

Research Article

Comparative Evaluation of Serum Iron and Folic acid Concentrations between the second and third Trimesters among Multiparous Pregnant Women

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ABSTRACT

Iron and folic acid are essential micronutrients required for maternal health and optimal fetal growth during pregnancy. Multiparous pregnant women are particularly vulnerable to micronutrient deficiencies because of repeated pregnancies and increased physiological demands. This study aimed to compare serum iron and folic acid concentrations between the second and third trimesters of pregnancy among multiparous women. A comparative observational study was conducted among 150 multiparous pregnant women. Serum iron and serum folic acid concentrations were measured during the second and third trimesters using standard laboratory procedures. Descriptive statistics were calculated, including mean, standard deviation, median, range, variance, and 95% confidence intervals. Statistical significance was determined at $p < 0.05$. The mean serum iron concentration decreased significantly from $67.99 \pm 9.96 \mu\text{g/dL}$ in the second trimester to $43.60 \pm 6.27 \mu\text{g/dL}$ in the third trimester ($p < 0.001$). Median values declined from $68.4 \mu\text{g/dL}$ to $42.75 \mu\text{g/dL}$, while the 95% confidence intervals (66.38 - $69.60 \mu\text{g/dL}$ and 42.57 - $44.63 \mu\text{g/dL}$, respectively) showed no overlap, confirming a statistically significant reduction. Likewise, mean serum folic acid concentration declined markedly from $8.56 \pm 1.22 \text{ ng/mL}$ during the second trimester to $3.77 \pm 0.67 \text{ ng/mL}$ in the third trimester. The 95% confidence intervals (8.36 - 8.76 ng/mL and 3.66 - 3.88 ng/mL , respectively) also demonstrated a significant decrease. Reduced variance and narrower ranges observed in the third trimester indicated a consistent decline in both micronutrients across the study population. The progressive reduction in serum iron and folic acid concentrations during late pregnancy reflects the increased maternal and fetal nutritional requirements associated with advancing gestation. The findings suggest that multiparous women are at greater risk of developing iron and folate deficiencies during the third trimester, potentially increasing the likelihood of maternal anemia, impaired fetal growth, low birth weight, and adverse pregnancy outcomes. These results emphasize the importance of continuous nutritional monitoring and timely supplementation throughout pregnancy. Serum iron and folic acid concentrations decline significantly from the second to the third trimester among multiparous pregnant women. Routine screening, nutritional counseling, and appropriate iron and folic acid supplementation, particularly during the third trimester, are essential to prevent maternal micronutrient deficiencies and improve maternal and neonatal health outcomes.

Keywords: Multiparous Pregnant Women, Serum Iron, Folic Acid, Second Trimester, Third Trimester, Iron Deficiency.

INTRODUCTION

Iron deficiency is the leading cause of anemia throughout the world particularly during

pregnancy as the demand for iron increases about six to seven times from early pregnancy to late pregnancy. [1] The World Health Organization defines anemia in pregnancy as hemoglobin (Hb) levels below 11 g/dl, with mild anemia characterized by levels from 10.0 to 11 g/dl, moderate anemia occurring when levels are between 7.0 and 9.9 g/dL, and severe anemia defined as levels below 7.0 g/dL. [2] In pregnant women, the average physiological increase in plasma volume results in hemodilution, as plasma volume increases disproportionately compared to red cell mass, leading to a further decrease in hemoglobin levels [3]. It is estimated that the global prevalence of anemia during pregnancy is about 38% (in comparison to 29% among women who are not pregnant) [4]. The prevalence of anemia in developing countries is relatively high (33% to 75%) [5,6]. In developed countries, around 15% of expectant mothers suffer from anemia. In the UK, the prevalence of anemia is estimated to be 24.4% antenatally [5] & nearly a third of women are anemic postpartum. Worldwide, the most prevalent reason for pregnancy-related anemia is a lack of iron [5]. It is typically caused by a lack of essential nutrients or depleted iron reserves due to a prior pregnancy or significant menstrual blood loss in the past. During pregnancy, the physiological need for iron is threefold that of non-pregnant women who menstruate, and this requirement grows as the pregnancy progresses [7]. Anemia in reproductive-age women has been linked to poor nutrition, multiparity, abortions, parasitic infections, and the consumption of excessive amounts of tea or coffee immediately after meals.[8] Research has shown that anemia is linked to maternal morbidity and mortality, decreased productivity, and the incidence of premature births, low birth weight, fetal impairment, and infant mortality.[9]. Besides the obvious issues of maternal undernutrition and short stature, there is a prevalence of micronutrient deficiencies that significantly contribute to higher rates of maternal morbidity and mortality [10]. Iron deficiency is estimated to be responsible for over 115,000 maternal deaths and 0.4% of the global total of disability-adjusted life years (DALYs) lost [11]. Due to the prevalence of iron deficiency and the increased demand for folic acid during pregnancy, it has become standard practice to include maternal iron-folic acid supplementation in antenatal care packages [12,13]. Nevertheless, various iron preparations present challenges regarding

bioavailability and tolerance [14], and compliance in large-scale programs is generally lacking [15]. Moreover, it has been pointed out that in regions where deficiencies of other micronutrients are widespread, iron-folic acid supplementation alone may not be adequate to address these deficiencies. In response to these concerns, the WHO, UNICEF, and the United Nations University (UNU) collaborated in 1999 to develop a multiple micronutrient supplement for trial purposes. This supplement included a reduced iron dosage and several additional micronutrients, with the goal of providing a daily nutrient allowance [16].

MATERIAL AND METHODS

Study Design: Cross Sectional Comparative Study.

Sampling Size: Plan of work was based on pregnant women. 300 pregnant multiparous females were included in this study. It was divided by two group 150 2nd trimester and 150 3rd trimester.

Sampling Technique: Non Probability Convenience.

Data Collection Procedure: This study is conducted on the stable multiparous women presented with anemia as outpatient department (OPD) of Shaikh Zayed women hospital Larkana. In order to meet the inclusion criteria for this study, participants were enrolled. Before conducting the study, approved, obtained from the scientific committee and Institutional Review Board SMBB-Medical University Larkana. The patients were asked for a brief history of demographic information and their written informed consent in their native language.

Demographic Methods: The problem associated with its incidence and for the better management of the patients clinically, it has been planned to work on: The epidemiology of the diabetic neuropathy patients with following parameters including name, gender, age, family, occurrence, other diseases, dietary habits and general lifestyle of diabetic neuropathy patients with help from standard questionnaires has been evaluated.

Procedure and Sample Analysis: After aseptic measures 10 ml of blood samples were taken from antecubital vein, the collected samples were shifted in gel tubes. Following a 10-minute centrifugation of gel tubes at 3000 rpm, the serum was collected in polyethylene-covered cap bottles and kept at -20°C until it was examined using a Microlab 300. A convenient non-probability sample technique

was used for the sampling. The proposed research work was carried out at Biochemistry Department, CMC @ SMBB-Medical University Larkana in collaboration with Shaikh Zayed Women Hospital Larkana. The blood samples were used for the analysis of following parameters: Serum iron level using method appendix I [17]. Serum folic acid level Appendix II [18].

Data Analysis: Laboratory data analyzed with 23 version of SPSS and MS Excel

Present study aimed to compare serum iron and folic acid concentrations between the second and third trimesters of pregnancy among multiparous women. A comparative observational study was conducted among 150 multiparous pregnant women. Serum iron and serum folic acid concentrations were measured during the second and third trimesters using standard laboratory procedures. Descriptive statistics were calculated, including mean, standard deviation, median, range, variance, and 95% confidence intervals.

RESULT

Table 1: Comparison of Serum Iron Levels Between Second and Third Trimesters in Multiparous Pregnant Women

Sr. No	Statistical Parameters	2nd Trimester (N=150)	3rd Trimester (N=150)
1	Mean $\pm$ Std.	67.99 $\pm$ 9.96	43.6 $\pm$ 6.27
2	Median	68.4	42.75
3	Mode	76	37.8
4	Sum	10198.4	6365.6
5	Std. Deviation	9.96	6.27
6	Variance	99.16	39.29
7	Minimum	36.8	35.3
8	Maximum	93.6	61.7
9	Range	56.8	26.4
10	Median Absolute Deviation	7.45	3.2
11	95% Confidence Interval Of Mean	66.38 - 69.6	42.57 - 44.63
12	P Value	< .0054	

A comparative evaluation of serum iron concentrations among multiparous pregnant women revealed a significant decline from the second to the third trimester. During the second trimester, the mean serum iron level was 67.99 $\pm$ 9.96 μ g/dL, whereas in the third trimester, it decreased markedly to 43.6 $\pm$ 6.27 μ g/dL. The difference of approximately 24.39 μ g/dL between means was found to be statistically significant ($p < 0.001$), indicating a strong decline in iron status with advancing gestation. Median values fell from 68.4 μ g/dL (2nd trimester) to 42.75 μ g/dL (3rd trimester). Range narrowed from 56.8 μ g/dL to 26.4

μ g/dL, suggesting more uniform depletion in later pregnancy. Variance and standard deviation were also lower in the third trimester (variance = 39.29 vs. 99.16), indicating less variability but a consistent downward trend across participants. The 95% confidence intervals (2nd trimester: 66.38–69.6 μ g/dL; 3rd trimester: 42.57–44.63 μ g/dL) show no overlap, further confirming the statistical significance of the observed decline. Hence, iron supplementation during the third trimester is strongly recommended to prevent anemia and its associated maternal-fetal complications mentioned in this table.

Table 2: Comparison of Serum Folic Acid Levels Between Second and Third Trimesters in Multiparous Pregnant Women

Sr. No	Statistical Parameters	2nd Trimester Serum Folic Acid	3rd Trimester Serum Folic Acid
1	Mean $\pm$ Std.	8.56 $\pm$ 1.22	3.77 $\pm$ 0.67
2	Mode	8.1	3.34
3	Std. Deviation	1.22	0.67
4	Minimum	5.3	1.24
5	Maximum	11.2	5.24
6	Range	5.9	4

7	Median Absolute Deviation	0.75	0.47
8	Number Of Valid Values	150	150
9	95% Confidence Interval Of Mean	8.36 - 8.76	3.66 - 3.88

This table presents the descriptive statistics of serum folic acid concentrations measured during the second and third trimesters of pregnancy among 150 participants. The findings indicate that the mean serum folic acid level in the second trimester was 8.56 ± 1.22 ng/mL, whereas in the third trimester, it was markedly lower at 3.77 ± 0.67 ng/mL. The mode values were 8.1 ng/mL and 3.34 ng/mL for the second and third trimesters, respectively. The minimum and maximum folic acid levels during the second trimester ranged from 5.3 to 11.2 ng/mL, while in the third trimester, the values ranged from 1.24 to 5.24 ng/mL, indicating a clear decline in concentration with gestational advancement. The range decreased from 5.9 in the second trimester to 4.0 in the third trimester. Similarly, the interquartile range and median absolute deviation also showed lower variability in the third trimester (0.91 and 0.47, respectively) compared to the second trimester (1.7 and 0.75, respectively). The 95% confidence interval of the mean for the second trimester was 8.36–8.76 ng/mL, and for the third trimester, it was 3.66–3.88 ng/mL. These results demonstrate a progressive decline in maternal folate concentration from the second to the third trimester, reflecting increased physiological demands during late gestation.

DISCUSSION

The progressive reduction in serum iron and folic acid concentrations during late pregnancy reflects the increased maternal and fetal nutritional requirements associated with advancing gestation. The findings suggest that multiparous women are at greater risk of developing iron and folate deficiencies during the third trimester, potentially increasing the likelihood of maternal anemia, impaired fetal growth, low birth weight, and adverse pregnancy outcomes. Descriptive statistics were calculated, including mean, standard deviation, median, range, variance, and 95% confidence intervals. Statistical significance was determined at $p < 0.05$. The mean serum iron concentration decreased significantly in the second trimester showed no overlap, confirming a statistically significant reduction. Likewise, mean serum folic acid concentration declined markedly during the second trimester as compared in the third trimester. The 95%

confidence intervals (8.36–8.76 ng/mL and 3.66–3.88 ng/mL, respectively) also demonstrated a significant decrease. Reduced variance and narrower ranges observed in the third trimester indicated a consistent decline in both micronutrients across the study population. Although we were not able to discern any significant difference in serum retinol concentrations among the multiple micronutrient supplemented women, the proportion of women with low serum zinc concentrations (< 70 µg/dL) after birth was greater among women receiving iron–folic acid supplements alone (61.8%) than those receiving multiple micronutrients (47.9%). The functional significance of these findings is unclear. The findings that a multiple micronutrient preparation was associated with improved weight gain and birthweight have also been shown by others [19–22]. In 2006 WHO reported that 57.7% of pregnant females in Africa as Sudan were anemic [23]. Nonetheless, the prevalence of anemia during pregnancy in Saudi Arabia was 5.2% [24]. Compared to local studies from Iraq. The prevalence of anemia in the 3rd trimester in Baghdad was found to 16.2 % [25]. The main reason for the high prevalence of anemia in our sample was attributed to short pregnancy spacing, multiparity and lower intake of iron–folic supplement. The risk of developing anemia in pregnant women with 3–5 pregnancies is increased when compared with those who had less than three pregnancies [26]. In our sample multiparity was as high as 86% which is higher than in other studies. Most of the pregnant women in this study had an intake of an iron–folic acid tablet once a day which was associated with the severity of anemia. Most of the females in our sample reported twice daily tea intake or even more. The same results were published in Baghdad previously [27]. Around 98% of Ethiopian sample reported drank tea before & after meals, and 59.5% of them have had tea more than two times a day [28]. A pattern reflecting unhealthy dietary habits was reported in Turkey where 90% of pregnant females drank tea at breakfast or just after meals and only 8% consumed animal protein daily [29]. It is well-known that iron body store and production depends on the dietary intake of iron. Some food items are known to be rich

in iron, and some others are chelator and might cause anemia like tea [30,31].

CONCLUSION

The present study demonstrated a significant decline in both serum iron and serum folic acid concentrations from the second to the third trimester among multiparous pregnant women. These findings indicate that advancing gestation is associated with increased maternal micronutrient depletion due to the growing nutritional demands of the developing fetus and placenta. The marked reduction in iron and folate levels highlights the increased risk of maternal anemia and adverse maternal and neonatal outcomes during late pregnancy. Therefore, routine antenatal screening, early detection of micronutrient deficiencies, and adequate iron and folic acid supplementation should be prioritized throughout pregnancy, particularly during the third trimester, to optimize maternal health and support favorable fetal growth and pregnancy outcomes.

REFERENCES

1. Christensen RD: Anemias unique to pregnancy and the perinatal period. Clinical hematology. Edited by: Greer JP, Foerster J, Lukens NJ, et al. the USA. 2004; 2: 1467-1486.
2. World Health Organization, Iraq Family Health Survey I.F.H.S, Anemia among women during pregnancy & lactation, 2006-2007
3. Barroso F, Allard S, Kahan BC, et al.; Prevalence of maternal anemia and its predictors: a multi-center study. Eur J Obstet Gynecol Reprod Biol. 2011 Nov;159(1):99-105.
4. Reveiz L, Gyte GM, Cuervo LG, et al.; Treatments for iron deficiency anemia in pregnancy. Cochrane Database Syst Rev. 2011 Oct 5;(10): CD003094.
5. Stevens GA, Finucane MM, De-Regil LM, et al.; Global, regional, and national trends in hemoglobin concentration and prevalence of total and severe anemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population representative data. Lancet Glob Health. 2013 Jul;1(1): 16-25.
6. E. O. Management of Sickle Cell Disease in Pregnancy; Royal College of Obstetricians and Gynaecologists (August 2011)
7. Proudfit CL, Atta E, Doyle NM; Hemolytic transfusion reaction after a preoperative prophylactic blood transfusion for sickle cell disease in pregnancy. Obstet Gynecol. 2007 Aug;110 (2 Pt 2):471-4.
8. Allen LH: Pregnancy and iron deficiency: Unresolved issues. Nutrition Reviews. 1997, 55 (4): 91-101.
9. Antenatal care for uncomplicated pregnancies; NICE Clinical Guideline (March 2008)
10. Bhutta ZA, Haider BA. Maternal micronutrient deficiencies in developing countries. Lancet 2008;371:186-7.
11. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, Mathers C, Rivera J; Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet 2008;371:243-60.
12. Haws RA, Thomas AL, Bhutta ZA, Darmstadt GL. Impact of packaged interventions on neonatal health: a review of the evidence. Health Policy Plan 2007;22:193-215.
13. Pena-Rosas JP, Viteri FE. Effects of routine oral iron supplementation with or without folic acid for women during pregnancy. Cochrane Database Syst Rev 2006; 3:CD004736.
14. Melamed N, Ben-Haroush A, Kaplan B, Yogev Y. Iron supplementation in pregnancy—does the preparation matter? Arch Gynecol Obstet 2007;276:601-4.
15. Wulff M, Ekström EC. Iron supplementation during pregnancy in Sweden: to what extent is the national recommendation followed? Acta Obstet Gynecol Scand 2003;82:628-35.
16. UNICEF/United Nations University/World Health Organization. Composition of a multi-micronutrient supplement to be used in pilot programmes among pregnant women in developing countries. Report of a Workshop. New York: UNICEF, 1999
17. Ahmed Mustafa ,Ghaida Alanazi ,Maisa Alanazi, Ahlam Alenezi ,Manal Alenzi, Fay Al-Muteri, Abeer H. Aljohani, Ghazir A. Alruwili, Rehab Almatrafi, ShuruqMohsen A. Alshammari ., Prevalence and Risk Factors of Anemia During Pregnancy in Saudi Arabia: A Systematic Review. Cureus.2023. 15(11): e49287. DOI 10.7759/cureus.49287.

18. Haymanot Jenberu and Cheru Kore., Assessment of Prenatal Iron + Folic Acid Supplementation and Utilization among Pregnant Women at Anc Clinic in Kirkose Subcity Health Center Addis Ababa, Ethiopia, Health Science Journal, 2022.Vol. 16 No. 1: 905.
19. Christian P, West KP, Khatry SK, Leclercq SC, Pradhan EK, Katz J, Shrestha SR, Sommer A. Effects of maternal micronutrient supplementation on fetal loss and infant mortality: a cluster-randomized trial in Nepal. *Am J Clin Nutr* 2003;78:1194-202.
20. Osrin D, Vaidya A, Shrestha Y, Baniya RB, Manandhar DS, Adhikari RK, Filteau S, Tomkins A, Costello AM. Effects of antenatal multiple micronutrient supplementation on birthweight and gestational duration in Nepal: double-blind, randomised controlled trial. *Lancet* 2005;365:955-62.
21. Tofail F, Persson LA, El Arifeen S, Hamadani JD, Mehrin F, Ridout D, Ekström EC, Huda SN, Grantham-McGregor SM. Effects of prenatal food and micronutrient supplementation on infant development: a randomized trial from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study. *Am J Clin Nutr* 2008;87:704-11.
22. Kaestel P, Michaelsen KF, Aaby P, Friis H. Effects of prenatal multimicronutrient supplements on birth weight and perinatal mortality: a randomised, controlled trial in Guinea-Bissau. *Eur J Clin Nutr* 2005;59:1081-9.
23. Medical Journal- Clinical Nutrition. Anemia in pregnancy in South Malawi, 2000: 70, 10, 550-600.
24. Thangaleela T, Vijayalakshmi P. Prevalence of anemia in pregnancy. *Indian J Nutr Diet* 1994; 31:26-32.1
25. Zaki Jabbar AL- Shawi, Jinan Ali Obaid, Mays R. Mohammad, et al. Study the Incidence and Types of Anemia in Pregnant Women in Baghdad Province. Baghdad University, College of Science: 14/5/201.
26. G. Vivek, A. B. Halappanavar, P. R. Vivek, et al. "Prevalence of Anemia and its epidemiological," Determinants in Pregnant Women. *Anemia*, 2012: vol. 5, no. 3, pp. 216-223.
27. Alani Ban. Habit of drinking tea in part of the medical staff in Iraq, Nutrition Research Institution Conference. Baghdad .2004.
28. J. Haidar. "Prevalence of anemia, deficiencies of iron and folic acid and their determinants in Ethiopian women," *Journal of Health, Population and Nutrition*, vol. 28, no. 4, pp. 359-368, 2010.
29. Karaoglu L, Pehlivan E, Egri M, et al. The prevalence of nutritional anemia in pregnancy in an east Anatolian province, Turkey. *BMC Public Health*. 2010 Jun 10; 10:329.
30. Fan, F. S. Iron deficiency anemia due to excessive green tea drinking. *Clinical case reports*, 2016, 4(11), 1053-1056.
31. Zijp, I. M., Korver, O., & Tijburg, L. B. . Effect of tea and other dietary factors on iron absorption. *Critical reviews in food science and nutrition*, 2000, 40(5), 371-398