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Original Article:

A Cross-Sectional Study of Hemoglobin Disorders in Pregnant Women Attending Two Urban Hospitals in Eastern Coast of Odisha, India.

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Abstract: Pregnant women are an important segment of the society. They bear the children and provide nourishment to them during the period of gestation of nine months. The health of a mother reflects the health of a child. No such study of prevalence of hemoglobinopathy in pregnant women from India is available. The study objectives were: to find the prevalence (genetic burden) of hemoglobin disorders in pregnant women belonging to urban setting; identify the communities at risk, and to determine the hematological profile of native pregnant women of coastal Odisha. A cross-sectional study of pregnant women visiting for antenatal care at two urban hospitals, Bhubaneswar and Berhampur in Coastal Odisha was investigated. A total of 178 pregnant women attending antenatal care check up at two urban hospitals in coastal Odisha were studied. Appropriate statistical tools were used for analysis of data. High prevalence of 13.5% for hemoglobin disorders was observed in urban pregnant women visiting two major hospitals in coastal Odisha. Mild to moderate anemia was recorded. Reduced values of hematological indices in women afflicted with hemoglobin disorders than the normal controls were noted. Major hemoglobinopathies detected were: β-thalassemia trait (5.6%), sickle cell trait (5.6%), hemoglobin E trait (1.1%), sickle cell-E-disease (0.6%), and hemoglobin H disease (0.6%). Mandatory awareness, comprehensive clinical management, and genetic/marriage counseling are highly essential to ameliorate the sufferings of afflicted pregnant women of coastal Odisha.

Key Words: Pregnant women; Hemoglobin disorders; Hemolytic anemia; Community health; Urban population; Coastal Odisha.

Introduction:

A healthy woman is an anchor of a happy and healthy family life and is an asset to the nation. The fundamental issues of public health concerning women and their health care are: sanitation, nutrition, pregnancy, childbirth, prenatal and postnatal care. Women face high risk of malnutrition, development and growth retardation, affliction of diseases, disabilities and even death at three critical stages of life – infancy, early childhood and reproductive phase. The high rates of maternal, neonatal, infant and child death are associated with early marriage in India. Women are basic health care providers yet they have limited access to health care. In every five minutes a woman somewhere in India dies as a result of complications attributed to pregnancy or delivery of childbirth.

Several epidemiological studies in public health have shown that both low and high hemoglobin (Hb) concentrations are associated with increased adverse birth outcomes, including fetal death, intrauterine growth retardation, preterm delivery, and low birth weight.[1-5] The key argument supporting anemia as an outcome measure is related to the fact that red blood cells contain hemoglobin, which is an essential component of the respiratory system for oxygen transport. Any substantial reduction in hemoglobin and red blood cells reflects a reduced capacity of oxygen transport to tissue. Such a reduction in oxygen transport can be regarded as an adverse health outcome; thus, iron deficiency has a definite effect on health because of anemia. High hemoglobin concentration in some women is attributed to pregnancy related complications.[5]

In looking at pregnancy-related or reproductive outcomes, anemia is an undesirable health outcome and a predictor or cause of other adverse outcomes, i e, maternal and fetal mortality, preterm birth, and low birth weight. Some studies found an association between anemia and adverse pregnancy outcomes[6,7] whereas other studies did not show a significant association.[8] Maternal nutritional state is an important predictor of perinatal results. This concept has gained more importance in the recent years as there is now growing acceptance of the 'fetal origin of adult disease' hypothesis. Understanding the effect of maternal hematological parameters on obstetric outcome has public health importance because these indicators are associated

with infant health and survival and influence intra-uterine life development and health in later life.

The prevalence of various hemoglobinopathies, i.e. sickle-cell disease, β-thalassemia syndrome, hemoglobin E or D disease, or hereditary persistence of fetal hemoglobin (HPFH), etc. varies from location/region to location/region and from community to community in India depending upon mating practices.[9-11] Marriage within the same clan (gotra) or title or blood relatives leads to increased probability of consanguinity and increased likelihood of having affected progeny. In India the symptoms of hemoglobinopathy start in some cases as early as 3 months of age, whereas in other cases remain asymptomatic even in the late thirties. No study pertaining to prevalence of hemoglobin disorders in pregnant women is available from anywhere in India. Keeping this state of affairs, this study was designed to know the prevalence (genetic burden) of hemoglobin disorders in urban pregnant women, identifying the communities at risk, and to determine the health and hematological profile of the native women in Odisha, India.

Materials and Methods:

This study was carried out at the Division of Human Genetics, Regional Medical Research Centre (Indian Council of Medical Research), Bhubaneswar, Odisha.

For this study, 2-3 ml intravenous blood samples were consecutively collected using disposable syringes and needles in disodium salt of Ethylene Diamine Tetraacetic Acid (EDTA) coated vials after taking informed consent from each pregnant woman visiting for antenatal check up at the Out Patient Department (OPD), Department of Obstetrics and Gynaecology, Capital Hospital, Bhubaneswar (116 samples) and from the Out Patient Department (OPD), Department of Obstetrics and Gynaecology, M.K.C.G Medical College & Hospital, Berhampur (62 samples) in coastal Odisha. Blood samples so collected were transported under wet ice-cold conditions to laboratory at Bhubaneswar within 24 h of collection for laboratory investigations and analysis following the standardized laboratory procedures and techniques. Hematological parameters were studied using an automated Blood Cell Counter (Model MS4, Melet Schloesing Laboratories, Cergy-Pontoise Cedex, France).

The sickling test was performed, using freshly prepared sodium metabisulphite solution using as a reducing agent for the presence or absence of sickle cell hemoglobin.[12] Routine hemoglobin lysate electrophoresis was carried out on cellulose acetate membrane (CAM) in Tris-EDTA-borate buffer at pH 8.9 and quantification of hemoglobin A2 fraction was done by elution method.[13] The value of more than 3.5% of hemoglobin A2 fraction of adult hemoglobin was taken as the cut off point for determining the β-thalassemia trait and more than 10% for hemoglobin E. Estimation of fetal hemoglobin was carried out as described by Weatherall.[13] For the confirmation of Hb-H disease, blood film was prepared with brilliant cresyl blue stain to examine the large Heinz body like inclusions.[13]

The diagnosis of sickle cell/β-thalassemia was based on findings of hemoglobin A, F, S and A2 on electrophoresis under acidic and alkaline media, elevated HbA2 levels (>3.5%), and family study. In view of the inverse relationship between HbA2 and Hb F levels, high levels of Hb F were very common in Indian sickle cell patients.[14] All the blood samples were further subjected to confirmation by hemoglobin variant analysis (made for Bio-Rad Diagnostics Group, Hercules, California, USA).

Each subject was requested to provide the background information such as name, age, residential address, reproductive history (abortion, miscarriage, stillbirth, etc) if any, month of gestation, history of hospitalization if any, blood transfusion or pregnancy related complications if any, etc. Out of a total of 178 pregnant women studied, one hundred fifty four (154) women who were free from any kind of hemoglobinopathies based on laboratory test findings were labeled as normal controls. Of the 24 pregnant women identified with hemoglobin disorders, 10 each were carriers of sickle cell disease and β-thalassemia syndrome, 2 carriers of hemoglobin E disease, and one each were sickle cell-E disease and hemoglobin H disease constituted the diseased group.

Results obtained were statistically tested by performing student t test to compare the normal with each diagnostic category for the difference between the two independent variables and significance, if any, also indicated.

Results:

Out of 178 pregnant women screened for hemoglobino-pathies, 24 showed hemoglobin abnormalities (Table 1). Thus, the prevalence of hemoglobin disorders in this cross-section of pregnant women attending the urban hospitals accounts for 13.5%, which is alarmingly high in the coastal state of Odisha. The women with β-thalassemia trait and sickle cell trait constituted 5.6% each of the pregnant women, 1.1 % hemoglobin E trait and 0.6% each sickle cell-E-disease and Hb H-disease consisted of the whole lot of pregnant women studied from urban hospitals of coastal Odisha.

Table 1: Prevalence of different hemoglobin disorders in pregnant women of coastal Odisha.

Diagnosis	No.	%
Normal (Hb AA)	154	86.5
β-thalassemia Trait	10	5.6
Sickle Cell Trait	10	5.6
Hemoglobin E Trait	2	1.1
Sickle Cell-Hemoglobin E Disease	1	0.6
Hemoglobin H Disease	1	0.6
Total	178	100.0

The pregnant women included in the study were in-between the age of 16 through 32 years (Table 2). However, the majority of them fell in the age of 23 (5), 26 (3), 31 (2), followed by 19, 20 and 22 years (2 each). Out of the 24 pregnant women who had hemoglobin abnormalities, 10 each were afflicted with β-thalassemia trait and sickle cell trait. Two had hemoglobin E trait.

Table 2: Age-wise distribution of 24 pregnant women with hemoglobin disorders in Odisha.

Age in years	β-thalassemia Trait	Hb AS	Hb AE	Hb SE	Hb H Disease
16		1			
17		1			
18		1			
19	1	1			
20	1	1			
21					
22	1	1			
23	2		2	1	
24	1				
25	1				
26	1	1			1
27		1			
28					
29		1			
30					
31	2				
32		1			
Total	10	10	2	1	1

Hemoglobinopathy afflicted 24 pregnant women were further categorized into the major caste groups (Table 3), i.e. general caste, other backward caste, scheduled caste, and scheduled tribe. The majority of them belonged to other backward caste (14), followed by general caste (5), scheduled caste (4) and scheduled tribe (1). Most vulnerable communities for hemoglobin disorders were: Khandayat (11), Karan (3), and so on in the decreasing order (Table 3). This distribution showed that hemoglobin disorders were not confined only to scheduled caste/tribe population but the defective gene had penetrated vigorously into the other backward and general castes in the state of Odisha.

Table 3: Caste/Tribe-wise distribution of 24 pregnant women with hemoglobin disorders

Communities	Caste/Tribe	β-thal. Trait	Hb AS	Hb AE	Hb SE	Hb H Disease
General Caste	Brahmin	1				
	Karan	1	1	1		
	Reddy	1				
Other Backward Caste	Khandayat	4	4	1	1	1
	Dumal		1			
	Teli		1			
	Barber		1			
Scheduled Caste	Gonda	1				
	Mala		1			
	Pana		1			
	Fisherman	1				
Scheduled Tribe	Haddi	1				

Table 4 shows the district-wise distribution of 24 defective hemoglobin afflicted pregnant women in coastal Odisha. A majority of the women with hemoglobin disorders came from Khurda (7), followed by Ganjam (5), Anugul (3), Boudh (2), Cuttack (2) district and so on.

Table 4: District-wise distribution of 24 pregnant women with hemoglobin disorders in Odisha.

District	β-thal.Trait	Hb AS	Hb AE	Hb SE	Hb H Disease
Anugul	1	2			
Boudh	1	1			
Cuttack	2				
Ganjam		5			
Kandhamal		1			
Khurda	3	1	2	1	
Mayurbhanj					1
Nayagarh	1				
Puri	1				
Shrikakulum (A.P.)	1				
Total	10	10	2	1	1

Of the 24 pregnant women afflicted with hemoglobinopathies were classified into different grades of anemia following the WHO Report[15] classification according to the level of hemoglobin (Table 5). A majority of the women had mild anemia, followed by moderate and severe anemia. A one-third of the afflicted pregnant women had normal hemoglobin level.

Table 5: Different grades of anemia in 24 pregnant women of Odisha

Grades of Anemia	β-thal.Trait N=10	Hb AS N=10	Hb AE N=2	Hb SE N=1	Hb H Disease N=1
Severe Anemia (< 7g/dl)	1	1	0	0	0
Moderate Anemia (7-10g/dl)	4	4	0	0	0
Mild Anemia (10.1-11g/dl)	2	2	2	0	0
Normal (>11.1g/dl)	3	3	0	1	1
Total	10	10	2	1	1

Table 6 illustrates the mean value of hematological indices of normal controls and the subjects with different diagnostic categories and the variations in different indices along with statistical significant level in 178 studied pregnant women.

Table 6: Mean value of hematological indices of different diagnostic categories in 178 pregnant women of Odisha

Parameters/ Hematological Indices	Hb AA (Normal) N=154 Mean±SD	β-thal.Trait N=10 Mean±SD	Hb AS N=10 Mean±SD	Hb AE N=2 Mean±SD	Hb SE N=1 Mean	Hb H Disease N=1 Mean
Age in years	23.7± 4.4	24.4±4.1	22.6±5.5	23.0±0.0a	23.0	26.0
Hb (g/dl)	9.9±2.2	10.1±2.0	9.5±2.2	10.8±0.3b	12.6	11.2
RBC (10^6/ml)	4.0±1.0	4.0±1.0	4.0±0.8	5.3±0.6a	2.4	5.9
MCV (fl)	80.7±10.5	74.2±9.2+	74.1±5.0**	82.2±0.9	61.9	66.1
HCT (%)	32.0 ± 8.6	29.8±8.5++	29.5±6.5	34.8±0.4c	14.7	38.9
MCH (pg)	26.8± 4.3	27.3±6.4	23.9±2.9*	25.5±0.1c	31.0	21.8
MCHC (g/dl)	32.5±3.0	33.3±2.2	32.7±3.3	30.8±0.1c	19.3	21.8
RDW (%)	9.5± 2.0	9.6±1.1	9.7±2.2	8.6±0.4a	13.4	12.3
WBC (10^3/ml)	8.1±3.6	8.4±2.7	6.4±3.4	9.1±1.6	12.9	10.5
Hb A2/E (%)	2.3± 0.7	4.1±0.3+++	1.9±0.3**	27.0±4.0c	26.2	2.7
Hb F (%)	0.8 ± 0.2	1.1±0.5	0.9±0.5	0.4±0.0	5.8	10.5
Hb S (%)	0.0± 0.0	0.0±0.0	25.3±6.2	0.0±0.0	68.0	0.0
Hb A (%)	96.6± 0.8	94.7±0.6+++	72.7±6.6**	72.2±3.3c	0.0	65.6

Hb AA vs Hb AE; a= p<0.05; b=p<0.02; c=p<0.001
Hb AA vs Hb AS; *= p<0.01; **=p<0.001
Hb AA vs β-thal. Trait; += p<0.05; ++=p<0.01; +++=p<0.001

Discussion:

The maternal nutritional parameters are associated not only with infant health and survival but may also influence development and health in later life. Understanding the effect of maternal adiposity on obstetric outcome has public health importance. Moreover, these are modifiable risk factors implicating that a large amount of morbidity (fetal and maternal) can be reduced by taking timely measures. Good health of an adolescent girl not only leads to better maternal health but also an indicator of giving birth to a healthy child as the fetus takes its all nutrients from the mother. On the other hand, an unhealthy mother will give birth to an abnormal child. In pregnant women, nutritional deficiencies can be overcome by taking balanced diet or additional dietary supplementations. But inherited (genetic) hemolytic disorders are life threatening and cannot be cured. This study has undertaken the latter most important public health aspect for investigations.

Healthy people can create a healthy society and the better future for mankind. Pregnant women constitute an important segment of the society. Their health and diseased conditions also determine the future of the progeny. Investigations pertaining to genetic burden of hemoglobin disorders in pregnant women of coastal Odisha, studied consecutively from two urban hospitals at Bhubaneswar and Berhampur, have revealed the prevalence of 13.5%, which is alarmingly high and needs intervention and prevention measures at grass root level in the vulnerable population (Table 1). Most common hemoglobin disorders detected in pregnant women

constituted β-thalassemia trait (5.6%), sickle cell trait (5.6%), hemoglobin E trait (1.1%), sickle cell-E-disease (0.6%), and hemoglobin H disease (0.6%). Most vulnerable communities identified were: Khandayat, Karan, Brahmin, Dumal, Teli, Pana, Haddi, etc. (Table 3) that hailed from Khurda, Ganjam, Anugul, Cuttack districts and so on (Table 4). Since a wide range of geographical scatter of abnormal genes was observed in the population, it is suggested to undertake a comprehensive clinical management, genetic counseling, educational and awareness campaigns to ameliorate the sufferings of affected pregnant women of coastal Odisha.

Anemia is the most common nutritional deficiency disorder in the world. The prevalence of anemia in pregnant women in developed and developing countries was 14% and 51%, respectively and 65-75% in India as per World Health Organization Report.[16] India contributed about 80% of the maternal deaths due to anemia in South Asia.[17] About 95% of anemia cases during pregnancy are due to iron deficiency. The cause is usually inadequate dietary intake (especially in adolescent girls), a previous pregnancy, or the normal recurrent loss of iron in menstrual blood (which approximates the amount normally ingested each month and thus prevents iron stores from building up). Early symptoms are usually nonexistent or nonspecific (eg, fatigue, weakness, light-headedness (fainting), mild dyspnea with exertion). Other signs and symptoms may include pallor and, if anemia is severe, tachycardia or hypotension. Anemia increases risk of preterm delivery and postpartum maternal infections. Treatment is directed at reversing the anemia. Transfusion is usually indicated for severe anemia or if the constitutional symptoms are present. In the present study, mild to moderate anemia was recorded in pregnant women afflicted with hemoglobin disorders (Table 5).

Preventing or treating anemia, whether moderate or severe, is desirable. Because iron deficiency is a common cause of maternal anemia, iron supplementation is a common practice to reduce the incidence of maternal anemia. Nevertheless, the effectiveness of large-scale supplementation programs needs to be improved operationally and, where multiple micronutrient deficiencies are common, supplementation beyond iron and folate can be considered.[6,7,18] The pathophysiologic mechanism of producing very high hemoglobin concentrations during pregnancy causes high blood viscosity, which results in both compromised oxygen delivery to tissues and cerebrovascular complications; and can produce such a high hemoglobin concentration because of reduced normal plasma expansion and cause fetal stress because of reduced placental-fetal perfusion. Thus, the high concentration of hemoglobin in some pregnant women is due to pregnancy related complications.[5]

Iron and folate supplementation during pregnancy is commonly practiced to prevent maternal anemia, which is often caused by iron deficiency. Part of the rationale for this practice is the high iron requirement during pregnancy, almost 3 times that required for nonpregnant women of childbearing years, which is difficult to meet from dietary sources.[7] Another reason for supplementation is that anemia caused by iron deficiency alone or in combination with other factors, eg, folate deficiency, vitamin A deficiency, and malaria, has been implicated as having several negative effects on maternal and fetal health. Therefore, anemia prevention through iron supplementation may help to improve reproductive outcomes. In the present study, low hemoglobin level in the normal pregnant women may be attributed to the above listed nutritional deficiency related factors. On the other hand, reduced hematological indices may be accounted for the hemolytic genetic defects of hemoglobin in the specified diagnostic categories of pregnant women (Table 6).

To conclude, several epidemiologic studies have shown that both low and high hemoglobin concentrations are associated with increased adverse birth outcomes, including fetal death, intrauterine growth retardation, preterm delivery, and low birth weight.[1-5] The most plausible explanation for the observed association between a high hemoglobin concentration and perinatal morbidity and mortality is that both conditions are often the result of hypertensive disorders of pregnancy or preeclampsia. The principal mechanism for perinatal morbidity and mortality due to preeclampsia is poor placental and fetal perfusion. The mechanism for the observed higher hemoglobin concentration is the failure of normal plasma expansion, hypovolemia, or hemoconcentration. Hypertension, hypovolemia, and poor placental perfusion is the part of physiologic disturbances of preeclampsia.[19] The known mechanism can explain the observed association, attributing the increased perinatal complications to the increased hemoglobin concentration in women with pregnancy. Existing evidence does not support the hypothesis that high hemoglobin concentrations during pregnancy result in poor pregnancy outcomes.[5]

Antenatal care is highly essential, especially with dietary supplementation[20] if needed, whereas, in case of affliction with hereditary disorders, the maintenance of an optimal status of pregnant women is desirable to avoid the adverse reproductive outcome in India.

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References:

1. Garn SM, Ridella SA, Petzold AS, Falkner F. Maternal hematological levels and pregnancy outcomes. *Semin Perinatol* 1981;5:155–162.
2. Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. Relations of hemoglobin levels in first and second trimesters to outcome of pregnancy. *Lancet*. 1986;1:992–995.
3. Lu ZM, Goldenburg RL, Cliver SP, Cutter G, Blankson ML. The relationship between maternal hematocrit and pregnancy outcome. *Obstet Gynecol* 1991;71:190–194.
4. Steer P, Alam MA, Wadsworth J, Welch A. Relation between maternal hemoglobin concentration and birth weight in different ethnic groups. *BMJ* 1995;310:489–491.
5. Ray Yip. Significance of an abnormally low or high hemoglobin concentration during pregnancy: special consideration of iron nutrition. *Am J Clin Nutr* 2000;72 (suppl):272-279.
6. Sharma A, Patnaik R, Garg S, Ramachandran P. Detection and management of anemia in pregnancy in an urban primary health care institution. *Indian J Med Res* 2008;128:45-51.
7. Susheela AK, Mondal NK, Gupta Rashmi, Ganesh Kamla, Brahmankar Shashikant, Bhasin Shammi, Gupta G. Effective intervention approach to control anemia in pregnant women. *Curr Sci* 2010;98:1320-1330.
8. Rush D. Nutrition and maternal mortality in the developing world. *Am J Clin Nutr* 2000;72(suppl):212–240.
9. Balgir RS. Spectrum of hemoglobinopathies in the state of Orissa, India: A ten years cohort study. *J Assoc Physicians India*. 2005;53:1021–1026.

10. Balgir RS. The spectrum of hemoglobin variants in two scheduled tribes of Sundargarh district in Northwestern Orissa. India. *Ann Hum Biol.* 2005;32:560–573.
11. Balgir RS. Scenario of hemoglobin variants in Central-East coast of India. *Curr Sci* 2006;90:1651–1657.
12. Dacie JV, Lewis SM. 1991. Practical hematology, 7th Edition. Edinburgh: Churchill Livingstone. 1991. pp 227–257.
13. Weatherall DJ. 1983. Hematologic methods. In: Weatherall DJ, editor. Vol. 6. Methods in hematology: The thalassemias. New York: Churchill Livingstone. 1983.
14. Balgir RS. The clinical and hematological profile of sickle cell disease cases in India. *Indian Practr.* 1995;48:423–432.
15. WHO Report. Preventing and controlling iron deficiency anemia through primary health care. Geneva: World Health Organization. 1989.
16. DeMayer EM, Tegman A. Prevalence of anemia in the world. *World Health Organization Quarterly*. 1998;38:302-316.
17. Ezzati M, Lopus AD, Dogers A, Vander HS, Murray C. Selected major risk factors and global and regional burden of disease. *Lancet* 2002;360:1347-1360.
18. Kalaivani K. Prevalence and consequences of anemia in pregnancy. *Indian J Med Res* 2009;130:627-633.
19. Gunningham FG. Hypertensive disorders in pregnancy. In: Eastman NJ, Hellman LM Editors. Williams Obstetrics. 19th Edition. Norwalk, CT: Appleton and Lange 1993. pp 763-818.
20. Balgir RS. Hematological profile of pregnant women with carrier status of hemoglobin disorders in coastal Odisha, India. *Int J Child Health Hum Develop.* 2011;4:325-332.