



## **Antioxidant vitamins status in anemic and non anemic Pakistani women in the third trimester of Pregnancy**

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Received: 20-08-2014 / Revised: 07-10-2014 / Accepted: 17-10-2014

### **ABSTRACT**

The pregnant women belonging from a poor socioeconomic class, in the third trimester of pregnancy suffer from iron deficiency anemia. The understanding of the variability of micronutrient status markers during pregnancy is limited. A cross sectional study was conducted during January 2010 to December 2010. Based on hemoglobin concentration the women in third trimester of pregnancy were divided into anemic group (Hb<11gm/dL) and non anemic group (Hb > 11 g/dl) as control. The anemic pregnant women had shown low levels of hemoglobin, PCV, RBC count, mean cell volume (MCV), and mean cell hemoglobin concentration (MCHC) as compared to normal control pregnant women. The RBC's were hypochromic and microcytic in anemic pregnant women. The anemic pregnant women also had shown low levels of serum iron and serum vitamins A, E, & C as compared to normal control pregnant women in the third trimester of pregnancy. Based on this study we suggest that in late pregnancy (3<sup>rd</sup> trimester) the pregnant women should not only be supplemented with iron, but they must be supplemented with antioxidant vitamins

**Key words:** Iron deficiency Anemia, Antioxidant Vitamins, Third trimester of Pregnancy

### **INTRODUCTION**

Iron deficiency anemia refers to anemia that is caused by lower level of iron. It occurs when the dietary intake or absorption of iron is insufficient, and hemoglobin cannot be formed.<sup>(1)</sup> Iron deficiency anemia during pregnancy is associated with increased risk of maternal mortality, premature delivery and low birth weight<sup>(2)</sup>. There is an association between maternal iron deficiency anemia and postpartum depression, with poor results in mental and psychomotor performance testing of offspring<sup>(3)</sup>.

In pregnancy red cell mass increases substantially however plasma volume expands more. Low level of hemoglobin or serum ferritin may be associated with high plasma volume expansion<sup>(4)</sup>. In iron deficiency the cells are hypo chromic & microcytic, depends on the severity of anemia<sup>(5)</sup>. Hamdy et al had demonstrated a strong relationship between serum levels of vitamin A & blood hemoglobin concentration during pregnancy in poor Egyptian families<sup>(6)</sup>. Vitamin A is involved in the pathogenesis of anemia by enhancing the growth

and differentiation of erythrocyte progenitor cells and mobilization of iron from tissues<sup>(7)</sup>. It was estimated that 10 million pregnant women are deficient of vitamin A, which is associated with increased maternal morbidity & mortality<sup>(8)</sup>. Ascorbic acid is important for the modulation of ferritin synthesis and iron storage<sup>(9)</sup>. Ascorbic acid supplementation increases dietary iron absorption<sup>(10)</sup>. Vitamin E is used to treat the iron deficiency anemia & has therapeutic advantage<sup>(11)</sup>. Vitamin E scavenges free radicals and may prevent destruction of RBC's in glucose –6 – Phosphate dehydrogenase deficient hemolytic anemia<sup>(12)</sup>. The maternal micronutrient deficiencies are wide spread in Pakistan and are potentially associated with maternal undernutrition & intrauterine growth retardation. Therefore the study was planned to reveal out the status of vitamins in anemic pregnant women in the third trimester of pregnancy residing in Karachi, Pakistan. The aim of the study is to evaluate the prevalence of iron deficiency anemia in anemic and non anemic pregnant women during 3<sup>rd</sup> trimester, in poor socio – economic class of the society and to correlate the level of essential micronutrients to anemia.

## MATERIAL AND METHODS

The pregnant women (after 24 weeks of gestation) having no symptoms of any disease were selected from the gynae department of Al – Khidmat Hospital, Karachi, Pakistan during January 2010 to December 2010. The pregnant women (after 24 weeks of gestation) were divided into two groups on the basis of the blood level of hemoglobin. The anemic pregnant women (after 24 weeks of gestation) had low hemoglobin (less than 11 gm/dl) and low hematocrit (less than 33%). The 20 control pregnant women (after 24 weeks of gestation) having no symptoms of anemia and Hemoglobin level above 11 g/dL were included in the study. The past and present history of anemic pregnant women and control pregnant women was taken by filling Performa. The study was approved by ethical committee of Department of Biochemistry, University of Karachi, Pakistan. The analysis of the blood samples were performed at Hematology Research unit, Department of Biochemistry, University of Karachi. About 10 ml. blood was taken from both control and anemic patients. Two ml. blood was collected in heparinised tubes & the blood was analyzed for hemoglobin level, Total blood cell count, platelet count by (sysmex XS – 1000) automated hematology analyzer. The blood was analyzed for serum iron and serum total iron binding capacity by Kit. Supplied by Firrimat Company. Blood Ascorbic acid was estimated by 2,4-dinitro-phenylhydrazine method<sup>(13)</sup>, Alpha tocopherol by Bakar & Frank Method<sup>(13)</sup>, Retinol was estimated by trifluoroacetic acid method,<sup>(13)</sup> random glucose was estimated by kit method supplied by Human diagnostic company.

**Statistical analysis:** For statistical analysis, statistical package for social science (SPSS version 11.0) was used. The anemic and control were analyzed by students t test.

## RESULT

Twenty control and fifty anemic pregnant women were included in the study. Table – 1 shows age, BMI and past history of control and anemic pregnant women. The abortion percentage was high in anemic pregnant women as compared to control pregnant women, where as none of the control & anemic women were suffering from Thalassemia. The percentage of PV bleeding was more frequent in the anemic pregnant women as compared to control women (Table – 2). The variation of hematological parameters in control and anemic pregnant women are summarized in (Table – 3) which shows low hemoglobin level in anemic pregnant women as compared to control pregnant women in the third trimester of pregnancy. The

morphology of RBC'S shows that most of the anemic women's RBC'S were anisocytotic, microcytic, and hypochromic. (Table – 4) The level of serum iron, Ascorbic acid, Retinol &  $\beta$  – Carotene was low in anemic pregnant women as compared to control pregnant women. The level of serum total iron binding capacity (TIBC) was high in anemic as compared to normal control pregnant women (Table – 5)

## DISCUSSION

Anemia during pregnancy and the post partum period is caused by iron deficiency and is a significant worldwide issue. In under developed countries anemia is a major contributory factor to maternal morbidity and mortality<sup>(14)</sup>. Inadequate antenatal care along with poor knowledge of dietary needs of pregnant women and over all poor socio economic conditions are responsible for this disease<sup>(15)</sup>. During pregnancy there are variation of hemoglobin at different trimester, the lowest values meeting this definition were, 11 gm/dl (first trimester), 10.5g/dl (second trimester), more than 11 g/dl (third trimester)<sup>(16)</sup>. In the present study low level of hemoglobin, packed cell volume (PCV), RBC's Count, mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), were found in anemic patients as compared to control pregnant women (Table – 3) as was found by Marahatta<sup>(17)</sup>. Morphologically RBCs of anemic pregnant women were hypochromic, and microcytic, (Table – 4) because of the deficiency of iron, & due to higher oxidative stress<sup>(18)</sup>.

Maternal glucose intolerance complicates up to 10% of pregnancies, because of either preexisting diabetes, or disease that begins during gestation<sup>(19)</sup>. Macrosomia, hypoglycemia, and iron deficiency result from worsening glycemic control late in pregnancy, during the last trimester<sup>(20)</sup>. There is no significant difference in the random glucose level between the anemic pregnant women and pregnant control subjects (Table – 5) in the third trimester of pregnancy. The pregnancy increases iron requirement to nearly 6 mg/day by second and third trimester due to high growth rates of the placenta & fetus, and the expansion of the maternal and blood cell mass. A sufficiently low hemoglobin level, and low hematocrit by definition make the diagnosis of anemia<sup>(21)</sup>. Iron is carried in the blood attached to the protein transferrin. The ratio of serum iron to TIBC is the most specific indicator of iron deficiency. In the present study a low percentage of transferrin saturation was found in anemic pregnant women as compared to control pregnant women (Table – 5), in the third trimester of Pregnancy. Synthesis of red blood cells requires an adequate

supply of nutrients including iron, folate, vitamin B<sub>12</sub> and ascorbic acid<sup>(22)</sup>. Vitamin A is involved in the pathogenesis of anemia, through diverse biological mechanisms, such as the enhancement of growth and differentiation of erythrocyte progenitor cells, potentiation of immunity to infection. Infections can lower the biochemical level of both iron and vitamin A<sup>(23)</sup>. In the present study a significant decrease in the level of vitamin A was found in anemic pregnant women as compared to control subjects, (Table – 6) as was found by Ma *et al*<sup>(24)</sup> and suhail *et al*<sup>(25)</sup>. Vitamin C is an important antioxidant which is necessary for the synthesis of red blood cells<sup>(26)</sup>. It improves hemoglobin & serum iron, and also influences the storage and transport of iron in the body. Ascorbic acid increases dietary iron absorption in iron depleted women<sup>(10)</sup>. In the present study a low level of blood vitamin C was observed, Ma *et al*<sup>(24)</sup> also found low level of vitamin C in anemic pregnant patients. The decrease in endogenous ascorbic acid may be due to its extensive use as antioxidant to protect the gastrointestinal tract from

the free radical damage during decreased iron level & increased level of lipid peroxidation products<sup>(27)</sup>. Deficiency of vitamin E in humans result a shortening of red cell life span<sup>(28)</sup>. Vitamin E provides a substantial improvement in the pathophysiology of hemolytic anemia<sup>(29)</sup>. Shamim *et al*<sup>(30)</sup> had found a positive association between  $\alpha$  – tocopherol and hemoglobin in early pregnancy. Serum  $\alpha$  – tocopherol level is low in anemic pregnant women as compared to pregnant control. Vitamin E is a chain breaking antioxidant, involved in the inhibition of propagation of free radicals generation during Iron deficient anemia and its level is decreased in the third trimester of pregnancy<sup>(31)</sup>. In the present study, a decline in the level of serum vit. A , C. & E had been found in anemic pregnant women as compared to control pregnant women (Table – 5) in the third trimester of pregnancy. It is concluded that in pregnancy women should not only be supplemented with iron, but the antioxidant vitamins such as vit. A, vit. C, & vit. E should also be given to anemic pregnant women to avoid anemia.

**Table – 1: Variations of Age, BMI and Past history of pregnant control and anemic pregnant women**

Parameters	Control (20)	Anemic (50)
Age (years)	27.05 ± 1.12	25.53± 0.74
B.M.I Kg / m <sup>2</sup>	24.12 ± 0.89	24.89± 0.61
Abortion	0%	13%
Thalassemia	0%	0%
Blood transfusion	6.6%	10%
Previous Baby weight (Lbs)	4.2±0.72	4.48±0.60

**Table – 2: Prevalence of Present history of anemic and control pregnant women**

Total Cases	Control (20)	Anemic (50)
Pv bleeding (percent)	5	16.6
Malena (percent)	15	50
Pregnancy month	7.0 ± 0.22	7.16 ± 0.17
Number of Children	2.35±0.28	2.0± 0.23

\* P<0.05 as compared to control pregnancy women

**Table – 3: Variation of Hematological parameters in control pregnant and anemic pregnant women.**

Parameters	Control (20)	Anemic (50)
Haemoglobin (gm/dL)	11.29± 0.17	8.32*± 0.16
Packed cell volume (%)	34.79 ± 0.90	27.57* ± 0.41
RCBCs (million/ml)	4.17 ± 0.12	3.71* ± 0.08
Mean cell volume (MCV) (fL)	83.96 ± 1.83	65.08*± 0.73
Mean Cell Hb (MCH) (Pg)	28.44± 0.70	24.11*± 1.01
Mean cell Hb. Concentration (MCHC) (Percent)	33.96± 0.47	30.45*± 0.31
W.B.C (Cell/μl)	9305.00± 458.0	11884.0± 2692.39
Neutrophils (%)	67.70 ± 1.38	73.06± 0.93
Lymphocytes (%)	23.20± 1.27	21.28± 0.93
Eosinophils (%)	3.05± 0.39	2.26± 0.20
Monocytes (%)	4.45± 0.34	3.18 ± 0.19
Platelet count- 10 <sup>3</sup> x cell/μl	251.35± 152.85	282.60± 231.60

\*P<0.05 as compared to control pregnant women

**Table – 4: Variation in the morphology of RBC's in control pregnant women & anemic pregnant women**

Morphology of RBC's in percentage	Control (Percent)	Anemic (Percent)
Anisocytosis (A) (%)	30	100
Hypochromic (H) (%)	10	100
Normocytic (N <sub>1</sub> ) (%)	90	0
Normochromic (N <sub>2</sub> ) (%)	70	0
Microcytosis (M) (%)	0	56

**Table – 5: Variation of serum iron, TIBC, Retinol, β – carotene and alpha tocopherol in control pregnant & anemic pregnant women.**

Parameters	Control (20)	Anemic (50)
Serum iron (µg/dL)	58.31± 1.04	40.91*± 1.47
Total iron binding capacity (TIBC) (µg/dL)	291.26 ± 1.59	359.0*± 4.13
Percentage of Transferrin saturation (%)	19.77± 1.05	11.46*± 1.04
Ascorbic acid (mg/dL)	1.35 ± 0.01	0.97* ± 4.13
Retinol (mg/L)	0.68 ± 0.01	0.52*± 0.01
β – carotene (mg/L)	0.84 ± 0.01	0.68*± 0.02
Alpha tocopherol (mg/L)	7.72 ± 0.10	6.89*± 0.20
Blood glucose (random) (mg/dl)	95.50± 3.22	97.94± 2.10

\* P &lt; 0.05 as compared to control pregnant women

**REFERENCE**

- Brady PG. Iron deficiency anemia: A call for aggressive diagnostic evaluation. *South Med. J.* 2007; 100 (10) : 966 – 967
- Gautam CS, Saha L, Sekhri K, Saha PK., Iron deficiency in pregnancy and the rationality of iron supplements prescribed during pregnancy. *Medscape J Med.* 2008; 10 : 12 – 14
- Perez EM, Hendricks MK, Beard JL, Murray Kolb LE, Berg A, Tomlinsou. Mother infants interactions and infant development are altered by maternal iron deficiency anemia. *J. Nutr.* 2005; 135 : 850 – 855.
- Allen LH., Anemia and iron deficiency effects on pregnancy outcomes. *Am. J. Clin. Nutr.* 2000; 71: 1280S – 1284S
- Miret S, Simpson RJ, Mckie., Physiology & molecular biology of dietary iron absorption. *Review of nutrition*, 2003 ; 23 : 283 – 301
- Hamdy A.M, Aleen, MM.A, EL-Shazly, A. A. Maternal vitamin A deficiency during pregnancy and its relation with maternal and Neonatal hemoglobin concentration among poor Egyptian families. *ISRN Pediatr.* 2013; 652148. PMC ID. PMC3763260.
- Gamble MV, Palafax NA, Dancheck B, Ricks MO, Briand K, Semba RD., Relationship of vitamin A deficiency, iron deficiency, and inflammation of anemia among preschool children in the republic of the Marshall Islands. *Eur. J Clin. Nutr.* 2004; 58 (10) : 1396 – 1401.
- Christian, P. and West, K.P. Nutrition: Vitamin A supplementation – Maternal & neonatal survival. *Nat. Rev. Endocrinol.* 2011; 7 (4) 190 –192
- Michel. R. Langlios, et al. Effect of hepatoglobin on the metabolism of vitamin C. *Am J: clin. Nutr.* 1997, 66:606 – 610
- Hunt, JR, Mullen, LM, Lykken. Gi, Gallagher SK, Nielsen FH; Ascorbic acid. Effect on going iron absorption and status in iron depleted young women. *Am. J. Nutr.* 1990: 51: 64955,
- Shapgina LA, Loseva MI, Sukharevskaja TM, Sharapov – VI, Sazonova OV, Tretiakov SV. Optimization of diagnosis and treatment of anemia under exposure to aromatic type organic solvents. *Terarkh;* 1996, 68 (12) : 15 – 19
- Sultana N, Begum N, Akhter S, Begum S, Quraishi, SB, Firdous, Ali, T. Role of vitamin E supplementation on serum levels of copper and Zinc in hemolytic anemia patients with G6. PD deficiency, *Mymensingh Med J;* 2008, 17: 584 – 590.
- Gowenlock, A. Varley's Practical clinical Biochemistry Publisher Heinemann. Medical Books 22 Bedford Square London: 1988; PP. 898, 902, 924.
- Saeed M, Khan TA, Khan SJ. Evaluation of risk factors in antenatal care. *Mother and child* 1996 : 34 : 139 – 142
- Nisar N, White F. Factors effecting utilization of antenatal care among reproductive age group women (15 – 49 years) in an urban squatter settlement of Karachi. *J. Pak. Med. Assoc.* 2003; 53 : 47 – 53
- Sukrat B, Suwathanapisate P, Siritawee, S, Pongthong, T, The prevalence of iron deficiency anemia in pregnant women in Nakh on sawan Thailand. *J. Med. Assoc. Thai,* 2010; 93 (7):765 – 770,
- Marahatta, R. Study of anemia in pregnancy and its outcome in Nepal Medical college teaching hospital, Khatmandu, Nepal. *Nepal. Med. Coll. J.* 2007; 9 : 270 – 274

18. Madhikarmi NL, Murthy KR. Antioxidant enzymes and oxidative stress in the erythrocytes of iron deficiency anemic patients supplemented with vitamins. *Iran. Biomed. J.* 2014; 18 (2): 82 – 87
19. Nold J, Georgieff MK. Infants of Diabetic mothers. In Rademacher R, Kliegman R, Eds. *Pediatric clinics of North America*, Philadelphia W.B. Saunders, 2004 : 51 : 619 – 637
20. Milley JR, Papacostas Js, Tabata BK. Effect of insulin on uptake of metabolic substrates by the fetus. *Am. J. Physiol.* 1986 : 251 : 349 – 359.
21. Nair KM. Iron absorption and its implications in control of iron deficiency anemia. *Nutr. News.* 1999, 20:1
22. Bothwell TH, Charlton RW, Cook JB, Finch CA. *Iron metabolism in man.* Oxford: Blackwell scientific 1979: 7 – 87
23. Bloem MW, Weddel M, Egger RJ et al. Iron metabolism and Vitamin A deficiency in Northeast Thailand. *Am. J. Clin. Nutr.* 1989; 50 : 332 – 338
24. Ma, AG, Schouten EG, Wang Y, XURX, Zheng MC, Li Y, Wang Q, Sun Y. Micronutrient status in anemic and non anemic Chinese women in the third trimester of pregnancy. *Asia Pac. J. Clin. Nutr.* 2009 : 18(1): 41 – 47
25. Suhail M, Patil S, Khan S, Siddiqui S., Antioxidant vitamins & lipoperoxidation in Non – pregnant, Pregnant and gestational diabetic women; Erythrocytes osmotic fragility profile. *J. Clin. Med. Res.* 2010: 11 : 2 (6) 266 – 273.
26. Behrman RE, Vanghan VC, Nelson W. E. eds. *Nelson Text book of pediatrics* WB. Saunders; 1987, PP 1039 –1044.
27. Viteri, FE, A new concept in the control of iron deficiency. *Biomed – Environ. Sci.* 1998: 1101: 46 – 60.
28. Frank A, Oski, MD. Anemia in infancy Iron deficiency & vitamin E deficiency. *pediatrics Review* 1980; 1 : 247 – 253.
29. Fibach E, Rachmilewitz EA. The role of antioxidants and iron chelators in the treatment of oxidative stress in thalassemia. 2010; 1202 : 10 – 16
30. Shamim AA, Kabir A, Merrill RD, Ali H, Rashid M, Sculze K, Labrique A, West KP, Christian P. Plasma zinc, vitamin B<sub>12</sub>, and  $\alpha$  – tocopherol are positively & plasma  $\delta$  – tocopherol is negatively associated with Hb concentration in early pregnancy in north west Bangladesh. *Public health Nutr.* 2013, 17: 1 – 18.
31. Chawala PK, Puri R. Impact of nutritional supplementation on hematological profile of pregnant women. *Indian Pediatr.* 1995; 32: 876 – 880.