IRON, FOLATE AND COBALAMIN DEFICIENCY IN ANAEMIC PREGNANT FEMALES IN TERTIARY CARE CENTRE AT RAWALPINDI

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Background: Anaemia in pregnancy is a common clinical problem contributing to increased maternal and foetal morbidity. This study was carried out to determine frequency of iron, folate and cobalamin deficiency and associated risk factors in the anaemic pregnant females who reported first time during second and third trimester for antenatal check-up in the tertiary care hospital at Rawalpindi. **Methods**: This case control study was carried out in a tertiary care hospital at Rawalpindi. Two hundred and fifty pregnant women (age: 19–43 years) consisting of 125 anaemic (Hb< 110 g/L) and 125 non-anaemic who reported first time at antenatal clinic were included. Data on socio-demographic characteristics, parity and dietary intake were collected. Complete blood counts were done. Serum ferritin, folate and cobalamin assays were performed by using DPC kits on Immulite-1000. Results: The pregnant women were categorised having mild (Hb up to 54%), moderate (Hb up to 36%), or severe (Hb up to10%) anaemia during antennal visit. They had significantly lower median (range) levels of haemoglobin 96 (40–110) g/L, ferritin 8 (3–142) g/L, folate 15 (3–54) ηmol/L and cobalamin 171 (111–629) ρmolL than controls (*p*=<0.01). Micronutrient analysis revealed secondary pregnancy related deficiency of Iron (57%), folate (20%), combined iron and folate (19%) and cobalamin (4%) in the female. Among the risk factors, low income (OR: 7.69), multiparty (OR: 2.93), lack of iron/folate supplementation (OR 2.91) and inadequate dietary intakes (OR 2.51) were associated with anaemia. Conclusion: The pregnant anaemic women had iron (57%); folate (20%), followed by combined iron folate (19%), and cobalamin (4%) deficiency during first antenatal visit. Low income, multiparty, poor diet and lack of supplements are the main contributor in development of anaemia during pregnancy.
Keywords: Anaemia, pregnancy, ferritin, cobalamin, iron, folate

INTRODUCTION

Anaemia in pregnancy is a common clinical problem contributing to increased maternal and foetal morbidity.[1] Nearly half the pregnant women in the developing countries including Pakistan are anaemic as compared to 23% in industrialized countries.[2] The reason for high prevalence of anaemia during pregnancy is nutritional deficiencies of iron and folate.[3] The relative contribution of inadequate intake of these micronutrients in the pregnant women varies greatly by dietary practices, socio-economical status, education and access to health care. Despite the measures taken by the health authorities to control anaemia during pregnancy, nutritional anaemia continues to be a reproductive health problem of great magnitude in Pakistan.[4–5]

Poor micronutrient intake during childbearing age may jeopardise the health of mother and infant.[5] Iron deficiency anaemia is common among pregnant women and its frequency varies in the pregnant women of Karachi (64%), Lahore (73%) and Multan (76%).[6–8] pregnant women are also prone to folate and cobalamin deficiency because of high physiological requirement during pregnancy.[9]

The deficiencies of specific micronutrients in these anaemic women need to be identified for appropriate treatment during pregnancy. Very limited data is available regarding biochemical deficiency of micronutrients in the pregnant women who have not been talking any iron/folate supplements and reported after first trimester for antenatal check-up.

This study was designed to estimate the frequency of iron, folate, and cobalamin deficiency in anaemic women who reported first time during second and third trimester for antenatal checkup in the tertiary care hospital at Rawalpindi. The secondary objective was to elucidate the risk factors associated with the deficiency of these micronutrients in the anaemic women.

SUBJECTS AND METHODS

This case control study was conducted at the department of Pathology, Army medical College in collaboration with a tertiary care hospital in Rawalpindi. This study was approved by Institutional Ethical Committee of Army Medical College, Rawalpindi.

A total of 250 pregnant women consisting of 125 anaemic (Hb<110 g/L) and 125 non-anaemic (controls) were included by convenient sampling technique after informed consent. They reported first time at the antenatal clinic during 12 to 36 weeks of pregnancy. The anaemic female patients had not yet started Iron/Folate supplements. Women having haemolytic anaemia, blood transfusions, any acute or chronic illness including diabetes mellitus, hypertension, hepatitis and gastro-intestinal diseases were excluded from the study.

The subjects were interviewed and data of socio-demographic characteristics, parity, gestational age, dietary habits was recorded. Dietary intakes were estimated by a food frequency questionnaire and two 24-h dietary recalls. An adequate diet was one which comprised the daily use of either meat, or dairy products and at least weekly use of fresh fruits and green leafy vegetables. Socio-economic status was assessed by the monthly income. Height and weight of all the patients were taken to assess the Body Mass Index (BMI). Medical history and gynaecological and obstetric examination was carried out. Anaemic women were provided haematinics and dietary advice.

Blood samples (2.5 ml) were collected in EDTA tubes (BD, USA). Complete blood count including RBC, WBC, platelets, Hb, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) was carried out on a haematology Analyser, SYXMEX KX-21 (Japan). According to WHO criteria the anaemia in pregnant women is classified into mild (haemoglobin 90–110 g/L); moderate (haemoglobin 70–90 g/L) and severe (haemoglobin <70 g/L). The anaemic females who had MCV <76 and >96 fL were label as microcytic and macrocytic respectively. The reference range of MCH and MCHC are 27–33 ρg and 33–36 g/dl respectively. Peripheral blood film was also examined.

Blood was collected in plane tube and serum was separated by centrifugation at 1000 G for 5 minutes. Serum ferritin, folic acid and cobalamin assays were performed by to chemeluminace method on kits issued by Diagnostic Product Corporation on IMMULITE 1000 (DPC,USA). Normal and abnormal controls were run with study samples for ferritin, folate and cobalamin assays and CVs were 3.6, 4.2 and 4.9% respectively.

Data was analysed by SPSS version-16 (Chicago IL, US). Mean, standard deviation, median and range were calculated for Hb, MCV, MCH, MCHC and micronutrients. Frequency and percentage of iron, folate and cobalamin deficiency were calculated based on lower limit of reference range based on non-anaemic pregnant women. The Mann Whitney U test was applied for comparison of micronutrients between anaemic and control groups. Risk factors were assessed by 'chi-square' tests. Spearman's co-relation was applied among ferritin, folate, cobalamin and haemoglobin levels. A *p*-value of less than 0.05 was considered as significant.

RESULTS

The demographic characteristics of pregnant women and control subjects revealed that most of them were young with an average age of 26 years (range: 19–43 years) and a BMI of 24.1 kg/m^2 (range: 17.8–30.1). The women were from second (68%) and third (32%) trimester of pregnancy. Gravidum ranged from 1–7 with a median of 4. The present study illustrated that anaemia was prevalent in women of child bearing age during their first visit to tertiary care hospital at Rawalpindi. Out of 125 anaemic pregnant women, mild anaemia (haemoglobin 90–110 g/L) was found in 54%; moderate anaemia (haemoglobin 70 to 90 g/L) in 36% and severe (haemoglobin<70 g/L) in 10% of patients.

The haematological parameters are summarised in Table-1. The mean haematological indexes including MCHC, MCV and MCH were significantly lower in anaemic women than controls (p<0.01). In the peripheral blood, RBC morphology revealed microcytic hypo chromic anaemia in about half patients and only three women had macrocytic anaemia with a few hypersegmented neutrophils. Micronutrient analysis revealed secondary pregnancy related significant deficiency of the serum concentrations of micronutrients including Iron, folate and cobalamin in the anaemic women as compare with control (Table-1). The results of this study also indicated that the fall in MCV, MCH and MCHC had a linear correlation (r=0.39, p<0.05) with serum ferritin level. The Hb concentration revealed a significant positive correlation with serum ferritin levels (r=0.47, p=0.01) and folate levels (r=0.28, p=0.05) in anaemic women.

Table-1: Comparison of haematological indices and micronutrients deficiency between anaemic and control group of women at Rawalpindi (n=250)

Parameters	Anaemic Women (n=125) Median (Range)	Non-anaemic Women (n=125) Median(Range)	*p*
Haemoglobin (g/L)	96 (40–110)	117 (110–134)	0.001
MCV (fL)	81 (63–100)	88 (76–96)	0.001
MCH (ρg)	24 (17–31)	29 (26–32)	0.001
MCHC (g/dl)	26 (22–35)	34 (30–36)	0.001
Ferritin (g/L)	8 (3–112)	36 (10–145)	0.001
Folate (ηmol/L)	15 (3–39)	20 (6–54)	0.012
Cobalamin (ρmol/L)	171 (111–629)	262 (131–687)	0.001

The major contributing factor to anaemia during pregnancy was iron deficiency followed by folate and cobalamin (Figure-1). Out of 125 anaemic women, 57 % of women had serum ferritin levels below the 10 µg/L. Biochemical evidence of folic acid deficiency was found in 20%. Combined Iron and folate deficiency was found in 19% of anaemic women. Cobalamin was relatively uncommon (4%) in the pregnant women in our medical setup.

The risk factors associated with the bio-chemical deficiencies in anaemic women are mention in Table-2. Majority of the anaemic women belonged to low socio-economic group as compared to the controls group and risk factors were calculated with odd ratio (OD). Monthly income less than PKR 7,000 (OD=7.69); multiparity (OD=2.93); lack of iron, folate and cobalamin supplement intake (OD=2.91); inadequate dietary iron intakes (OD=2.51) and **lack of education** (OD=1.47) were the main causative agents of micronutrient deficiency while educational status was not found to be contributory. Despite high prevalence of anaemia in our population, the tendency to use haematinic supplements is quite unsatisfactory in anaemic women ($p<0.01$).

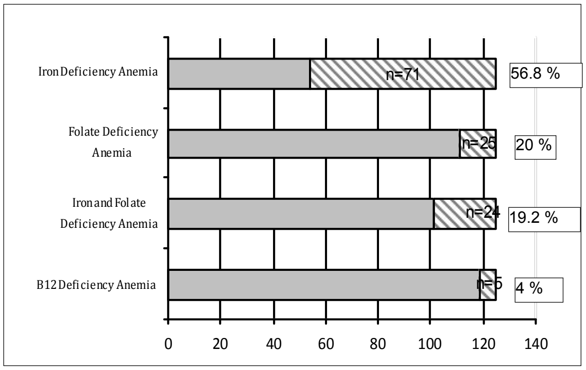

Figure-1: Biochemical deficiency of iron, folate and cobalamin among anaemic women (n=125)

Table-2: Comparison of risk factors for causation of biochemical deficiency in anaemic and control group of women at Rawalpindi (n=250)

Parameters	Anaemic women n (%)	Non-Anaemic women n (%)	Odds Ration	(95% CI)
Education				
Illiterate	65 (55.1)	53 (44.9)		
Primary school	60 (45.5)	72 (54.5)	1.47	(0.89–2.42)
Monthly Income (Pak Rupees)				
<7,000	64 (81.0)	15 (19.0)		
>7,000	61 (35.7)	110 (64.3)	7.69**	(4.04–14.64)
Dietary Intake				
Inadequate	71 (62.3)	43 (37.7)		
Adequate	54 (39.7)	82 (60.3)	2.51*	(1.50–4.18)
Iron and Folate Supplements				
No	70 (64.8)	38 (35.2)		
Yes	55 (38.7)	87 (61.3)	2.91*	(1.73–4.90)
Parity				
>2 Children	72 (64.9)	39 (35.1)		
<2 Children	53 (38.7)	84 (61.3)	2.93*	(1.74–4.92)

$^*p<0.005$, $^{**}p<0.001$

DISCUSSION

All women during the childbearing age are prone to develop anaemia. Measurements of routine haematological parameters during pregnancy are of relatively limited value for assessment of micronutrient deficiency in these patients. Detection of biochemical deficiency of iron, folate and cobalamin during pregnancy is important for both maternal and foetal well-being. Serum micronutrients assays are more sensitive for diagnosis of micronutrient deficiency anaemia; therefore considered better biochemical markers for detecting latent deficiency especially before the change of red cell morphology and indices takes place.

Only a few studies have measured micronutrient status, particularly among women of childbearing age in Pakistan. In the present study, anaemic patients had significantly lower concentration of serum ferritin, folate and B_{12} than the controls ($p<0.01$). Using ferritin level of 10 ηg/ml as the cut-off, we observed that 57% of the women were iron deficient. These findings are comparable with some previous studies which also revealed decrease in serum iron and ferritin in second and third trimesters.[10] Three different studies reported much higher frequency of iron deficiency anaemia in urban population of Karachi (64%), Lahore (73%) and Multan (76%) Pakistan (6–8). The frequency of iron deficiency anaemia is higher in our Pakistani women than reported in the anaemic pregnant women in Bhaktapur Nepal.[11] Anaemia which occurs in pregnancy is usually due to a deficiency of iron and folic acid which contributes to high maternal and infant mortality among women in developing countries.[12] We are still far behind the health status in the industrialised countries.[13]

Serum ferritin is considered as a better parameter for detecting latent iron deficiency especially before the change of red cell morphology and red cell indices. A high degree of correlation has been shown between serum ferritin concentration and bone marrow iron stores. During pregnancy, low serum ferritin concentrations in the presence of normal haemoglobin indicate deficient iron stores.[14] Supplementation with iron is generally recommended during pregnancy to meet the iron needs of both mother and foetus. Iron deficiency anaemia is associated with a >2-fold increase in the risk of preterm delivery.[15]

Micronutrient analysis also revealed that our women had biochemical evidence of folic acid, and less commonly, cobalamin deficiency anaemia. Chinese women of childbearing age had relatively more deficiencies of folic acid and, cobalamin than the pregnant women reported to anti-natal clinic in our tertiary care hospital.[9] Martí-Carvajal *et al* (2002) reported folate deficiency in 11.98% of pregnant anaemic women. However, combined anaemia (IDA and FDA) occurred in 11.52% which is higher than our study.[16] Serum and red cell folic acid levels are considered to be reliable direct indicators of folic acid status. Serum folic acid reflects the current intakes of folic acid while red cell folic acid is an indicator of long term status, as folic acid is incorporated during early development of red cells.[17] Folate intake and circulating serum concentrations are positively correlated during pregnancy.[18] Women who are vegetarians are most likely to develop Vitamin-B_{12} deficiency and 23% had cobalamin deficiency anaemia during pregnancy in rural

Southern Ethiopia.[19] An intervention strategy must address deficiencies of folic acid and other possible causal factors.

An imperative risk factor in the causation of anaemia during pregnancy was low socio-economic status reflected by less monthly income. It has a significant relationship with the causation of anaemia which is consistent with the study conducted in Lahore where the main reason for anaemia was poor socioeconomic status and increasing parity.[7] The pregnant women in our study ate meat occasionally. They used to take chapatti as an essential component of their regular meals which contains phytates, a known inhibitor of iron absorption.[20] The nutritional anaemia continues to remain a public health issue of great magnitude in India as well due to insufficient dietary intake.[21] Supplementation with iron is generally recommended during pregnancy to meet the iron requirements of both the mother and the foetus. Our findings show that poor nutrition and inadequate iron supplementation is probably sub-optimal during pregnancy. They also showed that maternal iron stores are depleted during the 2nd trimester especially in women who are not receiving supplemental iron. Lower concentrations of serum folate at week 28 were also associated with a greater risk of preterm delivery and low birth weight.[18] Supplement use during pregnancy was unsatisfactory which is also supported by different researchers.[8,20,22]

In our study, educational status of women did not significantly affect anaemia which is similar to a study done in Malaysia and contrary to what was reported from Lahore.[7,23]

Screening for anaemia, its treatment and fortification of food (wheat flour, milk, sugar and salt) with iron and folic acid to build long term iron stores remains the key to reduce anaemia during pregnancy. Women should be encouraged to report early in the antenatal clinics, preferably in the first trimester. Iron, folate and B_{12} supplements should be taken on a regular basis. This simple act can help save the life of mothers and babies throughout the nation. Prenatal supplement use appears to reduce the risk of low folate and B_{12} blood values but not biochemical iron status.[24]

Nutrition education components of the antenatal care and community health service should be intensified. Also, during the health education activities in the clinics, the importance of family planning, early bookings, improvement in nutritional status and micronutrient supplementation for antenatal care need to be stressed.

CONCLUSION

It is concluded that the pregnant anaemic women had iron (57%); folate (20%) followed by combined iron folate (19%) and cobalamin (4%) deficiency during first antenatal visit in the tertiary care hospital at Rawalpindi. Low income, multiparity, poor diet and lack of supplements are the main contributor in development of anaemia during pregnancy. Improvement in nutritional status and micronutrient supplementation should be stressed upon by lady health workers.

LIMITATIONS

High serum ferritin levels may be due to infection in anaemic patients. This is the only limitation in the use of serum ferritin as an indicator of iron deficiency.

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REFERENCES

1. Brabin BJ, Hakimi M, Pelletier D. An analysis of anaemia and pregnancy-related maternal mortality. J Nutr 2001;131(2S-2):604S–615S.
2. World Health Organization, United Nations Children's Fund, United Nation University. Iron deficiency anaemia: a assessment, prevention and control. A guide for programme managers. Geneva: WHO, 2001.WHO/NHD /01.3.9.
3. VanderJagt DJ, Brock HS, Melah GS, El-Nafaty AU, Crossey MJ, Glew RH. Nutritional factors associated with anaemia in pregnant women in Northern Nigeria. J Health Popul Nutr 2007;25(1):75–81.
4. Lone FW, Qureshi RN, Emmanuel F. Maternal anaemia and its impact on perinatal outcome in a tertiary care hospital in Pakistan. East Mediterr Health J 2004;10(6):801–7
5. Bakhtiar UJ, Khan Y, Nasar R. Relationship between maternal hemoglobin and perinatal outcome. Rawal Med J 2007;32:102–4.
6. Karim SA, Khurshid M, Memon AM, Jafarey SN. Anaemia in pregnancy--its cause in the underprivileged class of Karachi. J Pak Med Assoc 1994;44(4):90–2.
7. Sohail R, Zainab S, Zaman F. Prevalence of anaemia in obstetrical population. Ann King Edward Med Coll 2004;10(2):146–8.
8. Awan MM, Akbar MA, Khan MI. A study of anaemia in pregnant women of Railway Colony, Multan. Pak J Med Res 2004;43(1):11–4.
9. Ronnenberg AG, Goldman MB, Aitken IW, Xu X. Anaemia and deficiencies of folate and vitamin B-6 are common and vary with season in Chinese women of childbearing age. J Nutr 2000;130:2703–10.
10. Brian S, Alper, Kimber R, Reddy K A. Using ferritin levels to determine iron deficiency anaemia in pregnancy. Journal of Family Practice 2000;49:829–32.
11. Chandyo RK, Strand TA, Ulvik RJ, Adhikari RK, Ulak M, Dixit H, *et al*. Prevalence of iron deficiency and anaemia among healthy women of reproductive age in Bhaktapur, Nepal. Eur J Clin Nutr 2007;61:262–9.
12. Gopalan C. Women and nutrition in India. Ind J Nutr Dietet 1999;36:95–107.
13. Karimi M, Kadivar R, Yarmohammadi H. Assessment of the prevalence of iron deficiency anaemia, by serum ferritin, in pregnant women of Southern Iran. Med Sci Monit 2002;8:488–92.
14. Hyder SMZ, Persson LA, Chowdhury M, Lonnerdal B, Ekstrom EC. Anaemia and iron deficiency during pregnancy in rural Bangladesh. Public Health Nutr 2004;7:1065–70.
15. Scholl TO. Iron status during pregnancy: setting the stage for mother and infant. Am J Clin Nutr 2005;81(5):1218S–22S.

16. Martí-Carvajal A, Peña-Martí G, Comunian G, Muñoz S. Prevalence of anaemia during pregnancy: results of Valencia (Venezuela) anaemia during pregnancy study. Arch Latinoam Nutr 2002;52(1):5–11.
17. Seshadri S. Prevalence of micronutrient deficiency particularly of iron, zinc and folic acid in pregnant women in South East Asia. Bri J Nutr 2001;85(2):S87–S92.
18. Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of Pregnancy. Am J Cliii Nutr 1996;63:520–5.
19. Gibson RS, Abebe Y, Stabler S, Allen RH, Westcott JE, Stoecker BJ, *et al*. Zinc, gravida, infection, and iron, but not cobalamin or folate status, predict hemoglobin during pregnancy in Southern Ethiopia. J Nutr 2008;138:581–6.
20. Khalil AA, Jabbar T, Akhtar S, Mohyuddin S. Frequency and types of anaemia in an antenatal clinic in the third trimester of pregnancy. Pak Armed Forces Med J 2007;57:273–8.
21. Agarwal KN, Agarwal DK, Sharma A, Sharma K, Prasad K, Kalita MC et al. Prevalence of anaemia in pregnant & lactating women in India. Indian J Med Res 2006;124(2):173–84.
22. Asif N, Hassan K, Mahmud S, Zaheer HA, Naseem L, Zafar T et al. Comparison of Serum Ferritin Levels in Three Trimesters of Pregnancy and Their Correlation with Increasing Gravidity. Int J Pathol 2006;5(1):26–30.
23. Haniff J, Das A, Onn LT, Sun CW, Nordin NM, Rampal S, *et al*. Anaemia in pregnancy in Malaysia: a cross-sectional survey. Asia PAC J Clin Nutr 2007;16(3):527–36.
24. Gadowsky SL, Gale K, Wolfe SA, Jory J, Gibson R, O'Connor DL. Biochemical folate, B12, and iron status of a group of pregnant adolescents accessed through the public health system in southern Ontario. J Adolesc Health 1995;16(6):465–74.

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