimesters, respectively.

Blood draws

Approximately 4 mL of venous blood were drawn from the participant at each of the three time points during pregnancy. Trained phlebotomists at the hospitals/clinics collected the blood samples, and hemoglobin levels were determined on the spot via a hemocue (HB201; HemoCue America, Brea, CA, USA). The blood samples were stored on ice and delivered to the Cape Coast Teaching Hospital within 30 minutes of collection. Blood samples were centrifuged and serum aliquoted by a laboratory technician at the University of Cape Coast and subsequently stored in a -80^0 C freezer prior to shipping to The Pennsylvania State University, USA, where iron status biomarkers (serum iron, total iron binding capacity and serum Ft) and inflammatory biomarkers (alpha-1-acid glycoprotein (AGP) and c-reactive protein (CRP)) were determined. Serum ferritin was determined via ELISA (Ramco Laboratories TX, USA), calibrated against WHO standards. Serum iron and total iron binding capacity (TIBC) were determined using a colorimetric method [23]. Transferrin saturation (TSAT) was calculated [(serum iron/TIBC)×100]. AGP and CRP were measured using radial immunodiffusion tests (Kent Laboratories Inc., Bellingham, WA, USA) and used to adjust the Ft concentrations when inflammation was present using the Thurnham adjustment [24]. Ft values reported have therefore been adjusted for inflammation, where necessary.

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Assessment of psychosocial wellbeing

Depressive symptoms were assessed using the Center for Epidemiological Studies Depression Scale (CES-D) [25]. Anxiety was assessed using the Beck Anxiety Inventory (BAI) [26]. QoL was assessed using the RAND 36-Item Short Form Health Survey (SF-36) [27] at each of the three trimesters during pregnancy. A detailed description of these methods are described elsewhere [28]. In brief, pregnant women were asked to rate their depressive symptoms on a scale of 0-3 for each item. A cut-off ≥ 16 on the CES-D is indicative of elevated depressive symptoms [25]. Pregnant women rated their anxiety symptoms on a scale of 0-3 for each item. The cut-off point used for anxiety was ≥ 16 [26]. The RAND SF-36 measures 8 health constructs: physical functioning; role physical; bodily pain; social functioning; role emotional; general mental health (psychological distress and psychological wellbeing); vitality (energy/fatigue); and general health perceptions [29]. A cut-off of < 50 indicates low QoL.

Follow-up visits for 2nd and 3rd trimesters

During the second (13-27 weeks) and third trimester (28-36 weeks) visits, all measurements were repeated, with the exception of the SES questionnaire (which was only administered at the first visit).

Statistical Analysis

Demographic, food frequency, and clinical data were presented as frequencies for categorical data and means for continuous variables. Factor analysis was employed for the psychosocial data. This approach was adopted due to the origin of the psychosocial wellbeing questionnaires used in the study (CES-D, BAI, SF-36), which were developed within a Western cultural framework. Consequently, factor analysis was applied to the scales to evaluate the cultural suitability of these scales and ascertain the relevance of their scores and ensure the underlying constructs were valid within our population. A detailed description of the procedures can be found elsewhere [28]. In brief, exploratory factor analysis (EFA) with principal axis factoring and promax rotation was used to determine the CES-D, BAI, and RAND SF-36 factor solutions, using 1st trimester scores. Before factor analysis was considered, correlations between the items were checked and Kaiser-Meyer-Olkin Test (KMO) and Bartlett's test of sphericity were used to determine appropriateness for factor analysis [30]. Subsequently, scree plot, parallel analysis, and

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minimum average partials (MAP) were used to determine the number of factors to retain [31]. EFA was chosen since the psychometric measures have not been assessed for these scales in Ghana. For a factor to be considered adequate, pattern coefficients ≥ 0.30 were considered salient on a factor and a minimum of three salient items were required for each factor [32]. Internal consistency for each factor was examined using Cronbach's alpha ≥ 0.70 to determine adequacy, while communality showed the proportion of variance in each variable accounted for by each factor [33]. SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA) was used for data analysis. See supplemental Tables 1-3 for results on factor analysis.

Difference-in-difference analyses were used to determine the associations between change in iron status and change in psychosocial outcomes between trimesters. After calculating change in iron status between trimesters, participants were categorized as iron improvers or non-improvers, based on the known day-to-day variation for each iron biomarker. "Improvers" were those who experienced a positive change in iron status (Hb, Ft, Fe, TSAT or TIBC) greater than the known day-to-day variation and "non-improvers" were those who experienced changes in iron status less than the known day-to-day variation as well as negative changes in iron status beyond the known day-to-day variation [34]. To determine if change in psychosocial measures were associated with change in iron status, we controlled for covariates such as change in food insecurity, change in psychosocial outcomes (except the one used as predictor), parity, and marital status. Age, SES, income level, years of schooling, parity, marital status, and FIS were explored as potential covariates and those which correlated with the outcome variables assessed (anxiety, depression and QoL), were included in the analyses (FIS, parity, marital status, and other psychosocial outcomes (except the one used as predictor)). The 8-item version of the US Household Food Security Survey Module ranged from 0 (food secure) to a maximum of 8 (highest level of food insecurity). This was included as a continuous variable in analyses.

Results

The average age of participants was 27 ± 5 years with mean height of 1.59 ± 0.1 m. Mean weight was 61.7 ± 12.1 kg, 65.0 ± 12.9 kg, and 68.9 ± 11.9 kg for 1^{st} , 2^{nd} , and 3^{rd} trimesters, respectively (Table 1). About 68% of participants were either married or co-existing with a

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husband or partner while 32% were not married. About 8% of the women had no formal education, while 45% had up to a middle school education, with only 10% having a university degree. The majority (79%) of the women had an income-generating activity. Forty-seven percent of participants had children <5 years of age in their households, with 36% having two children <5 years in the household. Most participants (70%) had between 1-5 children, while 30% had no child prior to their current pregnancy. About 5% of the pregnant women had sickle cell traits (Table 1).

The prevalence of self-reported anemia was 4%, 3%, and 23%; while malaria was 12%, 17%, 17% for 1st, 2nd, and 3rd trimesters, respectively. No woman self-reported as diabetic or hypertensive in the 1st trimester. However, in the 2nd trimester, 1% reported hypertension and diabetes diagnoses while 1% reported hypertension but not diabetes diagnoses in the third trimester (Table 2). The mean systolic blood pressure (mean± SD) was 105±9 mmHg, 103±8 mmHg and 108±8 mmHg; and the mean diastolic blood pressure was 68±8 mmHg, 64±8 mmHg, and 69±8 mmHg for 1st, 2nd and 3rd trimesters, respectively (Table 2). About 23%, 90% and 58% of pregnant women reported taking iron supplements while 28%, 58% and 70% reported taking vitamin or mineral supplements during the 1st, 2nd and 3rd trimesters of pregnancy, respectively. About 50%, 30% and 25% of the women were food insecure in their 1st, 2nd and 3rd trimesters, respectively (Table 2).

Measured prevalence of iron deficiency

The prevalence of anemia (Hb <11.0 g/dL) was 37%, 63%, 58%; ID (Ft <15 μg/L) was 16%, 20%, 38%; ID (TIBC ≥ 400 μg/L) was 19%, 29% and 40%; ID (TSAT <16%) was 12%, 9% and 17%. About 30%, 22% and 32% of pregnant women had inflammation based on CRP (≥5.0 mg/L) while 29%, 6% and 2% had inflammation based on AGP (≥1.0 mg/L) for 1st, 2nd and 3rd trimesters, respectively. These values are based on assessment of iron status by the study, see Pobee et al., 2021.[35]

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Exploratory factor analysis

Factor analysis of the psychosocial scales revealed a one-factor solution with 13 items for the CES-D scale. This one-factor solution accounted for 100% of the variance, with a Cronbach's alpha of 0.84 and an eigenvalue of 8.78. The items included in this factor are: bothered, blues, mind, depressed, effort, restless, happy, lonely, unfriendly, enjoy, cry, sad, and dislike. These items describe depressive affect and interpersonal concerns, with two items reflecting positive affects (Supplemental Table 1).

A four-factor solution with 18 items was obtained for the BAI which accounted for 98.9% of the cumulative variance. Factor I had a Cronbach's alpha of 0.84 and an eigenvalue of 16.22, explaining about 57.75% of the variance. This factor was named the "fear-factor" because it included items such as scared, fear of dying, terrified, and fear of the worse. Factor II had a Cronbach's alpha of 0.70 and an eigenvalue of 5.42, explaining about 19.30% of the variance. This factor was named the "nervous-factor" because it included items such as numbness, wobbling legs, unable to relax, face-flushing, and hot/cold sweats etc. Factor III had a Cronbach's alpha of 0.76 and an eigenvalue of 4.10, explaining about 14.61% of the variance. This factor was named the "panic-factor" because it included items such as unsteady, shaky, choking, and fear of losing control. Factor IV had a Cronbach's alpha of 0.72 and an eigenvalue of 2.34, explaining about 8.35% of the variance. This factor was named the "somatic-factor" because it included items such as faint, dizzy, heart pounding, and hand trembling (Supplemental Table 2).

The RAND SF-36 had a four-factor solution with 26 items. The four-factor solution accounted for 100% of the cumulative variance. Factor I had a Cronbach alpha of 0.86 and an eigenvalue of 14.39 and it explained about 52.38% of the variance. Factor I was named the “physical health” factor because it included items such as performing vigorous activities, lifting, climbing stairs, etc. Factor II had a Cronbach alpha of 0.84 and an eigenvalue of 6.44 and it explained 23.45% of the variance. Factor II was named the “role physical” factor because it included items such as accomplish less, cut down on work, etc. Factor III had a Cronbach alpha of 0.79 and an eigenvalue of 3.62 and it explained 13.17% of the variance. Factor III was named the “role emotional” factor because it included items such as emotions limiting your work as a result of emotional problems or did not do work as carefully as usual as a result of emotional problems,

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etc. Factor IV had a Cronbach alpha of 0.73 and eigenvalue of 3.02 and it explained about 11% of the variance. Factor IV was named the "general health/vitality" (GHV) factor because it included items such as energy, pep, worn-out, excellent health (Supplemental Table 3). For additional information on factor analysis, see Pobee et al., 2022 [28].

Difference-in-difference analysis

We observed an improvement in depressive symptoms with improvement in Ft concentration. After controlling for covariates, women who experienced a significant increase in Ft concentrations between the 1st and 2nd trimesters (Ft "improvers") also experienced a significantly greater decrease in depressive symptoms during this time period, compared to women labeled as Ft "non-improvers" (-2.96 vs -0.58, p=0.028) (Table 3). Being an "improver" or "non-improver", based on changes in other iron biomarkers from 1st to 2nd trimester, was not associated with any difference in depressive symptom scores (Table 3). Between the 1st and 3rd as well as between the 2nd and 3rd trimesters, there were no significant differences observed in change in depressive symptoms scores between improvers and non-improvers for all iron biomarkers, after controlling for covariates (Table 3).

Regarding anxiety, and changes between the 1st and 2nd trimesters, fear scores decreased significantly more in Hb improvers, compared to Hb non-improvers (-2.62 vs -0.51, p=0.020). Changes in iron biomarkers between the 1st and 3rd trimesters were not differentially related to changes in anxiety for those classified as improvers vs. non-improvers (Table 4). However, between the 2nd and 3rd trimesters, total anxiety scores (1.56 vs -0.64, p=0.030) and its subscale, panic factor scores (0.45 vs -0.26, p=0.004), increased significantly more in TSAT improvers compared to TSAT non-improvers (Table 4).

Furthermore, associations between changes in iron biomarkers and QoL were observed. From 1st to 2nd trimester, physical health scores (-21.80 vs -3.75, p=0.005) decreased significantly more in Hb improvers compared to Hb non-improvers controlling for covariates (Table 5), whereas total

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QoL (13.99 vs 1.92, p=0.006), and its subscales, role physical (23.21 vs -2.55, p=0.025) and role emotional (27.50 vs 10.06, p=0.021) scores increased to a significantly greater extent in Ft improvers than Ft non-improvers, after adjusting for covariates (Table 5). Total QoL (-1.08 vs 7.94, p=0.017) and role physical scores (-10.98 vs 11.93, p=0.018) significantly decreased in TSAT improvers compared to TSAT non-improvers (Table 5). Between the 1st and 3rd and the 2nd and 3rd trimesters, no significant differences were seen between changes in QoL scores for those labeled as improvers or non-improvers, regardless of iron biomarker assessed (Table 5).

Discussion

To our knowledge, this is the first study to simultaneously examine the relation between iron status and depression and anxiety symptoms, and QoL throughout pregnancy. Relatively few studies have examined women's health in terms of iron status and psychosocial outcomes. Our study has added to the scarce literature available on the association between iron status and psychosocial outcomes during pregnancy and has shown that improvements in iron status at the early stages of pregnancy may have a greater impact on psychosocial wellbeing compared to improvements in iron status later in pregnancy. Our findings should help inform future programs aimed at iron supplementation during pregnancy to improve depressive symptoms and quality of life.

Association between iron status and depressive symptoms

Our study found an association between Ft and depressive symptoms. Pregnant women whose Ft levels improved from 1st to 2nd trimester experienced a decrease in depressive symptoms, suggesting that iron stores at the beginning of pregnancy may play a crucial role in the progression of depressive symptoms during pregnancy. Therefore, it is important for women to begin pregnancy with adequate iron stores to help prevent depressive symptoms.

So far, only one study, examined the association between iron status and depressive symptoms during pregnancy and found no correlation [36]. The contrasting findings could be due to the timing of the assessments; we examined the relation early in the first trimester (<13 week), while

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Armony-Sivan and colleagues assessed it during mid and late pregnancy, potentially missing the importance of early Ft stores. Additionally, while we assessed depressive symptoms using the CES-D scale, Armony-Sivan and colleagues used the Edinburgh Postnatal Depression Scale, which may also account for the differing results. Furthermore, we used psychometric analyses to validate our scores, which was not done by Armony-Sivan et al.

Of note, the literature on the association between iron status and depressive symptoms which focused on non-pregnant women of reproductive age (WRA), has mixed findings. Some studies did not find associations between iron status and depressive symptoms [19,20,37]) while others did [12,38,39]. Of those that found an association, most were intervention studies [17,18,38] and cross sectional. These studies reported either a significant negative association between Hb and depressive symptoms or between Ft and depressive symptoms [12,17,18]. Our finding of a negative association between Ft and depressive symptoms contributes to the existing literature and provides additional insight into this this association during pregnancy.

Association between iron status and anxiety symptoms

To our knowledge, no study has looked at the association between anxiety symptoms and iron status during pregnancy. We found an association between the fear factor subscale and hemoglobin concentrations, and between total anxiety scores, the panic subscale, and transferrin saturation. Pregnant women whose Hb improved from the 1st to 2nd trimester experienced a significantly greater decrease in fear compared to those whose Hb did not improve, and a higher Hb concentration during the 2nd trimester was associated with lower fear scores. On the other hand, pregnant women whose TSAT improved from 2nd to 3rd trimester experienced an increase in total anxiety and panic symptoms, while TSAT non-improvers experienced a decrease in these scores. This is in contrast with our hypothesis. The literature, however, suggests an association between anxiety and iron status among WRA, especially during the post-partum period [17,18,20]. These studies on anxiety have been conducted mostly in combination with assessments of depressive symptoms. Unlike our study, findings by Lever-van *et al.*, did not show an association between Hb and anxiety [20]. However, an intervention study by Verdon *et al*., found that anxiety symptoms decreased more in the iron treatment group [18], whereas Beard *et al*., did not find changes in anxiety symptoms with iron supplementation [17].

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Our findings regarding TSAT were unexpected; however, a possible explanation for the predominant finding being opposite our original hypothesis is that psychosocial outcomes have two components: physiological and psychological. The physiological component of anxiety may require a certain amount of energy. Therefore, a pregnant woman may need energy to experience anxiety, and those who are iron sufficient might report higher anxiety due to having more energy. This could explain why better iron transport was associated with increased anxiety, as observed in this study.

Association between iron status and QoL

Our results for QoL were similar in direction and variability to the results we obtained from our anxiety scores. For change between trimesters 1 and 2, we found that Hb improvers decreased significantly in physical health scores, while TSAT improvers decreased significantly in total QoL (TQoL) and role physical scores, compared to non-improvers, and Ft improvers increased significantly in TQoL, role physical and role emotional scores, compared to Ft non-improvers. The fact that our findings in relation to Hb and TSAT are in the opposite direction of our original hypothesis could be due to similar reasons as outlined for anxiety. We found that better iron stores are not beneficial to QoL in the 2nd and 3rd trimesters. However, Ft-improvers increased in TQoL, role physical and role emotional scores while Hb improvers decreased in physical health compared to non-improvers. If we are speculating that a pregnant woman needs energy to be anxious, then better iron stores may lead to more anxiety and if the pregnant woman becomes aware of her anxiety, then the emotional aspects of anxiety may set in, causing her to think and worry constantly about her anxiety, thus affecting her QoL. These are mere speculations, and more research is clearly warranted to better understand these associations.

When we compare our findings to existing literature (with the caveat that the previous studies were not conducted during pregnancy), we find that there is still much to understand about these relations. In a cross-sectional study Fordy and Benton, found no association between Ft and mood using the General Health Questionnaire among males and females in Wales, UK [(40)]. Their study was however cross sectional and involved male and female students and, as such, may not be generalizable. A similar study by Rangan *et al.,* involving female students in Australia found no relation between ID and QoL except for in anemic subjects [(41)]. Anemic subjects in this study,

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however, scored significantly higher on general health questions than non-anemic subjects. Another study in France, among a population of menstruating women, found no significant impact of iron deficiency on any component of the Duke score [42]. Two other studies in New Zealand among female students aged 18-44 years [26] and in Australia male and female blood donors who were between 26-53 years,[43] did not find a relationship between iron status and QoL. The New Zealand study authors reported that they used a convenience sample of students; as such, their results might not be generalizable. The Australian study authors, reported that there could be selection bias in their sample of blood donors chosen for their study, since only healthy blood donors may have reported for blood donation. Contrary to the studies above, two studies in Australia among women of reproductive age reported a relation between iron and QoL [14,44]. The first study was on a cohort of young and middle-aged women[44] while the other study was a randomized controlled trial involving women (18-50 years) who were given either supplements or a dietary regimen [14]. In the cohort study, iron deficiency was associated with decreased general health and wellbeing and increased fatigue. In the intervention group, mental component, and vitality scores were lower and physical functioning scores were higher for iron deficient women, compared to iron replete women. Similarly, a study by Grondin *et al*. in France among female students (17-38 years) found iron deficiency to be associated with lower perceived general health [13].

Our findings agree with the studies that found a relationship between iron status and QoL. In general, we did not find a relation between iron status and QoL in the 2nd and 3rd trimesters. However, between the 1st and 2nd trimesters, Ft-improvers increased in TQoL, role physical and role emotional scores while Hb improvers decreased in physical health scores compared to non-improvers . Similarly, TQoL and role physical scores decreased in TSAT improvers compared to TSAT non-improvers. Our findings show mixed results, and they must be interpreted with caution since our sample size was small and iron deficiency was not highly prevalent throughout pregnancy, except for the 3rd trimester. Moreover, this is the first study to look at the association between iron and QoL during pregnancy. As such, there is a need to conduct more studies in a highly prevalent ID population with a large sample size to confirm this finding throughout pregnancy.

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In summary, the most significant changes in psychosocial health between iron improvers and non-improvers (particularly in Ft and Hb) occurred between the 1st and 2nd trimesters. Ft improvers showed a decrease in depressive symptoms and an increase in total quality of life (TQoL), role physical, and role emotional scores compared to Ft non-improvers. Conversely, Hb improvers experienced a decrease in physical health compared to Hb non-improvers. No significant changes were observed between the 1st and 3rd trimesters or between the 2nd and 3rd trimesters for any psychosocial variables except for anxiety. TSAT improvers showed an increase in total anxiety and panic factors, whereas TSAT non-improvers showed a decrease.

The biological underpinnings of these findings may relate to changes in neurotransmitters as a result of iron deficiency and/or to changes in iron absorption as a result of chronic or severe psychological stress [11].

A few strengths of this study include its longitudinal design, which assesses both iron biomarkers and symptoms of depression and anxiety during pregnancy. Currently, iron supplementation is primarily aimed at treating iron deficiency and anemia. However, results of this study suggest that such supplementation should also be considered as a potential mechanism for improving psychosocial wellbeing. The findings from this study may be applicable to both high-income and low- and middle-income countries with similar social and economic backgrounds. Another strength is that this study was conducted in a population and culture that has not been extensively studied. Also, psychometric analyses were performed on the CES-D, BAI, and SF-36 scales, as such we are confident in the reliability of these questionnaires in Ghanaian pregnant women. We used factor loadings to examine the association between iron status and psychosocial variables, a method that is rarely used in nutrition studies. Typically, psychometric analyses are employed to test the reliability of questionnaires, but the factor loadings are often not utilized in subsequent statistical analyses. Using raw scores instead of factor loadings can undermine the nuanced insights provided by psychometric analyses.

The main limitation of this study was the high dropout rate of 37.8% from the 1st to 2nd trimesters, which reduced our sample size substantially during the 2nd and 3rd trimesters. Factors such as miscarriage, relocation, delivery, unanswered phone calls, and refusing participation led to this high dropout rate. Also, even though the intake of iron rich foods, particularly red meat,

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was assessed, assessing other iron sources such as chicken and fish may have been beneficial for a more comprehensive view of iron intake and is warranted for future studies.

Conclusions

Our data support the hypothesis that iron deficiency, as indicated by ferritin levels during early pregnancy, is associated with poorer psychosocial well-being. This suggests that iron supplementation in early pregnancy could potentially benefit women's mental health. These findings should alert stakeholders to the importance of screening for psychological issues during pregnancy—a practice that is not currently standard in many developing countries, including Ghana. We recommend replicating this study in areas with a higher prevalence of iron deficiency and with a larger sample size to address unanswered questions.

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Table 1: Socio-demographic characteristics of pregnant Ghanaian women in their first trimester (n=116)

Sociodemographic variables	n	%
Age (years)	116 (18-38)*	27.1 (5.2)†
Mean Height (m)	116	1.59 (0.1)†
Mean Weight (kg)		
1st trimester	116	61.7 (12.1) †
2nd trimester	71	65.0 (12.9) †
3rd trimester	71	68.9 (11.9) †
Marital Status		
Married or cohabitating	79	68.1
Not Married	37	31.9
Parity		
0	35	30.2
1-5	81	69.8
Educational Level		
No school	9	7.8
Primary	17	14.7
Middle	52	44.8
Secondary	26	22.4
University	12	10.3
Years of schooling		
0-6	12	11.2
7-9	56	52.4
>9	39	36.5
Income generating activity		
Yes	92	79.3
No	24	20.7
Number of children <5 years		
1	32	58.2
2	20	36.4
>2	3	5.5
Sickle cell trait		
Yes	6	5.2
No	107	92.2
Unknown	3	2.6

*n (range), †mean (SD)

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Table 2: Prevalence of disease, high blood pressure and supplement use among women during pregnancy

Variables	1st Trimester		2nd Trimester		3rd Trimester	
	n (range)	%	n (range)	%	n (range)	%
Gestational age, mean ± SD	116 (1-12)	8.29 ±3.33	71(16-27)	22.04±2.58	71 (28-36)	32.26±2.36
Anemia						
Yes	4	3.5	2	2.8	16	22.5
No	110	94.8	69	97.2	55	77.5
Don't know	2	1.7	-	-	-	-
Malaria						
Yes	14	12.1	12	16.9	12	16.9
No	102	87.9	59	83.1	59	83.1
Diabetes						
Yes	-	-	1	1.4	-	-
No	115	99.1	70	98.6	71	100.0
Don't know	1	0.9	-	-	-	-
High Blood Pressure						
Yes	0	0.0	1	1.4	1	1.4
No	116	100.0	70	98.6	70	98.6
Blood Pressure (mmHg**)***						
Systolic, mean ± SD	116 (75-134)	105±9	71 (84-122)	103±8	71 (92-133)	108±8
Diastolic, mean ± SD	116 (49- 90)	68±8	71 (49-90)	64±8	71 (51-89)	69±8
Iron supplement intake						
Yes	27	23.3	64	90.1	58	81.7
No	89	76.7	6	8.5	13	18.3
Don't know	-	-	1	1.4	-	-
Frequency of Iron supplement/week						
1 time	1	3.7	-	-	3	5.2
2-3 times	-	-	-	-	-	-
4+ times	26	96.3	64	100	55	94.8
N/a	-	-	-	-	-	-
Vitamin/Mineral supplement						
Yes	32	27.6	41	57.7	50	70.4
No	80	69.0	28	39.4	19	26.8
Don't know	4	3.4	2	2.8	2	2.8
Food Insecurity*	116		71		71	
Food Insecure	58	50.0	21	29.6	18	25.4
Food Secure	58	50.0	50	70.4	53	74.7

*Blood pressure and Food Insecurity were measured in the study, all other variables were based on self-report

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Table 3: Change in depressive symptoms scores relative to changes in iron biomarkers, comparing improvement versus non-improvement between trimesters.

			Depressive Symptoms		
Iron Biomarkers		n	Mean	SE	p-value
Trimester 2 versus Trimester 1					
Hemoglobin	Non-improvers	59	-0.79	0.64	0.746
	Improvers	11	-1.19	1.24	
Ferritin	Non-improvers	53	-0.58	0.67	0.028*
	Improvers	10	-2.96	1.05	
Transferrin Saturation	Non-improvers	43	-1.42	0.78	0.480
	Improvers	20	-0.76	0.84	
Trimester 3 versus Trimester 1					
Hemoglobin	Non-improvers	49	-1.88	0.69	0.920
	Improvers	18	-1.99	1.02	
Ferritin	Non-improvers	47	-2.05	0.66	0.953
	Improvers	13	-1.99	0.99	
Transferrin Saturation	Non-improvers	40	-1.87	0.72	0.701
	Improvers	19	-2.25	0.88	
Trimester 3 versus Trimester 2					
Hemoglobin	Non-improvers	31	-0.69	0.81	0.835
	Improvers	23	-0.90	0.91	
Ferritin	Non-improvers	34	-0.74	0.82	0.556
	Improvers	13	-0.10	1.09	
Transferrin Saturation	Non-improvers	30	-1.80	0.76	0.065
	Improvers	18	0.21	0.92	

Change in depressive symptoms versus change in iron biomarkers, controlling for covariates (psychosocial outcome at trimester 1, and iron variable trimester 1, change in food insecurity, change in anxiety, and quality of life), parity and marital status *p-value <0.05. Ferritin: adjusted for C-reactive protein and Alpha-1-acid glycoprotein using Thurnham adjustment (24).

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Table 4: Change in anxiety symptoms scores and their subscales relative to changes in iron biomarkers, comparing improvement versus or non-improvement between trimesters.

Iron Biomarkers			Anxiety Symptoms													
			Total Anxiety Factor Scores			Fear Factor			Nervous Factor			Panic Factor			Somatic Factor		
		n	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value
Trimester 2 versus Trimester 1																	
Hemoglobin	Non-improvers	59	-3.50	0.64	0.146	-0.51	0.43	0.020*	0.23	0.64	0.052	-0.86	0.39	0.451	-1.11	0.28	0.427
	Improvers	11	-5.42	1.34		-2.62	0.90		-1.11	0.31		-1.46	0.81		-1.57	0.59	
Ferritin	Non-improvers	53	-4.00	0.71	0.922	-0.75	0.50	0.888	-0.70	0.50	0.435	-0.84	0.39	0.451	-1.30	0.31	0.857
	Improvers	10	-3.88	1.15		-0.64	0.81		-0.85	0.35		-1.34	0.64		-1.21	0.50	
Transferrin Saturation	Non-improvers	43	3.16	0.76	0.261	-0.20	0.51	0.159	-1.09	0.40	0.579	-0.60	0.44	0.316	-1.51	0.32	0.123
	Improvers	20	-4.25	0.87		-1.12			-0.61	0.44		-1.16	0.50		-0.87	0.37	
Trimester 3 versus Trimester 1																	
Hemoglobin	Non-improvers	49	-3.35	0.76	0.684	-0.73	0.43	0.337	-0.57	0.71	0.957	-1.09	0.36	0.752	-1.17	0.35	0.947
	Improvers	18	-3.87	1.18		-1.41	0.64		-0.82	0.42		-0.88	0.59		-1.13	0.56	
Ferritin	Non-improvers	47	-3.97	0.71	0.471	-1.07	0.48	0.329	-0.42	0.80	0.618	-0.90	0.31	0.139	-1.38	0.32	0.731
	Improvers	13	-4.76	1.10		-1.97	0.91		-0.28	0.48		-1.59	0.46		-1.59	0.61	
Transferrin Saturation	Non-improvers	40	-4.19	0.81	0.492	-1.54	0.53	0.144	-1.26	0.67	0.220	-1.13	0.39	0.906	-1.38	0.36	0.790
	Improvers	19	-3.41	0.98		-0.22	0.75					-1.05	0.55		-1.22	0.50	
Trimester 3 versus Trimester 2																	
Hemoglobin	Non-improvers	31	-0.05	0.89	0.339	-0.24	0.41	0.786	0.15	0.40	0.385	-0.11	0.23	0.093	-0.05	0.21	1.000

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	Improvers	23	-1.11	1.02		-0.38	0.46		-0.29	0.46		-0.38	0.26		-0.05	0.24	
Ferritin	Non-improvers	34	-0.44	0.68	0.837	-0.38	0.29	0.768	0.24	0.35	0.500	-0.10	0.18	0.775	-0.19	0.17	0.810
	Improvers	13	-0.67	1.13		-0.24	0.49		-0.15	0.58		-0.02	0.30		-0.26	0.28	
Transferrin Saturation	Non-improvers	30	-0.64	0.74	0.030*	-0.42	0.36	0.070	0.20	0.38	0.445	-0.26	0.18	0.004*	-0.17	0.17	0.320
	Improvers	18	1.56	1.02		0.47	0.50		0.59	0.52		0.45	0.24		0.05	0.23	

Change in anxiety symptoms versus change in iron biomarkers, controlling for covariates (psychosocial outcome at T1 (continuous) and iron variable T1 (continuous), change in food insecurity, change in depressive symptoms and quality of life), parity and marital status). *p-value <0.05. Ferritin: adjusted for C-reactive protein and Alpha-1-acid glycoprotein using Thurnham adjustment (24).

Table 5: Change in quality-of-life scores and their subscales relative to changes in iron biomarkers, comparing improvement versus non-improvement between trimesters.

Iron Biomarkers			Quality of Life														
			Total QoL Factor Score			Physical Health			Role Physical			Role Emotional			General Health/vitality		
		n	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value
Trimester 2 versus Trimester 1																	
Hemoglobin	Non-improvers	59	4.06	2.54	0.429	-3.75	2.94	0.005*	1.57	6.66	0.623	11.71	4.58	0.326	6.71	1.63	0.709
	Improvers	11	-0.12	5.28		-21.80	6.13		-5.26	13.88		21.12	9.54		5.44	3.40	
Ferritin	Non-improvers	53	1.92	2.53	0.006*	-6.30	3.22	0.500	-2.55	6.73	0.025*	10.06	4.43	0.021*	6.48	1.79	0.626
	Improvers	10	13.99	4.18		-2.68	5.31		23.21	11.09		27.50	7.30		7.93	2.94	
Transferrin Saturation	Non-improvers	43	7.94	2.90	0.017*	-5.33	3.68	0.840	11.93	7.46	0.018*	17.72	5.38	0.112	7.44	1.98	0.620
	Improvers	20	-1.08	3.29		-6.27	4.17		-10.98	8.46		6.75	6.11		6.20	2.24	
Trimester 3 versus Trimester 1																	
Hemoglobin	Non-improvers	49	-6.88	3.12	0.659	-10.90	4.16	0.116	-15.43	5.59	0.219	-8.50	6.30	0.233	4.68	2.41	0.423
	Improvers	18	-4.37	4.96		-22.99	6.61		-4.4	8.2		5.30	10.01		8.21	3.83	

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Ferritin	Non-improvers	47	-5.51	2.99	0.717	-13.92	4.13	0.110	-9.68	5.13	0.673	-3.77	5.86	0.920	5.33	2.24	0.858
	Improvers	13	-7.62	5.76		-26.95	7.95		-5.46	9.87		-2.63	11.27		4.55	4.32	
Transferrin Saturation	Non-improvers	40	-2.02	3.32	0.101	-14.14	4.72	0.445	-9.63	5.87	0.282	2.93	6.66	0.463	6.26	2.49	0.617
	Improvers	19	-11.27	4.66		-20.17	6.62		-18.1	7.26		-5.23	9.33		4.18	3.49	
Trimester 3 versus Trimester 2																	
Hemoglobin	Non-improvers	31	-8.78	3.13	0.408	-6.80	4.98	0.541	-7.18	6.7	0.340	-21.65	6.52	0.848	0.52	2.19	0.323
	Improvers	23	-12.04	3.48		-10.63	5.53		-15.24	7.44		-20.09	7.23		-2.21	2.43	
Ferritin	Non-improvers	34	-8.50	2.76	0.896	-9.27	4.23	0.745	-7.78	5.67	0.646	-16.30	5.89	0.771	-0.64	1.93	0.617
	Improvers	13	-7.89	4.66		-6.95	7.15		-12.18	9.59		-13.41	9.97		0.99	3.26	
Transferrin Saturation	Non-improvers	30	-4.16	3.41	0.106	-4.38	5.22	0.099	-4.45	7.12	0.302	-10.17	7.34	0.698	2.38	2.37	0.158
	Improvers	18	-11.90	3.79		-16.49	5.81		-14.69	7.93		-14.11	8.17		-2.31	2.64	

Change in quality of life versus change in iron biomarkers, controlling for covariates (psychosocial outcome at T1 (continuous) and iron variable T1 (continuous), change in food insecurity, change in anxiety and depressive symptoms, parity, and marital status) *p-value <0.05. Ferritin: adjusted for C-reactive protein and Alpha-1-acid glycoprotein using Thurnham adjustment (24).

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