

## Cross-sectional study of nutritional markers in pregnancy

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### ABSTRACT

**Objectives:** To note the value of serum Vitamin B12, folic acid, and ferritin in normal and high-risk pregnancies (HRPs) in patients attending antenatal clinic at All India Institute of Medical Sciences (AIIMS). **Materials and Methods:** This is a cross-sectional study where a total of 282 patients attending Gynaecology Outpatient Department at AIIMS, New Delhi, India were recruited. Among the 282 subjects, 251 were pregnant, and 31 were controls. The serum was tested for serum Vitamin B12, serum folic acid, and serum ferritin levels using Beckman Coulter Access 2 immunoassay. **Results:** The median value of serum folic acid level in pregnant women was 12 pg/ml with range being 2–20 pg/ml in contrast to 8 pg/ml with range being 3–20 pg/ml in nonpregnant female. This difference was statistically significant. ( $P = 0.05$ ). There was no significant difference in the median level of serum Vitamin B12 and serum ferritin in pregnant and nonpregnant group. Serum Vitamin B12 level was lower in the third trimester (127 pg/ml) than in first trimester (171 pg/ml) and the difference is statistically significant ( $P = 0.03$ ). Serum ferritin levels were also significantly lower in the second trimester (16.4 pg/ml) than third trimester (24.55 pg/ml). Although the median serum folic acid level was lower in the first trimester (9.84 pg/ml) than in second trimester (10.8 pg/ml) and in the third trimester (13.18 pg/ml) but the difference was not statistically significant. There was no significant difference in Vitamin B12 level in HRPs (median value 134 pg/ml) as compared to low-risk pregnancies (149.5 pg/ml). **Conclusion:** Serum folic acid levels are significantly higher during pregnancy as compared to nonpregnant state. However, there was no significant difference in the median level of serum Vitamin B12 and serum ferritin in pregnant and nonpregnant group. Serum folic acid level and ferritin level were significantly higher in HRPs compared to low-risk pregnancies.

**Key words:** Ferritin, folic acid, high-risk pregnancies, Vitamin B12

### INTRODUCTION

Adequate maternal micronutrient status is especially critical during pregnancy. The main cause of multiple micronutrient deficiencies is a poor quality diet, often due to an inadequate intake of animal source foods especially

in developing countries. Women who avoid meat and/or milk in wealthier regions of the world are also at higher risk of micronutrient depletion during pregnancy. Gene polymorphisms can also cause micronutrient deficiencies through impaired absorption or altered metabolism. This usually results in suboptimal maternal status of single nutrients. One example is folate, where maternal polymorphisms may increase the risk of neural tube defects (NTDs) unless dietary intake of the vitamin is sufficient. In some diets high in unrefined grains and legumes the amount of nutrients consumed may be adequate, but dietary constituents, such as phytates

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and polyphenols, limit their absorption. Diseases such as malaria, and infection with intestinal parasites, also impair status and alter the metabolism of multiple micronutrients.<sup>[1]</sup>

Iron deficiency is the most commonly detected nutritional deficiency in pregnant women. It was postulated to be associated with poor pregnancy outcome and preterm delivery. During pregnancy, there is a significant increase in iron requirements due to increased red cell mass and fetoplacental growth.<sup>[2]</sup> Diet is also an important factor determining the preconceptional iron status in the reproductive age group.

Preconceptional supplementation of folic acid has been shown to reduce NTDs in the fetus.<sup>[3]</sup> Although some countries introduced folic acid fortification programs in time, fetal NTD still continues to affect about 6 in every 10,000 pregnancies.<sup>[3,4]</sup> There are probably other modifiable risk factors contributing to the prevalence of NTD. Vitamin B12 shows close metabolic association with folate. It has been demonstrated that the deficiency of Vitamin B12 may be an independent risk factor, almost triplicating the risk of NTD.<sup>[5,6]</sup>

Vitamin B12 includes a group of cobalt-containing compounds known as cobalamins. This vitamin is involved in myelin synthesis, fatty acid degradation, and protein and DNA synthesis.<sup>[7]</sup> All the natural Vitamin B12 is produced by microorganisms and it is only found in foods of animal origin and vegetables contaminated with Vitamin B12-synthesizing bacteria. Vitamin B12 is an animal source vitamin and deficiency is common in vegetarians due to low intake and in the elderly due to low absorption.<sup>[8]</sup> Therefore, vegetable based diets may lead to Vitamin B12 deficiency.

Most of the anemia in women of childbearing age and during pregnancy are related to iron deficiency.<sup>[9-12]</sup> However, both low serum folate or B12 have recently been reported in populations in developing countries, with particular respect to pregnant and lactating mothers and their infants.<sup>[13-18]</sup> There may be a higher incidence of folate and Vitamin B12 deficiency in the populations of developing countries than previously suspected.

In this study, we draw attention to micronutrient issues that are sometimes neglected in the context of pregnancy. Multiple micronutrient deficiencies are likely to be present in many situations, some of which have been insufficiently appreciated as contributors to poor pregnancy outcomes and infant development.

## MATERIALS AND METHODS

This cross-sectional study was done during November 2012 and March 2013. Ethical clearance was obtained from our Institutional Ethics Committee. A total of 282 patients were included in the study after taking informed consent. These were either pregnant ladies attending the antenatal clinic at All India Institute of Medical Sciences or healthy nonpregnant ladies presenting with simple gynecological complaints excluding malignancies or other major morbidities. We obtained blood samples from 251 pregnant ladies and another 31 nonpregnant women were included as controls. We drew 2 ml of venous blood in a plain vial and the serum was assayed for ferritin, folate, and Vitamin B12 levels.

All pregnant women irrespective of their age, gestational age, and high-risk status were recruited. High-risk pregnancies (HRPs) included gestation related high-risk factor (e.g., Preeclampsia) or preexisting medical disease (e.g., Cardiac disease). Pregnancies with intrauterine death, miscarriage, or sepsis were excluded.

The blood sample was centrifuged and the serum stored at  $-20^{\circ}\text{C}$ . Plasma folate was determined by radioisotope dilution assay with the dual count kit (Diagnostic Products, CA, USA), which also measures plasma Vitamin B12. Plasma ferritin levels were measured by radioimmunoassay using the Ferritin kit (Diagnostic Products, CA, USA).

### Statistics

As data obtained are nonparametric, i.e., does not follow the normal distribution curve, data are presented as median along with the minimum and maximum values (i.e., range). *P* value is calculated by rank-sum (Mann–Whitney) test.

## RESULTS

A total of 282 women were recruited, among whom 251 were pregnant, and another 31 were nonpregnant. The characteristics of women are given in Table 1.

There were 88 (35.1%) primigravidae and 163 (64.9%) multigravidae in the study. There were 71 (28.3%), 76 (30.3%), and 104 (41.4%) women in the first, second, and third trimester, respectively in the study.

Serum folic acid levels were significantly higher in pregnant females compared to nonpregnant healthy controls ( $P = 0.056$ ). There was no significant difference in serum Vitamin B12 level and serum ferritin level among pregnant subject and nonpregnant healthy control [Table 2].

No significant difference was noted between serum Vitamin B12 level, folic acid, and ferritin level in different age groups. Levels were comparable in all age groups [Table 3].

Serum Vitamin B12 levels were significantly decreased in the second and third trimester compared to the first trimester. Serum ferritin level was not significantly decreased in the second trimester compared to the first trimester. Serum ferritin level was significantly decreased in the third trimester [Table 4].

Serum folic acid and ferritin level were significantly increased in pregnancies having one high-risk factor compared to normal pregnancy. No significant difference is seen in Vitamin B12 level between HRP and normal pregnancy [Table 5].

There were 77 pregnancies with at least one high-risk factor in them. These are labeled as HRPs. Among the 61 HRPs, twenty had gestational diabetes, 16 had heart disease, 13 had intrahepatic cholestasis of pregnancy, nine had hypothyroidism, nine had coagulation disorders, and another nine had hypertensive disorders of pregnancy. Serum folate and ferritin level were significantly increased, and Vitamin B12 level was significantly decreased in gestational diabetes. No significant difference in serum ferritin, folate, and Vitamin B12 level in women, who had gestational hypertension, heart disease, hypothyroidism, intrahepatic cholestasis of pregnancy, and coagulation disorders [Table 6].

**Table 1: Characteristics of women**

Characteristics	Number of women	Percentage
Nonpregnant female	31	11
Pregnant female	251	89
Parity		
Primigravidae	88	35.1
Multigravidae	163	64.9
Gestation		
First trimester	71	28.3
Second trimester	76	30.3
Third trimester	104	41.4

**Table 2: Pregnant versus nonpregnant healthy controls**

	Pregnant (n=251)	Controls (n=31)	P
Vitamin B12	147 (8-1500)	171 (33-1500)	0.09
Folic acid	12 (2-20)	8 (3-20)	0.05
Ferritin	20.9 (1.3-507)	18 (2-65)	0.8

**Table 3: Age-wise distribution**

	Group 1 20-24 years (n=66)	Group 2 25-29 years (n=105)	Group 3 30-34 years (n=65)	Group 4 >35 years (n=15)	P
Vitamin B12	139.5 (26-529)	128 (3-788)	159 (41-564)	179 (12.27-1500)	0.5
Folic acid	11.59 (2.9-20)	12 (2-20)	13 (2-20)	18 (4-102)	0.6
Ferritin	22.7 (4-507)	20 (1.3-294.5)	18 (4-102)	24.1 (3.9-143)	0.9

Of the 77 HRPs with at least one high-risk factor, 13 had multiple high-risk factors. Serum folic acid and ferritin level were significantly increased in pregnancies having multiple high-risk factors compared to normal pregnancy [Table 7].

## DISCUSSION

Nutritional needs are increased during pregnancy and lactation for support of fetal and infant growth and development along with alterations in maternal tissues and metabolism. Mothers in developing countries often embark on pregnancy with low iron and other nutritional stores.

There are three main strategies for increasing maternal intake of multiple micronutrients. The first is to improve dietary quality. An easier and more common approach is to provide multiple micronutrient supplements to women on their first clinic visit. We provide all pregnant patients with iron and folic acid supplementation in second and third trimester.

We found that serum ferritin levels were not significantly decreased in the second trimester compared to the first trimester. Serum ferritin level was significantly decreased in the third trimester ( $P < 0.05$ ). Iron stores of most of the women were adequate at the beginning of pregnancy and were able to prevent iron deficiency anemia during gestation, but in the third-trimester iron stores dropped substantially regardless the use of iron supplements. During pregnancy, a sharp decrease in serum ferritin has been observed especially in the third trimester<sup>[19-21]</sup> even in women who started pregnancy with adequate iron reserves, with or without the use of iron supplements, although this decrease may be lower in iron supplemented women.<sup>[22]</sup> Although iron supplementation during pregnancy at the levels used by the women in this study did not affect iron stores at the end of pregnancy, it was effective in maintaining adequate circulating iron and might be beneficial for recovery of iron stores postpartum. There was no significant difference in serum ferritin level among pregnant subjects and nonpregnant healthy controls.

In our study, serum folic acid levels were significantly higher in pregnant females compared with nonpregnant healthy controls ( $P = 0.056$ ). Pregnancy is usually associated with a decrease in plasma and erythrocyte folate, especially in women of low socioeconomic level, multiparas, and with

**Table 4: Gestation wise distribution**

	T1 (n=71)	T2 (n=76)	T3 (n=104)	P
Vitamin B12	171 (26-1500)	135.5 (3-460)	127 (29-564)	0.03
Folic acid	9.84 (2.4-20)	10.8 (2-20)	13.18 (2-20)	0.1
Ferritin	17 (1.3-113.6)	16.4 (2.6-507)	24.55 (3.8-294.5)	0.02
<b>Testing the significance for Vitamin B12 and ferritin further</b>				
	<b>Vitamin B12</b>		<b>Ferritin</b>	
T1 versus T2	0.02		0.9	
T1 versus T3	0.02		0.01	
T2 versus T3	0.9		0.02	

**Table 5: Difference in Vitamin B12, folic acid and ferritin in pregnancies having one high-risk factor versus normal pregnancy**

	High risk pregnancy (n=77)	Normal pregnancy (n=174)	P
Vitamin B12	134 (26-564)	149.5 (3-1500)	0.74
Folic acid	14 (2-20)	11 (2-20)	0.01
Ferritin	27 (2.6-299)	19.4 (1.3-507)	0.02

low dietary intake of folate.<sup>[18,19,23]</sup> This decrease is mainly attributed to the intense placental transfer from mother to fetus, and to the increased catabolism and urinary excretion.<sup>[24]</sup> In this study, mean serum folate levels were adequate throughout pregnancy, as has been observed in well-nourished women in developed countries.<sup>[25]</sup> It is, therefore, possible that, in average, folate supplementation of the women in our study, are sufficient to maintain folate indices during pregnancy. Folate supplementation of nonanemic women during pregnancy in this study further improved folate indices, decreasing the risk of folate depletion and possibly increasing folate availability for maternal-fetal transfer. In addition, its long lasting effects in maintaining high maternal erythrocyte and plasma folate up to 2–3 months postpartum may also benefit the lactating women and their lactational performance.

In our study, serum Vitamin B12 levels were significantly decreased in the second and third trimester compared to the first trimester. Other authors have reported low B12 values in developing countries in the population in general and in mothers during pregnancy.<sup>[13-15]</sup> When maternal B12 status is low during pregnancy, fetal storage may be suboptimal and breast milk content reduced.<sup>[15,26]</sup> In Northern Pakistan, serum B12 levels in a healthy population were considerably lower than those described in the literature.<sup>[14]</sup> Low B12 values in rural Mexico were attributed to malabsorption of B12 associated with *Giardia*.<sup>[15]</sup> A 1994 national survey in Jordan reported *Entamoeba coli* in 14.3% and *Giardia* in 8.4% of stool samples of schoolchildren, and these may also reduce absorption of B12.<sup>[27]</sup>

In our study, serum folate and ferritin level were significantly increased, and Vitamin B12 level were significantly decreased

**Table 6: Risk factor wise distribution**

	Vitamin B12	Folic acid	Ferritin
HTN			
Present (n=9)	187 (97-564)	15.6 (2.8-20)	36 (4-299.5)
Absent (n=242)	141 (3-1500)	12 (2-20)	20.2 (1.3-507)
P	0.09	0.2	0.3
GDM			
Present (n=20)	128 (29-564)	16 (2-20)	35.35 (4-102)
Absent (n=231)	149 (3-1500)	12 (2-20)	19.9 (1.3-507)
P	0.04	0.04	0.005
Hypothyroidism			
Present (n=09)	87 (26-442)	15 (2.9-20)	29.4 (2.6-52.1)
Absent (n=242)	147.5 (3-1500)	1.2 (2-20)	20.2 (1.3-507)
P	0.47	0.26	0.37
ICP			
Present (n=13)	151 (31-455)	12.63 (4.8-20)	30.5 (10.7-77)
Absent (n=238)	145.5 (3-1500)	12 (2-20)	20 (1.3-507)
P	0.65	0.54	0.09
Heart disease			
Present (n=16)	147 (45-564)	11.5 (2.16-20)	24.85 (3.8-299.5)
Absent (n=235)	147 (3-1500)	12.31 (2-20)	20.3 (3-507)
P	0.28	0.6	0.5
Coagulation disorder			
Present (n=9)	123 (31-306)	13.08 (3.8-20)	15.3 (5-78)
Absent (n=242)	148.5 (3-1500)	12 (2-20)	20.95 (1.3-507)
P	0.32	0.43	0.95

HTN: Hypertension, GDM: Gestational diabetes mellitus, ICP: Intracranial pressure

**Table 7: Variation in nutritional markers in high-risk factor versus multiple high-risk factors in pregnancy**

	Normal pregnancy	1 high-risk factor	Multiple high-risk factor	P
Vitamin B12	149.5 (3-1500)	134 (29-455)	187 (26-564)	0.92
Folic acid	11 (2-20)	13.7 (2-20)	14.8 (4.8-20)	0.02
Ferritin	19.4 (1.3-507)	26 (2.6-102)	35.8 (4-299.5)	0.03
<b>Testing the significance further for folic acid and ferritin</b>				
		<b>Folic acid</b>	<b>Ferritin</b>	
One high risk versus normal pregnancy		0.05	0.11	
Multiple high risk versus normal pregnancy		0.02	0.02	
One versus multiple high risk		0.28	0.16	

in gestational diabetes. Serum folic acid and ferritin level were significantly increased in pregnancies having multiple high-risk factors compared to normal pregnancy. Tarim *et al.* also identified high maternal hemoglobin (Hb) and serum ferritin at the initial prenatal visit as a risk factor for gestational diabetes mellitus (GDM).<sup>[28]</sup> Soubasi *et al.* also concluded in their study that elevated maternal ferritin is not a reflection of excess iron stores, but is related to an increased risk of GDM or intrauterine growth restriction.<sup>[29]</sup>

In a recent study on Vitamin B12 levels during pregnancy and lactation, Bae *et al.*<sup>[30]</sup> observed that pregnancy and lactation altered Vitamin B12 status in a manner consistent with enhanced Vitamin B12 supply to the child. Consumption of the Vitamin B12 dose to 3 times, the recommended dietary allowance increased the bioactive form of Vitamin B12 suggesting that women in these



reproductive states may benefit from Vitamin B12 intakes exceeding current recommendation.<sup>[30]</sup>

Morton *et al.*<sup>[31]</sup> performed a study on maternal and perinatal predictors of newborn iron status by observing cord blood samples for serum ferritin and Hb concentration and its association of serum ferritin and Hb with maternal and birth characteristics. They observed iron deficiency in 7% of newborns in New Zealand. They observed lower median serum ferritin concentrations in newborns whose mothers consumed more milk during pregnancy.

In a systemic review and meta-analysis of randomized controlled trials of prenatal iron use and prospective cohort study prenatal anemia, Haider *et al.*<sup>[32]</sup> observed increased maternal mean Hb concentration with iron use compared with controls and significantly reduced the risk of anemia, iron deficiency, iron deficiency anemia, and low birth weight in them. They found decreased incidence of anemia with an increase in iron dose. There was an increase in birth weight and decrease in risk of low birth weight for every 10 mg increase in dose/day. They also observed increase in birth weight by 14 g for each 1 g/L increase in mean Hb concentration. However, there was no evidence of significant effect on duration of gestation small for gestational age birth and birth length with mean Hb concentration.

In a study from Rawalpindi Pakistan, Khan *et al.*<sup>[33]</sup> observed 57% prevalence of iron deficiency anemia, 20% prevalence of folate deficiency, 19% combined iron and folate deficiency and 4% cobalamin deficiency during pregnancy. They also observed low-income multiparity, poor diet and lack of supplements to be the main contribution to the development of anemia during pregnancy.<sup>[33]</sup>

In another study on serum ferritin, Vitamin B12 and folic acid level, Kösüs *et al.*<sup>[34]</sup> observed that in addition to folic acid supplementation, deficiencies of ferritin and Vitamin B12 must be corrected in patients considering pregnancy or during early first-trimester pregnancy to obtain more accurate serum screening results.

In a previous study on iron, folate, and Vitamin B12 stores among pregnant women in a rural area of Haryana state, India, Pathak *et al.*<sup>[35]</sup> observed poor iron, folate, and Vitamin B12 stores in 67.7%, 26.3%, and 74.1% pregnant women, respectively. They observed concomitant deficiencies of iron, folate, and Vitamin B12 supplementation in 16.2% of women. They also observed that 59.9% women were consuming <75% of the recommended daily caloric allowance indicating an overall poor food intake. They observed Vitamin B12

deficiency to play an important role in causing anemia apart from iron and folate.

Both folate and cobalamin deficiencies are characterized by megaloblastic anemia and elevated homocysteine level which lead to cardiovascular problem and adverse pregnancy outcome as recurrent abortions and preeclampsia.<sup>[36]</sup>

Karabulut *et al.* estimate serum iron, folate, and Vitamin B12 levels in first trimester pregnancies in the Southwest region of Turkey. They concluded that iron and Vitamin B12 deficiencies were relatively common in a pregnant population consuming vegetable based diet. Iron and Vitamin B12 supplementation in addition to folate must be considered for the well-being of fetus in pregnant female.<sup>[37]</sup>

## CONCLUSION

Iron, folate and B12 status should be monitored during pregnancy/lactation in developing countries, where nutritional deprivation is more prevalent, and women of childbearing age often have a high fertility rate and inadequate interpregnancy interval to replenish body stores. Along with routine iron and folic acid supplementation, Vitamin B12 supplementation should also be considered.

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## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Allen LH. Multiple micronutrients in pregnancy and lactation: An overview. *Am J Clin Nutr* 2005;81:1206S-12S.
2. Allen LH. Pregnancy and iron deficiency: Unresolved issues. *Nutr Rev* 1997;55:91-101.
3. Lumley J, Watson L, Watson M, Bower C. Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. *Cochrane Database Syst Rev* 2001;3:CD001056.
4. Honein MA, Paulozzi LJ, Mathews TJ, Erickson JD, Wong LY. Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA* 2001;285:2981-6.
5. Ray JG, Blom HJ. Vitamin B12 insufficiency and the risk of fetal neural tube defects. *QJM* 2003;96:289-95.
6. Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR, Cole DE. Association of neural tube defects and folic acid food fortification in Canada. *Lancet* 2002;360:2047-8.
7. Ray JG, Wyatt PR, Thompson MD, Vermeulen MJ, Meier C,

- Wong PY, *et al.* Vitamin B12 and the risk of neural tube defects in a folic-acid-fortified population. *Epidemiology* 2007;18:362-6.
8. Shane B. Folic acid, Vitamin B12 and Vitamin B6. In: Stipanuk M, editor. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia: W.B Saunders Company; 2001. p. 483-518.
  9. Yip R. Iron-deficiency: Contemporary scientific issues and international programmatic approaches. Symposium: Clinical nutrition in developing countries. *J Nutr* 1994;124:1479S-90S.
  10. Jilani I, Qazaq HS, Al-Arabi ZA. A study on anemia among pregnant at mother and childhood (MCH) centres in Jordan for the year 1990 and the first half of 1991. Jordan: Ministry of Health; 1992.
  11. Mawajdeh S, Badran O, Haddadin A, Abu-Laban A, Idris M. Prevalence and Determinants of Anaemia and Iron-deficiency Among Jordanian Women 15-49 Years of Age: A National Study. UNICEF, Jordan Country Programme and the Ministry of Health, Jordan; 1996.
  12. Dallman PR, Yip R, Oski FA. Iron deficiency and related nutritional anemias. In: Nathan DG, Oski FA, editors. *Hematology of Infancy and Childhood*. 4<sup>th</sup> ed. Philadelphia: Saunders Company; 1993.
  13. Chanarin I, Malkowska V, O'Hea AM, Rinsler MG, Price AB. Megaloblastic anaemia in a vegetarian Hindu community. *Lancet* 1985;2:1168-72.
  14. Modood UM, Anwar M, Saleem M, Wiqar A, Ahmad M. A study of serum Vitamin B12 and folate levels in patients of megaloblastic anaemia in Northern Pakistan. *J Pak Med Assoc* 1995;45:187-8.
  15. Allen LH, Rosado JL, Casterline JE, Martinez H, Lopez P, Muñoz E, *et al.* Vitamin B-12 deficiency and malabsorption are highly prevalent in rural Mexican communities. *Am J Clin Nutr* 1995;62:1013-9.
  16. Almadan MS, al Awamy BH, al Mulhim IA. Nutritional Vitamin B12 deficiency in infancy. *Indian J Pediatr* 1993;60:683-5.
  17. Specker BL, Black A, Allen L, Morrow F. Vitamin B-12: Low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. *Am J Clin Nutr* 1990;52:1073-6.
  18. Neumann CG, Harrison GG. Onset and evolution of stunting in infants and children. Examples from the Human Nutrition Collaborative Research Support Program. Kenya and Egypt studies. *Eur J Clin Nutr* 1994;48 Suppl 1:S90-102.
  19. Herberg S, Bichon L, Galan P, Christides JP, Carroget C, Potier de Courcy G. Iron and folacin status of pregnant women: Relationships with dietary intakes. *Nutr Rep Intern* 1987;35:915-30.
  20. Zamorano AF, Arnalich F, Sánchez Casas E, Sicilia A, Solis C, Vázquez JJ, *et al.* Levels of iron, Vitamin B12, folic acid and their binding proteins during pregnancy. *Acta Haematol* 1985;74:92-6.
  21. Bartels PC, Helleman PW, Soons JB. Investigation of red cell size-distribution histograms related to folate, Vitamin B12 and iron state in the course of pregnancy. *Scand J Clin Lab Invest* 1989;49:763-71.
  22. Qvist I, Abdulla M, Jägerstad M, Svensson S. Iron, zinc and folate status during pregnancy and two months after delivery. *Acta Obstet Gynecol Scand* 1986;65:15-22.
  23. Chanarin I, Rothman D, Ward A, Perry J. Folate status and requirement in pregnancy. *Br Med J* 1968;2:390-4.
  24. McPartlin J, Halligan A, Scott JM, Darling M, Weir DG. Accelerated folate breakdown in pregnancy. *Lancet* 1993;341:148-9.
  25. Ek J, Magnus EM. Plasma and red blood cell folate during normal pregnancies. *Acta Obstet Gynecol Scand* 1981;60:247-51.
  26. Allen LH. Vitamin B12 metabolism and status during pregnancy, lactation and infancy. In: Allen LH, King J, Lönnerdal B, editors. *Nutrient Regulation during Pregnancy, Lactation and Infant Growth*. New York: Plenum Press; 1994.
  27. Shuqaidef SW. Study of intestinal parasites and anemia in 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> grade school-children in Jordan. Jordan: United Nations Children's Fund/Ministry of Health; 1995.
  28. Tarim E, Kilicdag E, Bagis T, Ergin T. High maternal hemoglobin and ferritin values as risk factors for gestational diabetes. *Int J Gynaecol Obstet* 2004;84:259-61.
  29. Soubasi V, Petridou S, Sarafidis K, Tsantali CH, Diamanti E, Buonocore G, *et al.* Association of increased maternal ferritin levels with gestational diabetes and intra-uterine growth retardation. *Diabetes Metab* 2010;36:58-63.
  30. Bae S, West AA, Yan J, Jiang X, Perry CA, Malysheva O, *et al.* Vitamin B-12 status differs among pregnant, lactating, and control women with equivalent nutrient intakes. *J Nutr* 2015;145:1507-14.
  31. Morton SB, Saraf R, Bandara DK, Bartholomew K, Gilchrist CA, Atatoa Carr PE, *et al.* Maternal and perinatal predictors of newborn iron status. *N Z Med J* 2014;127:62-77.
  32. Haider BA, Olofin I, Wang M, Spiegelman D, Ezzati M, Fawzi WW; Nutrition Impact Model Study Group (anaemia). Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: Systematic review and meta-analysis. *BMJ* 2013;346:f3443.
  33. Khan DA, Fatima S, Imran R, Khan FA. Iron, folate and cobalamin deficiency in anaemic pregnant females in tertiary care centre at Rawalpindi. *J Ayub Med Coll Abbottabad* 2010;22:17-21.
  34. Kösüs N, Kösüs A, Hizli D, Bitirgen EÜ, Turhan NÖ. Can serum ferritin, Vitamin B (12) and folic acid levels affect serum screening tests during pregnancy? *J Matern Fetal Neonatal Med* 2012;25:1674-7.
  35. Pathak P, Kapil U, Yajnik CS, Kapoor SK, Dwivedi SN, Singh R. Iron, folate, and Vitamin B12 stores among pregnant women in a rural area of Haryana State, India. *Food Nutr Bull* 2007;28:435-8.
  36. Mahmoud A, Elkattan EA, Eldaly AA, Omran EF, Mandour I. A comparative study of folate and Vitamin B12 serum levels in preeclamptic versus normotensive pregnant women in correlation with uterine and umbilical artery Doppler findings and pregnancy outcome. *J Turk Ger Gynecol Assoc* 2009;10:152-7.
  37. Karabulut A, Sevket O, Acun A. Iron, folate and Vitamin B12 levels in first trimester pregnancies in the Southwest region of Turkey. *J Turk Ger Gynecol Assoc* 2011;12:153-6.