Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

THE STUDY OF FOLIC ACID AND VITAMIN B_{12} LEVELS IN ANAEMIA IN PREGNANCY

YOGENDER P[1], BALRAM SINGH THAKUR[2], RANGASWAMY R[3] AND SREEKANTHA[4*]

[1] Assistant Professor, Department of Pathology,NMC,Raichur.
[2] Professor, Department of Pathology, NMC,Raichur.
[3]Professor, Department of Pathology, KIMS,Bangalore.
[4]Associate Professor, Department of Biochemistry, NMC, Raichur.

ABSTRACT

In developing countries the prevalence of anaemia among pregnant women averages 56%, ranging between 35 to 100% among different regions of the world. Various studies from different regions of the country (India) have reported the prevalence of anaemia to be between 33 and 100%.In India, anaemia is the second most common cause of maternal deaths, accounting for 20% of total maternal deaths. Anaemia affects mainly the women in child bearing age group, young children and adolescent girls. Association of anaemia with adverse maternal outcome such as puerperal sepsis, ante-partum haemorrhage, post-partum haemorrhage and maternal mortality is no longer a debatable subject. Folic acid and vitamin B_{12} are essential in the synthesis of nucleoprotein, particularly of erythropoietic cells. Hence this study is taken to study the serum levels of Folic acid and vitamin B_{12} in pregnant ladies and correlate with seviarity of anaemia.

KEY WORDS: Foloic acid, Vitamin $B_{12,}$ Pregnancy.

SREEKANTHA
Associate Professor, Department of Biochemistry, NMC, Raichur.

INRODUCTION

In ancient Indian medicine it is reported that Sushruta described anemia as a form of 'Pandu roga' or Jaundice. It was believed to be due to the derangement of kapha or phlegma and was manifested by the whiteness of the eyes, skin and finger nails. Charaka described another form of 'Pandu roga' associated with the eating of clay[1]. John Lange for the first time gave a concise and clinical picture of hypochromic anemia. In a letter written to his friend whose daughter had extreme pallor, he described the symptomatology-palpitations, dyspnea and swelling of the ankles. He used the classical name "marbus vigineus". Joseph Leinttand [1703-1780], a physician of versaills devoted a chapter on "Anemias" in his text book. In France, by the middle of the seventeenth century, iron salt and other remedies were used in its treatment.. Anemia is defined as haemoglobin level in the blood is below the lower extreme of the normal range for the age and sex of the individual.[1]Normal Range is for Men- 13.6 – 17.2 g/dl and for Women- 12- 15 g/dl[2]. There are many causes of anaenia;mainly nutritional ;Deficiencies affecting DNA synthesis : B_{12} and Folate deficiencies is considered as preventive importance. The specific vitamins that are required in the maturation process are vitamin B_{12} , Folic acid and vitamin C. Folic acid and vitamin B_{12} are essential in the synthesis of nucleoprotein, particularly of erythropoietic cells. Vitamin B_{12} acts at an early stage in the synthesis of RNA but Folic acid acts at a later stage in the synthesis of DNA. Thus deficiency of Vitamin B_{12} results in defective synthesis of both RNA and DNA, while the deficiency of the Folic acid results in the defective synthesis of DNA only. Vitamin C is required for conversion of folic acid to folinic acid. The second type of anemia in the pregnancy is of megaloblastic type due to folic acid or vitamin B12 deficiency. In about 40-50 % cases there is associated folic acid deficiency. The role of vitamin B12 contribution to incidence of anemia is still debated.The megaloblastic anemia present with symptoms of slowly progressive anemia similar to IDA. The laboratory evaluation should include a complete blood count, peripheral blood smear, serum folic acid level, serum vitamin B12[3,5]. In peripheral blood smear study, the outstanding feature is the presence of macrocytic red cells, many of which are oval. Cells of normal size and microcytes are also present and occasionally small fragmented and distorted cells are prominent. A moderate leucopenia may be present. Hypersegmented neutrophils are always present. A moderate thrombocytopenia is usual[1,3]. The anemia is a macrocytic with an elevated MCV (110 to 140 fl) but commonly normochormic with increased MCH and normal MCHC. Serum folate levels are usually lower during pregnancy, but fasting serum folate of less than 3 ng/ml is considered diagnostic for FAD (Folic acid deficiency)[3].On Bone marrow examination, marrow is moderately to markedly cellular with proliferating erythroid precursors replacing fat. There is Megaloblastic erythropoiesis which shows Megaloblasts with asynchrony of nuclear and cytoplasmic maturation. Myelopoiesis is normal with Giant metamyelocytes. Megakaryocytes are decreased in number. Marrow Iron is moderately increased[4,5]. Hence,the study of serum levels of folic acid and Vitamin B_{12} is taken and to study in detail and the relationship of above mentioned parameters Vs iron deficiency anaemia.

MATERIALS AND METHODS

The present study was carried out in pregnant women who attended the out patient and inpatient department of OBG in recent 2years at KIMS Hospital and Research Centre, Bangalore.A detailed clinical history of each patient was recorded and a thorough clinical examination was performed. Venous blood was collected in all women with aseptic precautions in EDTA anticoagulant for hematological investigations. Separate blood sample was collected for biochemical investigations. Serum was separated on the same day of blood collection and stored in a refrigerator between 2 to 8 degree centigrade. Biochemical study was carried out within three days of blood collection.

Inclusion Critera

Pregnant women whose haemoglobin levels are less than 11gm%.

Exclusion Criteria

-Pregnant women who had already received blood transfusion. -Anemias of chronic disorders and haematological malignancies.
-Consent refusal.

1-Estimation of Vitamin B_{12} BY CLIA (CHEMILUMMINESCENCE IMMUNOASSAY) :

PRINCIPAL OF THE TEST :

Vitamin B_{12} assay is a competitive immunoassay using direct The assay uses Releasing Agent (sodium hydroxide) and DTT to release the vitamin B12 from the endogenous binding proteins in the sample and cobinamide to prevent rebinding after the Solid Phase is added to the sample.

- The procedure of the test is same as described above for Serum Ferritin procedure.

2-The Serum Folic acid was also estimated by CLIA (CHEMILUMMINESCENCE IMMUNO ASSAY).

STATISTICS

Chi Square test was applied for testing the statistically significance of the results.

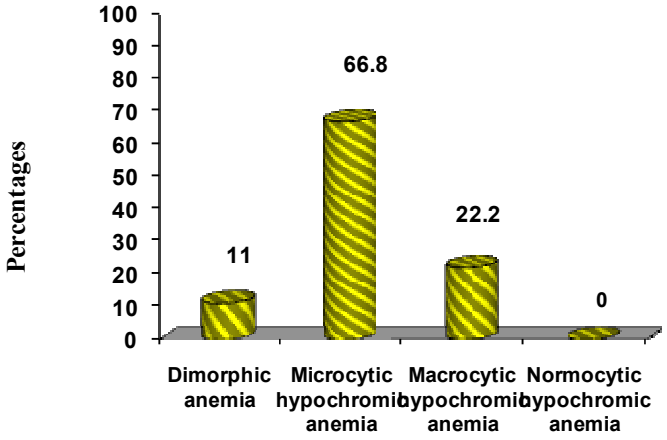
Chi-Square Test

$\chi^2 = \frac{\sum(Oi - Ei)^2}{Ei}$, Where Oi is Observed frequency and Ei is Expected frequency.

RESULTS

Study Design: Prospective clinical non –controlled study with 500 pregnant women is undertaken over a period of recent 2 years in the department of pathology, KIMS , Bangalore of pregnant women attending OPD and IPD in the department of Obstetrics and Gynaecolgy, KIMS Hospital and Research centre,Bangalore to study the various types of anemia

Graph 1
Peripheral smear Examination



Peripheral smear examination

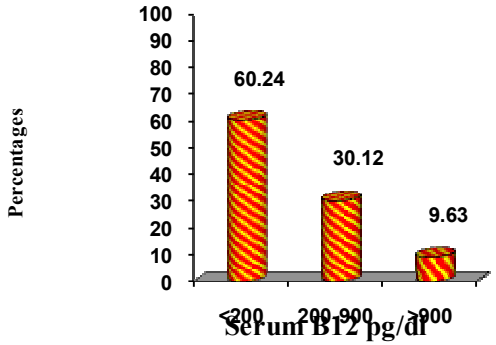
In the study of 500 cases of anemia in pregnancy, on Peripheral smear examination following types of anemia were found. Microcytic Hypochromic anemia was 334 cases(66.8%), Macrocytic anemia was 111 cases(22.2%), Dimorphic anemia was 55 cases(11%).

Distribution of Serum Serum B12 and Serum folic acid.

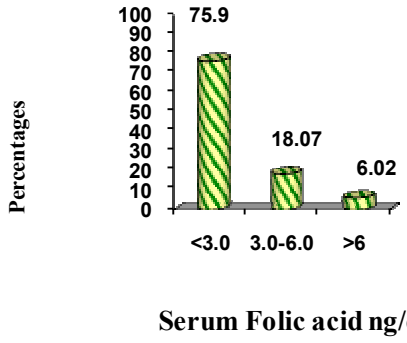
Table 1
Distribution of Serum Ferritin, TIBC, Transferring Saturation, Serum B12, Serum folic acid, MCV and RDW

Investigation	Criteria	Number of patients	%	95%CI
Serum vitamin B12 pg/dl (n=166)	<200	100	60.24	75.59-87.66
	200-900	50	30.12	8.04-18.47
	>900	16	9.63	2.66-9.92
Serum Folic acid ng/dl. (n=166)	<3.0	126	75.90	74.98-87.11
	3.0-6.0	30	18.07	9.10-19.95
	>6.0	10	6.02	2.22-11.08

Graph 2
Distribution of serum Vitamin B_{12}.



Graph 3
Distribution of Serum Folic acid.



Serum Vitamin B_{12} was done in 166 cases(including Dimorphic anemia cases) out of which 100 cases had < 200 pg/dl, 50 cases had in between 200 to 900 pg/dl and 16 cases had >900 pg/dl. Serum Folic acid was done in 166 cases(including Dimorphic anemia cases) out of which 126 cases had <3 ng/dl, 30 cases had in between 3 to 6 ng/dl and remaining 10 cases had >6 ng/dl.

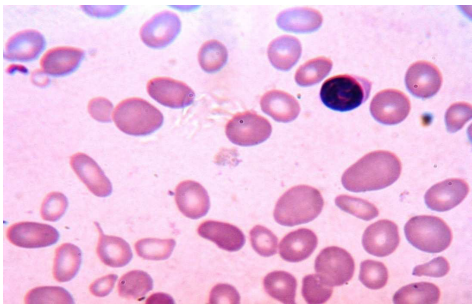


FIGURE 1
Dimorphic Anemia showing macrocytes(Black arrow), microcytes(Red arrow) and a small lymphocyte for comparision with RBC'S(Yellow arrow).[Leishman's stain, oil immersion(x100)].

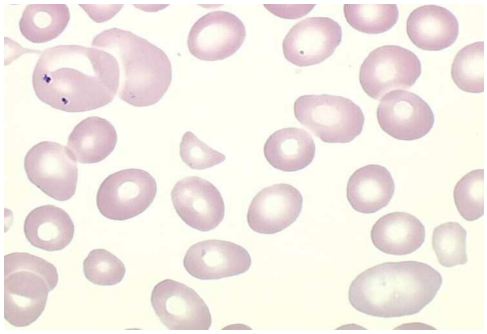


FIGURE 2
Megaloblastic Anemia showing macrocytes (Green arrow). [Leishman's stain, oil immersion(x100)].

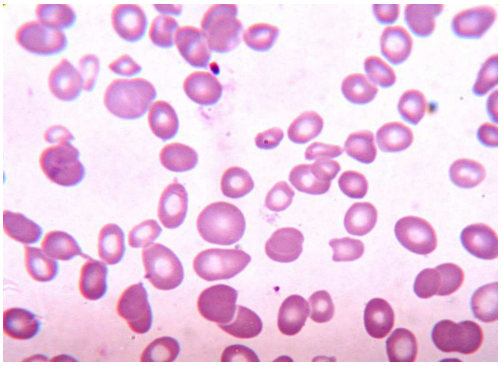


FIGURE 3
Dimorphic Anemia showing macrocytes(Blue arrow) and microcytes(Black arrow). [Leishman's stain, oil immersion(x100)].

DISCUSSION

The present study is a prospective study over a period in recent 2 years in the department of pathology, Kempegowda Institute Of Medical Sciences,Bangalore. Total no:of 500 cases of pregnant women attending OPD and IPD in the department of Obstetrics and Gynaecolgy, KIMS Hospital and Research centre,Bangalore were included in the study. Anemia in pregnancy constitutes a major public health problem in developing countries,perticularly in pregnancy. Anemia is one of the most common medical condition met during pregnancy. This was noted in most of the women attending the antenatal clinic. Iron deficiency anemia accounted for 72.4%, and dimorphic anemia 17%, and megaloblastic anemia accounts for 25.2% in our present study.

Table No 2
Showing Percentage distribution of Type of Anemia in comparision with other studies.

Authors	*Iron Deficiency*	*Dimorphic*	*Megaloblastic*
Ratnam R [6]	84%	9%	6%
Present study	72.4%	2.4%	25.2%

Our present study shows 72.4% as Iron deficient, 2.4% as Dimorphic and 25.2% as Megaloblastic anemia. Our present study correlates with Ratnam R[6] found 84% Iron deficiency, 9% as Dimorphic and 6% as Megaloblastic anemia in his study.

Table No 3
Showing Serum Folic Acid in Macrocytic Anemia in comparision with other studies.

Authors	*<3 ng/ml*	*3-6ng/ml*	*>6 ng/ml*
Casal MNG [7]	89.85%	55.33%	54.81%
Moffatt ME [8]	49%	43%	8%
Thoradeniya.T [9]	62.1%	22.7	-
Present Study	75.90%	18.07%	6.02%

In our study Serum Folic acid was less than 3 ng/ml in 75.90% and Our present study correlates with the studies done by Casal MNG [7](89.85%), Thoradeniya.T[9](62.1%) and Moffat M.E[8](49%).

Table No 4
Showing serum Vitamin B_{12} in Macrocytic anemia in comparision with other studies

Authors	*<200 pg/ml*	*200-900 pg/ml*	*>900pg/ml*
Casal MNG [7]	61.34%	37.33%	1.33%
Metz J [10]	80%	85%	-
Present study	60.24%	30.12%	9.63%

In our study Serum Vitamin B_{12} levels were less than 200 pg/ml in 60.24% cases and our present study correlates with Casal MNG[7] (61.34%) and Metz J[10] (80%).
According to our present study objective we categorized anemia under improved classification of anemia based on MCV and RDW[11] . So we have categorized our cases according to the above classification.

1.) MCV Low, RDW Normal (Microcytic homogenous) : NIL
2.) MCV Low, RDW High (Microcytic heterogenous) : Iron deficiency anemia(362 cases).
3.) MCV Normal, RDW Normal (Normocytic homogenous) : NIL
4.) MCV Normal, RDW High (Normocytic heterogenous) : Dimorphic anemia (12 cases).
5.) MCV High, RDW Normal(Macrocytic homogenous) : NIL
6.) MCV High, RDW High(Macrocytic heterogenous) : Folate and Vitamin B_{12} Deficiency (126 cases).

CONCLUSION

- The incidence of anemia in pregnancy is high. Moderate to severe anemia is common in third trimester.
- In 500 cases studied, 362 cases(72.4%) belonged to Iron deficiency anemia.
- One hundred twenty six cases(25.2%) were diagnosed as Megaloblastic anemia.
- Twelve cases(2.4%) were diagnosed as Dimorphic anemia.
- Anemia is common in the age group between 21 to 30 years due to repeated and short interval of pregnancies.

- In 500 cases of anemia, 334 cases(66.8%) were Microcytic Hypochromic anemia, 111 cases(22.2%) were Macrocytic anemia, 55 cases(11 %) were Dimorphic anemia and 1 case(0.20%) was Normocytic Hypochromic anemia on Peripheral smear examination.

REFERENCES

1. Firkin F, Chesterman C , Penington D, Rush B. de Gruchy's Clinical Hematology in Medical Practice, 5th ed. Blackwell Science Ltd ; 2008.
2. Gautam VP, Bansal Y, Taneja DK, Saha R. Prevalence of Anemia Amongst Pregnant Women and its Socio-Demographic Associates in a Rural Area Of Delhi. Indian Journal Of Community Medicine 2002; 27(4):157-160.
3. Meda N, Mandelbrot L, Cartoux M, Dao B, Ouangre A, Dabis F. Anaemia during pregnancy in Burkina Faso, West Africa, 1995-96:prevalence and associated factors. Bulletin of the World Health Organization 1999;77(11):916-922.
4. Dutta DC. Text Book of Obstetrics including Perinatology and Contraception, 6th ed. New Central Book Agency (P) Ltd ; 2006.
5. Gupta R, Kalia M. Diagnosis of anaemia in Expectant Mothers. J.Hum.Ecol 2004; 15(1): 69-71.
6. Ratnam R. Prevalence of anemia and Iron deficiency in the third trimester in rural vellor district South India. Trop Doct 2001; 31(2) : 86-89.
7. Casal MNG, Osorio C, Landaeta M, Leets I, Matus P, Fazzino F et al. High prevalence of folic acid and vitamin B12 deficiencies in infants, children, adolescents and pregnant women in Venezuela. European Journal of Clinical Nutrition 2005;59: 1064–1070.
8. Moffatt ME. Current status of nutritional deficiencies in Canadian aboriginal people. Can J Physol Pharmacol 1995; 73(6): 754-758.
9. Thoradeniya T, Wickremasinghe R, Ramanayake R, Atukorala S. Low folic acid status and its association with anaemia in urban adolescent girls and women of childbearing age in Sri Lanka. British Journal of Nutrition 2006; 95:511–516.
10. Metz J, McGrath K, Bennett M, Hyland K, Bottiglieri T. Biochemical Indices of Vitamin B, Nutrition in Pregnant Patients With Subnormal Serum Vitamin B_{12}, Levels. American Journal of Hematology 1995; 48:251-255.
11. Bessman JD, Gilmer PR, Gardener FH. Improved Classification of Anaemias by MCV and RDW. Am J Clin Pathol 1983; 80 : 322-326.