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Study of hemoglobin levels among pregnant women in different trimesters at a tertiary hospital

Sradhamoni Kumbang[1], Archana Khanikar[1], Rumi Konwar[1] and Siddhartha Sankar Konwar[1,*]

[1] Department of Physiology, Assam Medical College, Dibrugarh, Assam, India.

* Correspondence: sskonwar@gmail.com

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Abstract: Background: Pregnancy increases the demand for nutrients and hemoglobin. Besides these, physiological changes in blood parameters also occurs during pregnancy. Anemia in pregnancy is a serious condition contributing to increased maternal and fetal morbidity and mortality. Present study has been undertaken with the knowledge of hemoglobin values and its distribution in different trimesters of pregnancy.

Material and Methods: Present study was single-center, prospective, comparative, parallel-group, observational study, conducted in pregnant women attended the ANC OPD or admitted in antenatal ward and sixty healthy non-pregnant women of same age group.

Results: The mean hemoglobin value was $12.23 \pm 1.32gm$ % throughout pregnancy. In control group the mean value was $12.59 \pm 2.50gm$%. Statistically significant variation was observed between control and each trimester of pregnancy ($p < 0.05$). Significant difference observed between 1st and 2nd trimester ($p < 0.05$) and 2nd and 3rd trimester ($p < 0.05$). When compared between 1st and 3rd trimester, difference was not significant ($p > 0.05$) Hemoglobin values are lower in pregnant cases as compared to non-pregnant subjects ($p < 0.05$). Lowest hemoglobin value is observed in 2nd trimester in maximum number of cases. Mean hemoglobin level was found lower in pregnant women with gravidity more than 1 as compared to primigravidae.

Conclusion: A significant association was observed between hemoglobin level and trimester of pregnancy. High occurrence of low hemoglobin level in pregnant women was found belonging to lower socioeconomic class. Maternal hemoglobin value decreases with increase in gestational age.

Keywords: Hemoglobin level; Pregnancy; Trimester of pregnancy; Anemia.

1. Introduction

It has been known to mankind from ancient times that blood is the essence of life. Blood is a special type of connective tissue [1] which plays a vital role in our body as it helps in maintaining a series of physiological needs, the most important being the exchange of respiratory gases: oxygen and carbon dioxide.

The oxygen carrying capacity of blood is due to the presence of hemoglobin, the iron containing metalloprotein [2] inside the red blood cells or erythrocytes. Oxygen carried by hemoglobin is one of the vital mechanisms by which tissue survives. The level of hemoglobin in blood is dependent on nutrients, especially Iron, Folic Acid and Amino Acids [3] which are required for hemoglobin synthesis. The importance of hemoglobin lies in the fact that deficiency of hemoglobin leads to a condition known as anaemia, which can be caused by either too few red blood cells or too little hemoglobin in the cells [4], i.e., qualitative and quantitative reduction of hemoglobin and red blood cells in the circulation in relation to age and sex of the individual.

Pregnancy increases the demand for nutrients, hemoglobin. Besides these, physiological changes in blood parameters also occurs during pregnancy. Factors leading to anemia in obstetric cases are multiparity and blood loss in antepartum, intrapartum and postpartum period. Lactation, malnutrition and malabsorption are the additional factors. Anemia in pregnancy is a serious condition contributing to increased maternal and fetal morbidity and mortality [5].

The hemoglobin level in different trimesters have not been studied extensively in this part of the country. So the present study has been undertaken with a view that knowledge of hemoglobin values and its distribution in different trimesters of pregnancy in the study population will prove useful towards management of pregnant mother.

2. Material and methods

Present study was single-center, prospective, comparative, parallel-group, observational study, conducted in department of Physiology, Assam Medical College and Hospital, Dibrugarh, India. Study duration was of 1 year (September 2009 to August 2010). Study approval was obtained from institutional ethical committee.

Inclusion criteria

- Patients attended the Outpatient Department of Obstetrics and Gynaecology or admitted in antenatal ward (for routine antenatal check up) without having other ailment, Willing to participate in present study.

Exclusion Criteria

- Those subjects with medical illness like Diabetes, renal diseases, heart or lung disease.
- Cases with prevailing illness or disease which is documented.

Study was explained to patients in local language & written consent was taken for participation & study. One hundred eighty (180) pregnant women, sixty (60) from each trimester of pregnancy belonging to the age group of 20-40 years and sixty (60) healthy non-pregnant women of same age group were taken as control. Details of cases & controls such as socio-demographic profile, obstetric history, antenatal care history, clinical history (complaints, past medical/surgical history), examination findings, laboratory reports were noted in case record proforma.

Hemoglobin Estimation was done by Cyanmethaemoglobin Method, 2 ml of venous blood is collected using EDTA as anticoagulant, 20 microliter of blood is then added to 5 ml of cyanide Ferricyanide solution. It is mixed thoroughly inverting the tube several times. After that the tube is allowed to stand at room temperature for 10 minutes for completion of the reaction then optical density of the solution is measured in a photoelectric colorimeter using a 540 nm filter and taking a reagent solution as black. The value of hemoglobin in gram percent is obtained with the help of standard table and curve prepared from Hemoglobin standard obtained from a reputed commercial firm.

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi- square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

3. Results

The study was carried out in 240 subjects out of which 60 being non-pregnant control and the remaining 180 in three different trimesters of pregnant (60 in each trimester). The mean hemoglobin value was 12.23 $\pm$ 1.32 gm % throughout pregnancy.

Table 1. Comparison of hemoglobin in different trimesters of pregnancy

	Trimesters					
	1st	2nd	2nd	3rd	3rd	1st
Range	8.4-16.2	9.4-13.5	9.4-13.5	11.2-13.3	11.2-13.3	8.4-16.2
Mean $\pm$ SD Hemoglobin (gm%)	12.58 $\pm$ 2.01	11.91 $\pm$ 0.83	11.91 $\pm$ 0.83	12.20 $\pm$ 0.55	12.20 $\pm$ 0.55	12.58 $\pm$ 2.01
p Value	<0.05		<0.05		>0.05	

In the 1st trimester of pregnancy, the range was from 8.4-16.2gm%. The mean value was 12.58 $\pm$2.01 gm%. In the 2nd trimester of pregnancy the range was from 9.4-13.5gm%. The mean value was 11.91 $\pm$0.83

gm %. In 3rd trimester of pregnancy the range was from 11.2-13.3 gm%. The mean value was 12.20 ±0.55 gm%. Significant difference observed between 1st and 2nd trimester ($p < 0.05$) and 2nd and 3rd trimester ($p < 0.05$). When compared between 1st and 3rd trimester, difference was not significant ($p >0.05$), see Table 1.

Table 2. Comparison of mean hemoglobin of pregnant and control

	Pregnant women (n =180)	Control (n = 60)	p value
Mean Hemoglobin ± SD (gm%)	12.23 ± 1.32	12.59 ± 2.50	<0.05

From Table 2, it can be observed that the mean hemoglobin value was 12.23 ± 1.32 gm % throughout pregnancy. In control group the mean value was 12.59 ± 2.50 gm%. Statistically significant variation was observed between control and each trimester of pregnancy ($p <0.05$).

Table 3. Comparison of hemoglobin in each trimesters with controls

	Trimesters					
	1st	Control	2nd	Control	3rd	Control
Range	8.4-16.2	7.2-16.8	9.4-13.5	7.2-16.8	11.2-13.3	7.2-16.8
Mean ± SD Hemoglobin (gm%)	12.58 ± 2.01	12.59 ± 2.50	11.91 ± 0.83	12.59 ± 2.50	12.20 ± 0.55	12.59 ± 2.50
p Value	<0.05		<0.05		<0.05	

Table 3 shows that the Hemoglobin values are lower in pregnant cases as compared to non-pregnant subjects ($p <0.05$). Lowest hemoglobin value is observed in 2nd trimester in maximum number of cases.

Table 4. Age wise distribution and hemoglobin in cases and controls

Age group (in years)	1st Trimester		2nd Trimester		3rd Trimester		Controls	
	No.	Mean ± SD Hb (gm%)	No.	Mean ± SD Hb (gm%)	No.	Mean ± SD Hb (gm%)	No.	Mean ± SD Hb (gm%)
20-25	8	12.58 ± 2	8	11.44 ± 0.83	12	12.09 ± 0.60	10	12.36 ± 2.69
26-30	25	12.84 ± 2.11	25	11.87 ± 0.76	23	12.32 ± 0.55	24	12.85 ± 2.70
31-35	21	12.38 ± 2.09	20	12.21 ± 0.76	19	12.15 ± 0.53	18	12.56 ± 2.64
36-40	6	11.17 ± 1.68	7	11.7 ± 1.04	6	11.17 ± 1.68	8	12.20 ± 1.43

It was observed from Table 4 that maximum number of cases belong to 26 to 30 years of age range. Out of 180 pregnant women 25 in the 1st and 2nd trimester each and 23 in the 3rd trimester of pregnancy. Mean hemoglobin values was 12.84 gm%, 11.87 gm% and 12.32 gm% in 1st, 2nd and 3rd trimester respectively in the same age range, i.e., 26-30 years. Age wise distribution was found to be statistically not significant.

Table 5. Gravidity wise distribution of hemoglobin level in different trimesters of pregnancy

Gravida	First Trimester		Second Trimester		Third Trimester		Total (%)
	No. of cases (%)	Mean ± SD Hb (gm%)	No. of cases (%)	Mean ± SD Hb (gm%)	No. of cases (%)	Mean ± SD Hb (gm%)	
1	25 (41.67 %)	13.81± 1.85	26 (43.33 %)	12.24± 0.72	24 (40 %)	12.73± 0.22	75 (41.67 %)
2	9 (15 %)	12.42 ± 2.09	14 (23.33 %)	11.49± 0.86	9 (15 %)	12.27± 0.05	32 (17.78 %)
3	11 (18.33 %)	11.95 ± 1.50	8 (13.33 %)	11.61± 1.13	12 (20 %)	11.92± 0.26	31 (17.22 %)
4	13 (21.67 %)	11.03 ± 1.21	10 (16.67 %)	11.78± 0.47	12 (20 %)	11.85± 0.20	35 (19.44 %)

Table 5 shows that the maximum number of cases were primigravidae comprising 41.67% of total cases. Mean hemoglobin value of 13.81 gm% and SD was 1.85, 12.24 gm% and SD 0.72 and 12.73 gm% and SD 0.22 in the 1st, 2nd and 3rd trimester respectively. It was observed that there was progressive decrease in mean hemoglobin level in pregnant women with increasing number of gravidity. Mean hemoglobin level was found lower in pregnant women with gravidity more than 1 as compared to primigravidae.

Table 6. Distribution of hemoglobin in different socio-economic class in pregnancy

Socioeconomic Class	No of cases	Mean Hbgm (%)	SD(±)
Upper	0	-	-
Upper Middle	10	13.04	1.52
Lower Middle	76	12.38	1.40
Upper Lower	59	12.11	1.34
Lower	35	11.87	1.26

It was observed from Table 6 that maximum number of cases (76 out of 180) belongs to lower middle strata of socioeconomic class as per Kuppuswamy's classification. There was not a single case from high socio-economic strata. In lower socioeconomic group mean hemoglobin level was found lower as compared to higher economic group.

Table 7. Distribution of hemoglobin according to dietary habit

	CASES Mean ± SD Hb (gm%)	CONTROL Mean ± SD Hb (gm%)	P Value
Vegetarian	12.30 ± 1.22	12.53 ± 2.59	>0.05
Non vegetarian	12.21 ± 1.63	12.89 ± 2.16	

It is observed from Table 7 that mean hemoglobin value in vegetarian was 12.30 gm%, SD 1.22 and 12.53 gm% and SD 2.59 in cases and control respectively. For non-vegetarian the values was 12.21 gm% and SD 1.63 and 12.89 gm%, SD 2.16. This variation in mean hemoglobin value in non-vegetarian is statistically not significant ($p > 0.05$).

4. Discussion

Anaemia in pregnancy is an important preventable cause of maternal and perinatal morbidity and mortality. Pathological anaemia of pregnancy is mainly due to nutritional deficiency and among them iron deficiency anaemia is most common.

There are marked physiological changes in the composition of the blood in healthy pregnancy, mainly to combat the risk of hemorrhage at delivery. Plasma volume and red cell mass increases by 50% and 18.25% respectively, resulting in dilutional decrease in hemoglobin concentration called the physiological anaemia of pregnancy maximum at 32 weeks of gestation. WHO has recommended a cut off value of 11 gm/dl in 1st and 3rd trimester and 10.5 in 2nd trimester to define anaemia during pregnancy.

In India incidence of anaemia [6] during pregnancy ranges from 40-90%. In India, out of pregnant population of 22 million women [7] it affects 13 million pregnant women. About 0.5 million women die annually in India7 as a result of pregnancy and its complications. Anemia is the leading contribution to this high maternal mortality rate. Maternal consequences of anemia include cardiovascular symptoms, reduced physical and mental performance, reduced immune function and increased risk for blood transfusion in the postpartum period.

A detailed compilation of prevalence of anemia in women published by Brabin [8] estimated that maternal mortality ranges is 27 per 100,000 live births in India. Fetal consequences of iron deficiency anemia are an increased risk of growth retardation, premature birth, intra uterine death, amnion rupture and infection.

Women often become anemic during pregnancy because the demands for iron and other vitamins is increased. The mother must increase her production of red cells and in addition the fetus and placenta need their own supply of iron, which can only be obtained from the mother. In order to have enough red cells for the fetus, the body starts to produce more red cells and plasma.

In normal pregnancy there is a gradual and progressive fall in hemoglobin level up till the 32nd to 36th week [9] after which the level shows a gradual rise up till term. This initial fall in hemoglobin concentration has been attributed to the greater increase in plasma volume compared to increase in red cell volume. This phenomenon is known as physiological anemia of pregnancy.

Anemia may be pathological in some conditions when there is inability to meet the extra nutritional demands for hemoglobin synthesis during pregnancy. Some workers consider the fall in hemoglobin as a part

of physiological anemia to be abnormal and have shown that some, if not all of this fall can be prevented by providing hematinic supplements. Deleterious effects occurs in mother as well as baby as a result of anaemia, which is multifactorial in our community.

In the present study, the mean hemoglobin concentration was 12.23 $\pm$ 1.32 gm% throughout gestation. The results show that hemoglobin level falls progressively as the period of gestation progresses uptil 2nd trimester followed by slight rise up to term but still lower than 1st trimester. It was also seen that maximum number of cases had their lowest hemoglobin level in 2nd trimester of pregnancy. This is probably due to increasing requirements of iron and other nutritional factors for hemoglobin synthesis as the pregnancy progresses coupled with the fact that iron stores are exhausted in most women in the 2nd trimester. Moreover, maximum physiological hemodilution occurs in the 2nd trimester further exaggerating the condition.

It was observed that this trend of mean hemoglobin values of the present study was comparable with figures obtained by a number of researchers, see for example [10–13] in which the authors found a progressive fall in mean hemoglobin level from 1st trimester through the 3rdtrimester, but mean hemoglobin values were lower in the present study in comparison to study by other researchers.

Another study done by Iyenger *et al.,* [6] obtained similar finding but mean hemoglobin values were comparatively higher than our study. This difference in hemoglobin values from the present study is probably due to varying no of cases, higher socio-economic and better nutritional status, higher level of education, health care delivery system of the place, and difference in race and ethnicity of the study population.

Another finding of the present study was that hemoglobin values of pregnant women were lower when compared to that of non-pregnant counterpart who had mean hemoglobin value of 12.59 gm%. This finding is correlated with study done by Knight *et al.,* [11] who observed 79-82% variation of hemoglobin value from the non-pregnant status during 3 trimesters of pregnancy. This data is also consistent with findings of study done by Pulokka *et al.,* [14] who observed that mean hemoglobin value is lower in pregnant women even if iron supplements are given during pregnancy. The possible explanation could be higher nutritional need for increased metabolic demand of pregnancy period as compared to non-pregnant group.

In the present study, it was observed that there was progressive decrease in mean hemoglobin level in pregnant women with increasing number of gravidity. On analysis of hemoglobin distribution according to parity it was observed that high parity rate is inversely related to hemoglobin level in pregnant women. This finding of the present study is consistent with the findings of previous studies done by a number of authors, see [10,12,15]. This finding is due to exhaustion of nutritional factors which depletes progressively with childbearing and may be due to previous illness and complications related to previous pregnancies.

In the present study, mean hemoglobin values were higher in vegetarian but statistically not significant. Conflicting results are observed in other studies which is probably due to the fact that in the present study vegetarian group belonged to higher socio-economic class as compared to non-vegetarian. In this part of the country proper study that described the pattern of hemoglobin concentration among pregnant women has not been done extensively.

The findings of the present study was parallel with the established relation between hemoglobin and trimester of pregnancy. Limitation of the study was that prevalence of Anaemia was not assessed in pregnant group and the their values were obtained in a wide range. As per WHO guidelines hemoglobin level of $<$ 11 gm% during 1st and 3rd trimester and $<$ 10.5 gm% in 2nd trimester of pregnancy is considered as anaemia. In the present study, a few cases had hemoglobin level in the range of anaemia as per WHO criteria. But to confirm anaemia further investigations were not performed among the study group. In this part of the country proper study that described the pattern of hemoglobin concentration among pregnant women has not been studied extensively.

Socioeconomic and demographic characteristics of a pregnant women have potential influence on hemoglobin distribution and other hematological changes during pregnancy. High frequency of low hemoglobin level in this study group reflects inadequate access to antenatal care, low socioeconomic background and nutritional deficiency and other socio-cultural behaviour of the women.

A knowledge of these hematological values and trends in the study population group would prove useful towards management of pregnant mothers and planning of maternal health services. A comprehensive community based intervention with iron supplementation, helminth treatment and increase in knowledge regarding information, education and communication through effective strategies, to improve the hematological status of pregnant women in each trimester, is needed.

5. Conclusion

A significant association was observed between hemoglobin level and trimester of pregnancy. High occurrence of low hemoglobin level in pregnant women was found belonging to lower socioeconomic class. Maternal hemoglobin value decreases with increase in gestational age. Distribution pattern of hemoglobin in pregnant women in this part of the country which is lower than that of non-pregnant counterparts correlates with the finding of studies in other parts of India.

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