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Anaemia during Pregnancy and its Association with Adverse Maternal and Foetal Outcomes

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ABSTRACT

Background: Nutritional iron deficiency is the main cause of anemia throughout the world. It is especially common in women of reproductive age and particularly during pregnancy. Anemia during pregnancy is associated with adverse perinatal outcomes in the form of preterm birth, stillbirth, small for gestational age babies and increased perinatal mortality. There is persistently high prevalence of anemia in Indian mothers despite a national program in place. Objective: To assess the prevalence of anaemia, its different types and their association with maternal and perinatal outcomes. **Methods:** This study was conducted on 100 pregnant patients with severe anemia. The study population was divided into 3 groups based on basis of peripheral smear picture i.e. microcytic, macrocytic and dimorphic anemia. These pregnant mothers were followed up until their time of delivery and associations between anaemia types and maternal as well neonatal adverse outcomes were explored. All the data collected was analyzed using statistical computer software (STATA Version 13.0). **Result:** Microcytic hypochromic anemia was most commonly present (45 patients, 45%). Intrauterine growth restriction was seen in 20% of total study population and out of these, majority were born to mother with macrocytic anemia (32.5%). The same trend was also seen for gestational hypertension with 8.9%. 32.5% and 6.7% patients having gestational hypertension in microcytic, macrocytic and dimorphic anemia groups respectively (p=0.009). A total of 23 patients had low birth weight babies; 65.2% patients with low birth weight babies had macrocytic anemia and this difference was statistically significant (p=0.024). Overall 15% of babies were small for gestational age, out of which 66.7% babies were in macrocytic anemia group. Low Apgar score at one minute after birth was seen in four babies born to mothers having macrocytic anemia and this was a statistically significant relationship (p=0.044). **Conclusion:** Anemia is increasingly becoming prevalent and is associated with poor maternal and perinatal outcomes. Public health interventions should be made by way of appropriate food fortifications to reduce prevalence of anemia Early diagnosis should be made by appropriate investigations and proper therapy should be started as soon as possible according to type of anemia, in order to prevent adverse maternal and foetal outcomes.

Keywords: Maternal, Fetal, Pregnancy.

INTRODUCTION

Anemia is defined as hemoglobin level that is less than two standard deviation below the median value for a healthy matched population.[1] WHO had accepted up to 11gm% as the normal hemoglobin level in pregnancy.[2] Therefore any hemoglobin level below 11gm% in pregnancy should be considered as anemia. Pregnancy itself leads to anemia by causing a state of hydraemic plethora. There is disproportionate increase of plasma volume as compared to red blood cell mass during pregnancy leading to apparent reduction of red cell mass,

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hemoglobin and hematocrit value. The peripheral blood smear shows normochromic normocytic red cells. This is so called physiological anemia of pregnancy. In the only well-conducted longitudinal study that could be found of the 'hydraemia' of pregnancy in iron-replete healthy pregnant women, 10.4 g/dl was the lowest recorded value3. This physiological situation can be further complicated by acquired nutritional problems which include iron, folic acid, vitamin B12 and protein deficiency. Anemia may also be due to other reasons like hemolysis or hemorrhage.

Nutritional iron deficiency is the main cause of anemia throughout the world. It is especially common in women of reproductive age and particularly during pregnancy. The demand for iron increases about six to seven times during entire duration of pregnancy4. Most women begin their pregnancy with little or no iron reserve, which is

further compounded by repeated and closely spaced pregnancies and prolonged periods of lactation. Anemia in pregnancy has been almost synonymous with iron-deficiency anemia but there appears to be a changing trend with emerging evidence of macrocytic anemia. Although supplementation of diets with Iron and Folic Acid (IFA) tablets has been a part of Indian Government programmes for over three decades, levels of IFA intake remain low and prevalence of anemia remain high. National Family Health survey (2005-06) reported that only 22 percent of pregnant women consumed IFA tablets for 90 days or more during pregnancy.5 The Ministry of Health, Government of India has also recommended intake of 100 mg elemental iron with 500 µg of folic acid in second half of pregnancy for a period of at least 100 days. Government of India in collaboration with WHO, UNICEF and FOGSI had also launched 12 by 12 initiative in 2007 to combat the problem of anemia. Despite these measures anemia continues to be a major problem.

Available literature suggests that anemia during pregnancy is associated with adverse perinatal outcomes in the form of preterm birth, stillbirth, small for gestational age babies and increased perinatal mortality.6,7,8,9 Lower birth weights in anemic women have been reported in several studies.10,11 Anemia has been frequently associated with other obstetric conditions such as twins and antepartum hemorrhage which are independent risk factors for low birth weight, prematurity and increased perinatal and maternal morbidity and mortality.12 Iron deficiency may increase the risk of maternal infections and low hemoglobin may cause a state of low-grade chronic hypoxia that induces maternal and fetal stress. In the background of these available evidence and also the persistently high prevalence of anemia in Indian mothers, the current study was done to assess the prevalence of anaemia, its different types and their association with maternal and perinatal outcomes.

Aims and objectives of the study:

1. To study different types of anemia in cases of severe anemia during pregnancy.
2. To document maternal and fetal outcome in patients of severe anemia.

MATERIALS AND METHODS

Study design: Prospective Longitudinal study.

Study setting: Departments of Obstetrics and Gynaecology, Pathology and Pediatrics, Military Hospital Ahmednagar, Maharashtra.

Sample size considered for the study: Using a reference of 35% prevalence of macrocytic anemia amongst cases of severe anemia 13 with 5% precision and 95% confidence interval, number of patients required to be studied were 80. We finally included 100 patients for our study

Inclusion criteria:

- Singleton pregnancy
- Hemoglobin <7.0 gm%.
- No previous treatment for anemia

Exclusion criteria:

- Cases of moderate and mild anemia i.e. Hb ≥ 7gm %.
- Associated obstetric complications such as gestational diabetes mellitus and antepartum hemorrhage which may alter perinatal outcome.
- Associated medical complications such as essential hypertension, hemoglobinopathies, bleeding disorders, hemolytic anemia, liver disorders, renal dysfunction, cardiac disorders, auto immune disease and epilepsy.
- Not willing for participation in the study.

Study processes

Written informed consent was taken from all the subjects fulfilling the inclusion criteria. All patients were admitted to the hospital. A detailed history was taken followed by thorough general physical and systemic examination. History included information about the socioeconomic status (Modified Kuppuswamy scale), age, parity, interval since last delivery, associated excessive bleeding during menses and bleeding from any other site. Information regarding dietary habits, calorie and protein intake was also collected. Subjects were asked for any history of symptoms suggestive of anemia such as loss of appetite, easy fatigability, breathlessness, palpitations and decreased working capacity.

A thorough general physical examination was done to identify the features of anemia such as pallor, koilonychia, edema, jugular venous pressure and presence of any lymphadenopathy. Associated features such as stomatitis, angular cheilosis were also looked for. In cardiovascular and respiratory system examination, haemic murmurs and pulmonary basal crepts were looked for. Abdominal examination was done for presence of hepatosplenomegaly. Routine obstetric examination included assessment of gestational age, fetal growth and well being.

Examination of peripheral smear was done to diagnose type of anemia. Estimation of red cell indices, serum levels of iron, ferritin, folate and cobalamin was also done. At enrolment, approximately 4 ml venous blood was taken from each patient and divided into 2 parts. 1 ml of blood was transferred to a vacutainer containing EDTA solution for the estimation of hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and for peripheral blood smear evaluation. Hemoglobin, hematocrit, MCV, MCH and MCHC were estimated by automated counters. A peripheral blood smear was stained with

Leishman's stain to determine the morphology of the blood cells.

The other part of the sample (3 ml) was used for the estimation of serum ferritin, iron, folate and cobalamin value. It was centrifuged @ 2000 rpm for 10 minutes in a centrifuge machine. The separated serum was then transferred to aliquots and the aliquots were stored at -80°C for later measurement of serum ferritin, iron, folate and cobalamin in the Biochemistry Laboratory, Maulana Azad Medical College. Serum ferritin, folate and cobalamin were measured by electrochemiluminescence method using automated clinical immunoanalyzer in a closed system (Elecys 2010, Roche Diagnostics). Serum iron estimation was done using fully automated clinical chemistry analyzer (Olympus) by spectrophotometric method using ready to use Randox reagent kits.

All patients were followed till delivery. Delivery was conducted in hospital for all the patients included in the study. After delivery maternal and neonatal status was assessed. Maternal outcome was recorded in terms of mode of delivery, gestational age at delivery, peripartum and postpartum complications and need for blood transfusions. Neonatal outcome was assessed in terms of Apgar score, birth weight, period of gestation, admission to neonatal intensive care unit (NICU), neonatal morbidity and mortality.

Statistical analysis

All the data collected was analyzed using statistical computer software (STATA Version 13.0). Mean and standard deviation was calculated for continuous variables and proportions for categorical variables. Significance for continuous variables was calculated using ANOVA while for proportions, Chi-square tests were used. Quantitative data was analyzed by Mann Whitney test. Correlation was done by Pearson's coefficient. A p-value of 0.05 was considered statistically significant.

RESULTS

This study was conducted on 100 pregnant patients with severe anemia. The study population was divided into 3 groups based on basis of peripheral smear picture. Microcytic hypochromic anemia was most commonly present (45 patients, 45%). A significant number of patients were found to have macrocytic anemia (40 patients, 40%). Dimorphic anemia was present in only a small number of women amongst these cases of severe anemia (15 patients, 15%). Diagnosis of microcytic anemia was made by presence of microcytosis and hypochromia in peripheral blood smear. [Table 1] presents the characteristics of the study population according to the three anaemia groups. Baseline characteristics of the study population [Table 1] presents the characteristics of the study population according to the three anaemia groups. The mean age of total study population was 25.6 years. The results of analysis of variance (ANOVA) shows that there was no statistically significant difference between the age of patient and occurrence of different types of anemia (P=0.089). Among study population, 74% patients were from lower socioeconomic class. The study population was comparable with respect to socioeconomic status as no statistically significant relationship was found between different types of anemia and socio-economic status (P=0.19). Out of the total study population, 57% of the patients belonged to Hindu community and 43% of patients were Muslims. In the study population, 86% patients were multigravidae and only 14% were primigravidae. Majority of patients i.e. 83% of total study population were recruited in advanced pregnancy i.e. gestational age of 32 weeks and more. The mean gestational age at time of recruitment in the microcytic anemia, macrocytic anemia and dimorphic anemia groups were 34.05 ± 3.69, 35.38 ± 2.93 and 35.14 ± 3.25 weeks respectively. The results of analysis of variance shows that no statistically significant difference was seen in mean gestational age at time of recruitment in different types of anemia (p= 0.171).

The median time elapsed since last gestation was less in patients of microcytic anemia as compared with patients who had macrocytic anemia and dimorphic anemia and this difference was found to be statistically significant (p=0.024). Amongst individual groups, statistically significant relationship was found between microcytic and macrocytic anemia (p= 0.019); and microcytic and dimorphic anemia (p=0.038). It means that mean interval since last gestation was significantly less in patients of microcytic anemia in comparison to patients with macrocytic and dimorphic anemia. The difference between macrocytic and dimorphic anemia was not statistically significant. In patients with macrocytic anemia, intake of non-vegetarian items in diet was less (22.5%) as compared to patients of microcytic anemia and dimorphic anemia (37.8% and 33.3% respectively) but the difference was not found to be statistically significant (p = 0.308).

Table 1: Characteristics of the study patients.

	Microcytic anemia n=45	Macrocytic anemia n=40	Dimorphic anemia n=15	Total n=100
	N(%)	N (%)	N (%)	N (%)
Age (years)				
<20	0(0)	1 (2.5)	0 (0)	1(1)
20- 25	16 (35.6)	10 (25)	10 (66.7)	36 (36)
25-30	21 (46.7)	27 (67.5)	4 (26.7)	52 (52)

>30	8 (17.8)	2 (5.0)	1 (6.7)	11 (10)
Mean ± SD	26.11 ± 3.49	25.65 ± 2.66	24.07 ± 2.79	p=0.089
Socio-economic status				
Lower-middle	11 (24.4)	5 (12.5)	0 (0)	16 (16)
Upper- lower	3 (6.7)	5(12.5)	2 (13.3)	10 (10)
Lower	31 (68.9)	30 (75)	13 (86.7)	74 (74)
Religion				
Hindu	25 (55.6)	25 (62.5)	7 (46.7)	57 (57)
Muslim	20 (44.4)	15 (37.5)	8 (53.3)	43 (43)
Gravidity				
1	5 (11.1)	6 (15)	3 (20)	14 (14)
2-3	25 (55.6)	29 (72.5)	10 (66.7)	64 (64)
≥4	15 (33.3)	5 (12.5)	2 (13.3)	22 (22)
Gestational age (weeks)				
<28	5 (11.1)	2 (5)	1 (6.7)	8 (8)
28-32	5 (11.1)	2 (5)	2 (13.3)	9 (9)
32-36	21 (46.7)	16 (40)	4 (26.7)	41 (41)
>36	14 (31.1)	20 (50)	8 (53.3)	42 (42)
Mean ± SD	34.05 ± 3.69	35.38 ± 2.93	35.14 ± 3.25	p=0.171
Time elapsed since previous gestation				
Median (Range)**	1.50 (0.50- 7.00)	2.00 (0.67- 7.00)	2.25 (1.00- 4.50)	0.024
Type of food consumed				
Vegetarian	28 (62.2)	31 (77.5)	10 (66.7)	69 (69)
Non-Vegetarian	17 (37.8)	9 (22.5)	5 (33.3)	31 (31)

**P-value<0.05

Effect on maternal and neonatal outcomes

[Table 2 and 3] presents data on the effect of maternal anaemia types during pregnancy on maternal neonatal outcomes. Intrauterine growth restriction was seen in 20% of total study population at time of recruitment, this percentage in macrocytic anemia was 32.5% and in microcytic and dimorphic anemia were 13.3% and 6.7% respectively. The same trend was also seen for gestational hypertension with 8.9%. 32.5% and 6.7% patients had gestational hypertension in microcytic, macrocytic and dimorphic anemia groups. Intrauterine fetal demise was seen in only two patients, both patients were from macrocytic anemia group.

Table 2: Comparison of obstetric complications in different types of anemia.

Complications	Microcytic anemia n=45	Macrocytic anemia n=40	Dimorphic anemia n=15	Total n=100	p value
	N (%)	N (%)	N (%)	N (%)	
Gestational hypertension	4 (8.9)	13 (32.5)	1 (6.7)	2 (18)	0.009**
Pre-eclampsia	1 (2.2)	1 (2.5)	0 (0)	2 (2)	0.832
IUGR	6 (13.3)	13 (32.5)	1 (6.7)	20 (20)	0.033**
IUD	0 (0)	2 (5)	0 (0)	2 (2)	

IUGR- Intrauterine growth restriction; IUD- Intrauterine death; **P-value<0.05

Table 3: Comparison of birth outcomes in different types of anemia.

Complications	Microcytic anemia n=45	Macrocytic anemia n=40	Dimorphic anemia n=15	Total n=100
	N(%)	N (%)	N (%)	N(%)
Birth weight in Kg				
<2.5	7 (15.6)	15 (39.5)	1 (6.7)	23 (23)
2.5- <3	34 (75.6)	21 (55.3)	14 (93.3)	69 (69)
≥3	4 (8.9)	2 (5.2)	0 (0)	6 (6)
Mean ±SD (gm)	2733 ± 223.31	2628 ± 305.48	2653 ± 232.02	p=0.175
Neonatal outcomes				
Prematurity	4 (8.9)	8 (20)	1 (6.7)	13 (13)
SGA	4 (8.9)	10 (25)	1 (6.7)	15 (15)
Low Apgar score (<9 at 1 min)**	0 (0)	4 (10)	0 (0)	4 (4)
Admission to NICU	0 (0)	3 (7.5)	1 (6.7)	4 (4)
Intrauterine death	0 (0)	2 (5)	0 (0)	2 (2)

SGA- Small for gestational age; NICU- Neonatal intensive care unit

The difference was statistically significant for gestational hypertension (p=0.009) and intrauterine growth restriction (p=0.033). Two patients having macrocytic anemia had intrauterine death, however

numbers were too small to calculate statistical significance. Both gestational hypertension and intrauterine growth restriction were more frequently seen in patients of macrocytic anemia and this higher incidence of these two obstetric complications was statistically significant when compared with microcytic anemia.

In total study population, 23 patients had low birth weight babies. 65.2% patients with low birth weight babies were having macrocytic anemia and this difference was statistically significant. Out of these 23 low birth weight neonates, 11 were SGA, eight were premature and four were both. The mean birth weight was also lower in macrocytic anemia patients but the difference was not statistically significant. In the study population, 13% of babies were premature i.e. delivered before 37 weeks. 15% of babies were small for gestational age, out of which 66.7% babies were in macrocytic anemia group. Low Apgar was noticed only in neonates of mothers with macrocytic anemia. Of the total study population, four neonates were admitted to NICU. Two babies were admitted for low birth weight, one for respiratory depression and one baby had transient tachypnea of newborn. Intrauterine death occurred in two patients with macrocytic anemia.

DISCUSSION

The present study was designed to study details of different types of anemia i.e. microcytic anemia, macrocytic anemia and dimorphic anemia in cases of severe anemia during pregnancy and their association with maternal and fetal outcomes. This study was a prospective longitudinal study involving 100 pregnant women with severe anemia i.e. hemoglobin <7gm/dl, recruited from the antenatal clinic and gynae casualty of Lok Nayak Hospital, New Delhi. They were divided into three groups on the basis of peripheral smear picture. Microcytic hypochromic anemia was most commonly present (45%) but a significant number of patients were found to have macrocytic anemia (40%). Dimorphic anemia was present in only a small number of women amongst these cases of severe anemia (15%). This is in accordance with results of a study conducted in the same department three years back where 40% of overall patients had macrocytic anemia.[13] In another study conducted in 2005, Patra et al observed that amongst 130 pregnant women with severe anemia, having hemoglobin concentration less than 5g/dl, prevalence of microcytic anemia was 49%, followed by dimorphic (35%) and macrocytic anemia (10%).[14] In western countries, prevalence of macrocytic anemia is comparatively less. In an American study, only 3-4% of pregnant women with anemia were found to have macrocytic anemia.[15] The reason for this low incidence could be abundance of vitamin B12 and folate in American diet. Prevalence of vitamin B12 deficiency is much higher in countries like India because majority of individuals consume purely vegetarian diet which is likely to be vitamin B12 deficient.[16-18] The increasing prevalence of vitamin B12 in different groups of population is highlighted in various studies19-26 but there is paucity of literature regarding prevalence of macrocytic anemia in pregnancy.

We found a high prevalence of anaemia in the age group of 20-30 years, similar to other studies.[27-29] Occurrence of anemia in younger age group observed in present study suggests that these women had iron and other micronutrient deficiency from the adolescent period. National Nutrition Monitoring Bureau (NNMB) survey report stating that 70% of adolescent girls are anemic in our country also supports this hypothesis.[30] In this study, 86% patients were multigravidae and only 14% were primigravidae reflecting that incidence of severe anemia increases with increase in gravidity but this increase did not reach level of statistical significance. Multiparity is a separate risk factor for anemia as it leads to reduction in maternal iron reserves in every successive pregnancy.[31,32] This is in accordance with other studies which showed increased incidence of anemia in multigravid women.[28,29] Presence of severe anemia in primigravid women suggests that these women entered the pregnancy in a pre-existing nutritionally deprived state.

In this study, 83% of patients presented to hospital in advanced gestation i.e. gestational age of 32 weeks and more. Other studies also reported similar findings with majority of patients with anemia presenting in hospitals in advanced pregnancy.[13,14,28,29] The explanation for this observation is that most of the patients were from lower socioeconomic strata and they usually either did not have routine antenatal care or had poor level of antenatal care leading to delayed diagnosis. This is further complicated by the fact that most of these women had chronic anemia as prevalence of anemia in non pregnant females is also very high in our country.[33,34] Due to the presence of chronic anemia, these women are rarely symptomatic during stage of moderate anemia and therefore, present to hospital in late stages when they had already developed severe anemia. This is further compounded by occurrence of maximum hemodilution at this time.[35]

The mean time elapsed since last pregnancy was 2.25 years in present study. 61% of overall patients conceived within three years of previous pregnancy, reflecting that severe anemia is more common in women with repeated pregnancies at short intervals. Repeated pregnancies result in decreased iron stores due to iron requirements of both mother and fetus during pregnancy. Further, due to closely spaced pregnancies, body gets no time to regain its iron stores and the woman enters the next pregnancy in iron deficient state. The mean interval since previous

gestation in microcytic, macrocytic and dimorphic anemia was 1.90 ± 1.52, 2.49 ± 1.38 and 2.54 ± 1.13 years respectively. The difference between mean intervals since previous gestation was found to be statistically significant in different types of anemia with mean interval significantly lesser in patients with microcytic anemia as compared to macrocytic and dimorphic anemia. This shows that iron stores are affected more by closely spaced repeated pregnancies. Similar results were seen in anemic women in other studies also.[14,28]

It has been described in various studies that anemia is associated with obstetric complications such as gestational hypertension,[14,36,37] pre-eclampsia, cardiac failure and their incidence increases with severity of anemia but only one study documented association of these obstetric complications with macrocytic anemia.[13] In present study, macrocytic anemia was found to have statistically significant relationship with various obstetric complications such as gestational hypertension, intrauterine growth restriction and intrauterine fetal demise. Gestational hypertension was present in 18% (18/100) of overall patients, 72.2% (13/18) of which had macrocytosis. Intrauterine growth restriction was also seen more frequently in patients of macrocytic anemia and this difference was statistically significant. Pair wise comparison between groups revealed that statistically significant relationship was present only between microcytic and macrocytic anemia and not between other groups. Intrauterine fetal demise was seen in only two patients and both patients had macrocytic anemia. Pre-eclampsia was present in two patients, one patient from microcytic and the other from macrocytic anemia group but the difference was not statistically significant. These results show that obstetric complications were more commonly associated with macrocytic anemia. These findings corroborate with findings of previously published studies.[13,14,36]

Of the total 100 patients, 15 patients (15%) had very severe anemia at time of recruitment. In this subgroup of very severely anemic women, as many as 73.3% (11/15) patients were found to have macrocytosis. Mean hemoglobin levels in microcytic, macrocytic and dimorphic anemia were 5.838 ± 0.91, 5.310 ± 1.21 and 5.880 ± 0.94 respectively. The mean hemoglobin levels were also significantly lower in patients of macrocytic anemia. Macrocytic anemia was found to be associated with very severe anemia in previous study also.[13] Literature is deficient in studies dealing with the problem of macrocytic anemia in pregnancy. Only one study could be identified in literature documenting association of adverse maternal and perinatal outcomes with macrocytic anemia in pregnancy.[13] In this study, neonatal morbidity was seen more frequently in patients with macrocytosis as compared with patients of microcytic and dimorphic anemia.[13] As all patients were having severe anemia the increase in neonatal morbidity in patients with macrocytic anemia can be attributed to type of anemia. In present study also, 23% of all babies were low birth weight i.e. birth weight <2500 gm, out of which 65.2% babies were born to mothers having macrocytic anemia and this difference was statistically significant. In macrocytic anemia, 39.5% of patients had low birth weight babies whereas in microcytic anemia, only 15.6% patients had low birth weight babies. Out of these 23 low birth weight neonates, 11 were small for gestational age, eight were premature and four were both small for gestational age and premature.

CONCLUSION

The prevalence of anemia in pregnancy is still high in our country despite various preventive programmes launched by Government of India. Further, a significant number of women present with severe anemia reflecting poor antenatal care in our country and high prevalence of anemia in women even before pregnancy. Iron deficiency anemia was the most common (45%) but a significant proportion of patients with severe anemia in pregnancy had macrocytic anemia (40%) whereas dimorphic anemia was present in 15% patients. Lower socioeconomic status and closely spaced pregnancies without supplementation of iron and multivitamins were found to be associated risk factors for anemia. Severity of anemia also had association with macrocytosis with 73% of patients with very severe anemia having macrocytic anemia. Neonatal morbidity measured in terms of prematurity, low birth weight, low Apgar score, admission to neonatal intensive care unit were also more commonly observed in patients of macrocytic anemia. Therefore, patients with anemia should be investigated properly to diagnose types of anemia for initiation of appropriate treatment. Public health interventions should be made by way of appropriate food fortifications to reduce prevalence of anemia in both pregnant and non pregnant women. Efforts should be made to educate women regarding the importance of antenatal care. Early diagnosis should be made by appropriate investigations and proper therapy should be started as soon as possible according to type of anemia.

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