Introduction

Every individual has the liberty and right to make choice about sexual and reproductive health. Reproductive and sexual health is a complete physical, mental and social well-being in all matters related to reproduction which implies that the people are able to have a satisfying safe sex life, capability to reproduce, and freedom to decide when, and how often to do. When a couple decides to have a child, the woman must have access to services for a viable and fit pregnancy, safe delivery and, safe and healthy baby. Parents must have enough resources to look after themselves and to support survival of their family. Anemia in pregnancy is one of the causes of maternal morbidity and, maternal and fetal mortality in India. Hemoglobin transports oxygen to different parts of the body. Any defect in hemoglobin structure leads to its adverse functions. Screening of pregnant women for hemoglobinopathies helps in early intervention for reducing morbidity and mortality. Although the prevalence of hemoglobinopathies especially of the sickle cell disorders is high in Madhya Pradesh but any study on pregnant women is lacking. This study had set the objectives to find the prevalence of anemia and hemoglobin disorders in pregnant women, and to determine the health status through hematological indices profile in central India.

Hospital based a cross-sectional study showed 14.8% prevalence of hemoglobinopathies among 527 pregnant women, the sickle cell trait being 9.1%, followed by sickle cell disease (3%), β-thalassemia trait (2.3%), and hemoglobin E trait (0.4%). About 85% of the pregnant women/mothers were found free of hemoglobinopathies. Of the 12.1% pregnant women were suffering from sickle cell disorders. However, the overall 56.4% anemia was observed in pregnant women, severe anemia ranging in between 8.3% to 62.5%. A comparison of hematological indices of pregnant women afflicted with and without sickle cell disorders have revealed much reduced hemoglobin level, RBC count, MCV, HCT, and MCH; and raised leukocytosis and fetal haemoglobin in sickle cell disorder cases than among the normal pregnant women. Further, due to faulty reproductive health practices as well as neglect of reproductive and child health care facilities available to pregnant women/mothers, the pooled mothers with hemoglobinopathies in this study showed statistically significant higher number of stillbirths (p<0.01), infant mortality (p<0.05), childhood mortality (p<0.02) and less surviving offspring (p<0.001) than in the normal controls. A more vigorous and realistic campaign of prophylactic regime of supplementations for these pregnant women and child health care has been suggested.

Keywords: Prevalence; Hemoglobinopathies; Pregnant women/mothers; Reproductive health; Hemolytic anemia; Hematological indices; Madhya Pradesh; Central India
and child health practices and enough efforts are not done to create a movement for improving the health of women [1]. The Central part of India is predominantly poor, low-birth-weight babies prevalent with undernourishment (Protein Energy Malnutrition, PEM) and anemia, poor child-feeding practices, food insecurity, vulnerability to infectious environment, limited access to basic health care services, and overwhelming neonatal/infant mortality [2]. The people of Central India are at a higher risk for being carriers of hemoglobinopathies (genetic burden) in pregnant women/mothers attending a tertiary hospital in central India; ii) to compare the reproductive outcome of normal control mothers with those afflicted with hemoglobinopathies, and iii) to compare the health status and reproductive history of pregnant mothers with and without hemoglobinopathies during April 2012 to March 2014 in a tertiary hospital in central India.

Table 1: Prevalence of hemoglobinopathies in pregnant women/mothers during April 2012 to March 2014 in a tertiary hospital in central India.

<table>
<thead>
<tr>
<th>Diagnosis of pregnant mothers</th>
<th>Total conception (N=449)</th>
<th>Live birth (N=449)</th>
<th>Abortion (N=449)</th>
<th>Stillbirth (N=449)</th>
<th>Neonatal Death (N=449)</th>
<th>&lt; 1 year Death (N=449)</th>
<th>&lt; 10 years Death (N=449)</th>
<th>Surviving Offspring (N=449)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell trait (N=48)</td>
<td>55 9 5 3 4 31 35</td>
<td>40 72.7</td>
<td>6 10.9</td>
<td>9 16.4</td>
<td>3.5 5.5</td>
<td>4.7 3.3</td>
<td>5.9 1.1</td>
<td>34 61.8</td>
</tr>
<tr>
<td>Sickle cell disease (N=16)</td>
<td>18</td>
<td>12 66.7</td>
<td>2 11.1</td>
<td>4 22.2</td>
<td>1.5 6.6</td>
<td>2.1 1.1</td>
<td>3.6 7.4</td>
<td>9 50.0</td>
</tr>
<tr>
<td>β-thalassemia trait (N=12)</td>
<td>10</td>
<td>9 90.0</td>
<td>1 10.0</td>
<td>0 0.0</td>
<td>1.0 10.0</td>
<td>2.0 20.0</td>
<td>2.2 20.0</td>
<td>7 70.0</td>
</tr>
<tr>
<td>Hemoglobin E trait (N=2)</td>
<td>4</td>
<td>4 100.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0.0 0.0</td>
<td>1.2 5.0</td>
<td>1.2 5.0</td>
<td>3 75.0</td>
</tr>
<tr>
<td>Hemoglobinopathies (Pooled)</td>
<td>87</td>
<td>65 74.7</td>
<td>9 10.4</td>
<td>13 14.9</td>
<td>5.7 5.7</td>
<td>9.1 10.4</td>
<td>11 12.8</td>
<td>53 60.9</td>
</tr>
<tr>
<td>Normal</td>
<td>310</td>
<td>251 81.0</td>
<td>35 11.3</td>
<td>17 5.5</td>
<td>14 4.5</td>
<td>15 4.8</td>
<td>15 4.8</td>
<td>243 78.4</td>
</tr>
</tbody>
</table>

WHO, World Health Organization, Hb hemoglobin, Anemia in normal control versus sickle cell disease cases is highly significant at P<0.001.

Table 3: Comparison of reproductive history of pregnant mothers with and without hemoglobinopathies during April 2012 to March 2014 in a tertiary hospital in central India.

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<tr>
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<td>0 0.0</td>
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<td>4 100.0</td>
<td>0 0.0</td>
<td>0 0.0</td>
<td>0.0 0.0</td>
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*Birth to 28 days (neonatal mortality) +Birth to 365 days or within 1 year (Infant mortality) #Childhood mortality

Difference between normal controls versus different diagnostic categories of pregnant mothers significant at aP<0.05; bP<0.02; cP<0.01; dP<0.001.
Subjects and Methods

This is a prospective cross-sectional study carried out in the Outpatient Department of Obstetrics and Gynecology, NSCB Medical College and Hospital, Jabalpur in central India. Pregnant women/mothers who visited for antenatal care check up were investigated. 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. Confirmed cases of sickle cell disorders or other hemoglobinopathies were formed our study group and the negatives (without hemoglobinopathies) were taken as control group. Cases suffering from other abnormalities were not included or referred to us by the concerned doctor. All those pregnant women who had iron or folic acid deficiency, etc. were treated and were given iron-folic acid, multi-vitamins, and other dietary supplemenations by the attending and referring doctor of Gynecology Department of NSCB Medical College and Hospital, Jabalpur for improving the hematological indices, maternal health of the pregnant women/mothers, and for the proper development of the fetus. The study was carried out according to the ethical guidelines for biomedical research on human subjects. Intravenous 2-3 mL blood samples of a cross-section of 527 pregnant women/mothers were collected using disposable syringes and needles in disodium salt of ethylene diamine tetra acetic acid (EDTA) coated vials after taking informed consent from each pregnant woman/mother during the period from April 2012 to March 2014. Blood samples so collected were transported under wet ice-cold conditions to National Institute for Research in Tribal Health (formerly Regional Medical Research Centre for Tribals), Jabalpur for laboratory investigations and were analyzed in Biochemistry laboratory following the standardized laboratory procedures and techniques [16, 17]. Hematological parameters were studied using an automated blood cell counter (Model MS59, Melet Schloesing Laboratories, Cergy-Pontoise Cedex and France). The sickling test was performed by using freshly prepared 2% sodium metabisulphite solution as a reducing agent for the absence or presence of sickle cell hemoglobin [16]. Routine hemoglobin (Hb) lysate electrophoresis was carried out on cellulose acetate membrane in Tris-EDTA-borate buffer at pH 8.9 and quantification of hemoglobin A2 fraction was done by elution method [17]. The value of more than 3.5% of hemoglobin A2 fraction of adult hemoglobin was taken as cut off point for determining the β-thalassemia trait; and more than 10% of A2, as hemoglobin E. Estimation of fetal hemoglobin was carried out as described by Weather all [17]. 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 sickle cell/β-thalassemia was based on findings of hemoglobin A, F, S and A2 on electrophoresis under acidic and alkaline media, elevated Hb A2 levels (>3.5%), and family study. Anemia was defined as per the WHO Report Guidelines [18]. Before giving the biochemical and hematological investigations report to each pregnant woman; all the carriers/affected ladies were given genetic counseling. This study presented a comparative hematological picture of pregnant women/mothers against normal controls reflecting the status of reproductive health care in India. Results obtained were statistically tested by performing student t test (for hematological indices) and chi-square analysis (for reproductive history parameters) to compare the normal with each diagnostic category for the difference between the two independent variables, and significance if any, was also indicated.

Results

In a cross-sectional study of 527 pregnant women/mothers screened for hemoglobinopathies referred from the Department of Gynecology and Obstetrics, Netaji Subhash Chandra Bose Medical College and Hospital, Jabalpur during the period from April 2012 to March 2014, alarmingly high prevalence of 14.8% of hemoglobinopathies was found in the present study, and the affliction with sickle cell trait being 9.1%, followed by sickle cell disease (3%), β-thalassemia trait (2.3%), and hemoglobin E trait (0.4%). About 85% of the pregnant women/mothers were found free of hemoglobinopathies. The women with sickle cell disorders constituted 12.1% of the total pregnant women/mothers investigated and reported for the first time from central India. There were total 16 (3%) pregnant women (9 were mothers) aged between 20 to 32 years, who were suffering from the homozygous sickle cell disease (Table 1). All the pregnant women/mothers were graded for anemia according to the classification of WHO Report [18] and data were presented in Table 2. It was apparent from table that the average or overall 56.4% of the pregnant women/mothers had different grades of anemia, and severe anemia range falling in between 8.3% to 62.5%. However, 100% anemia persisted in those pregnant women/mothers who were suffering from homozygous sickle cell disease. The frequency of severe anemia was recorded to be 8.3% (β-thalassemia trait), 16.7% (normal), 31.3% (sickle cell trait) and 62.5% (homozygous sickle cell disease) in pregnant women/mothers and the difference between normal controls and different diagnostic categories was statistically highly significant (p<0.001). Reproductive outcome history profile of pregnant mothers is presented in Tables 3. The proportional difference in outcome between normal controls and different diagnostic categories of mothers was found statistically
significant. It is observed that the proportion of stillbirths is higher in sickle cell disease mothers (p<0.001) than in the controls. Similar results were obtained for the deaths under 10 years of age (p<0.05). Surviving offspring was lower in both sickle cell trait (p<0.01) and sickle cell disease (p<0.01) mothers as compared to controls. The pooled mothers for hemoglobinopathies showed statistically higher number of stillbirths (p<0.01), infant mortality (p<0.05), childhood mortality (p<0.02) and less surviving offspring (p<0.001) than in the normal controls. Similar comparisons for reproductive history outcome per 1000 live-births are presented in Table 4. Table 5 shows the comparison of hematological indices of pregnant women/mothers with and without hemoglobinopathies. The values for hematological indices such as Hb, red blood cell (RBC) and hematocrit (HCT) were much reduced and elevation of leukocytosis and fetal hemoglobin indices such as Hb, red blood cell (RBC) and hematocrit (HCT) were much reduced and elevation of leukocytosis and fetal hemoglobin were observed. The results were obtained for hematological parameters between normal-control and sickle cell trait, sickle cell disease, and hemoglobin E trait cases have been indicated in Table 5.  

**Discussion**

The present study was focused on the prevalence of hemoglobinopathies in pregnant women/mothers attending a tertiary hospital in central India. For the first time, the study has revealed alarmingly high prevalence of genetically determined hemoglobinopathies (14.8%), with affliction of sickle cell trait (9.1%), sickle cell disease (3%), β-thalassemia trait (2.3%), and hemoglobin E trait (0.4%). About 85% of the pregnant women/mothers were found free of hemoglobinopathies (Table 1). The sickle cell disorders constituted 12.1% in pregnant women/mothers, thus, showing the high magnitude of hereditary hemolytic disorders in central India. Hereditary hemolytic anemia, nutritional deficiencies (iron-folic acid deficiency, vitamin B12, protein energy malnutrition, etc.), parasitic infections (malaria) and parasitic infestations (worms) play a major role in determining the pregnancy outcome in both underdeveloped and developing countries of the world. Among the hereditary hemolytic disorders: β-thalassemia syndrome, sickle cell disease, and G6PD enzyme deficiency, are the significant contributors to anemia in pregnant women in tropical and subtropical countries including India. It is interesting to note the lower or reduced prevalence of anemia (56.4%), and severe anemia with a range of (8.3%-62.5%) among the pregnant women/mothers attending a tertiary hospital in central India than the Indian average of 72%. However, in those pregnant women/mothers who are suffering from homozygous sickle cell disease, 100% anemia is still existing (Table 2) due to the failure to raise the hemoglobin level even with iron-folic acid supplementations, ascribable to inherited hemoglobin structural abnormalities. The frequency of severe anemia in pregnant women/mothers afflicted with β-thalassemia trait (8.3%), normal (16.7%), sickle cell trait (31.3%) and homozygous sickle cell disease (62.5%) is still very high. For the distribution of different grades of anemia, the difference between normal (without hemoglobinopathies) and homozygous sickle cell disease pregnant women/mothers was highly significant (p<0.001). It is alarming to learn that due to poor reproductive and child health practices as well as neglect of reproductive health care facilities available to pregnant women/mothers, a higher reproductive wastage, higher infant mortality, higher childhood mortality and less survival of offspring is encountered in central India. This has been revealed by the reproductive outcome study carried out in a tertiary hospital in central India. It was noted that the proportion of stillbirths is higher in mothers with sickle cell disease (p<0.001) than in the controls. Similar results were obtained for deaths under 10 years of age (p<0.05). Surviving offspring was less in both sickle cell trait (p<0.01) and sickle cell disease (p<0.01) mothers as compared to controls. The pooled mothers for hemoglobinopathies showed statistically higher number of stillbirths (p<0.01), infant mortality (p<0.05), childhood mortality (p<0.02) and less surviving offspring (p<0.001) than in the normal controls (Table 3). Similar results were obtained for comparison of reproductive history outcome per 1000 live-births between normal controls and different diagnostic categories of mothers in relation to hemoglobinopathies (Table 4). In general, in the normal pregnancy, blood volume increases in a concomitant hemodilution. Although RBC mass increases during pregnancy, plasma volume increase is greater, resulting in a relative anemia. This hemodilution results in a physiologically lowered level of hemoglobin, value of HCT, and RBC count, but it has no effect on the mean corpuscular volume (MCV). A comparison of
hematological indices of these pregnant women/mothers has revealed that afflicted mothers with sickle cell disorders have much reduced hemoglobin level, RBC count, MCV, HCT, and mean corpuscular hemoglobin; and raised leukocytosis and fetal hemoglobin than in the normal (without hemoglobinopathies) pregnant ladies (Table 5). Low profile of hematological indices in general do not show significant variations between pregnant women/mothers (carrier cases) and without hemoglobin disorders and indicate very poor health status. Even normal pregnant women/mothers had reduced red cell indices due to iron-foie acid deficiency, mal- and under nutrition, and other parasitic infections/infestations, etc. However, there was a statistically significant reduction in hemoglobin level (p<0.001), RBC counts (p<0.001), and HCT (p<0.001); and elevation of leukocytosis (p<0.01), and fetal hemoglobin (p<0.001) in patients of homozygous sickle cell disease than in the normal control (without hemoglobinopathies) pregnant women/mothers (Table 5). Quantitatively the hemoglobin A2 was raised in pregnant nutrition, and other parasitic infections/infestations, etc. However, health status. Even normal pregnant women/mothers had reduced cases) and without hemoglobinopathies and indicate very poor significant variations between pregnant women/mothers (carrier 5). Low profile of hematological indices in general do not show the normal (without hemoglobinopathies) pregnant ladies (Table hemoglobin; and raised leukocytosis and fetal hemoglobin than in 5). Low profile of hematological indices in general do not show the normal (without hemoglobinopathies) pregnant ladies (Table hemoglobin; and raised leukocytosis and fetal hemoglobin than in the normal (without hemoglobinopathies) pregnant ladies (Table and normal pregnant women/mothers affected with hemoglobin disorders than the normal (without hemoglobin disorders) controls. Anemia in pregnancy, in general, is associated with the adverse consequences for both the mother and the fetus. Adverse consequences of maternal anemia may affect not only the neonate and infant for poor intrauterine growth, prematurity and low birth weight and high perinatal mortality but also increase the risk of non-communicable diseases when the child grows into an adult life and risk of low birth weight even in the next generation. Immuno-depression in anemic women renders them more susceptible to infections especially to urinary tract infections, and to increased morbidity affecting the course and outcome of pregnancy. Low profile of hematological indices do not show significant variations between pregnant women with (in carrier cases) and without hemoglobinopathies, showing pathetic health conditions of pregnant women/mothers, in general. Due to poor reproductive health practices as well as negligible reproductive health facilities available to pregnant women/mothers, encountering a higher reproductive wastage, higher infant mortality, higher childhood mortality and less survival of offspring is a compensated price in central India. Prenatal screening or screening during the first trimester or as early as before 12th week is very useful, which includes identification of carriers, prenatal diagnosis of at risk couples and, subsequently, termination of pregnancy, if needed, to prevent the birth of sickle cell disease/thalassemic child. Mandatory awareness instructions for consumption of balanced diet, comprehensive clinical management, and genetic/marriage counseling after prenatal diagnosis are highly essential to ameliorate the sufferings of afflicted (especially pregnant) women/mothers. Poverty, ignorance, non-availability and/or failure to utilize the available medical health facilities are shown to be associated with maternal anemia, and maternal and perinatal morbidity, and mortality in the rural population of central India. A more vigorous and realistic campaign of prophylactic regime of supplementation for these pregnant women and child health care has been suggested.

Acknowledgements

The author is grateful to Dr. V.M. Katooch, Ex-Secretary, Department of Health Research, Government of India and Ex-Director General, Indian Council of Medical Research, New Delhi for providing the necessary research facilities and the subjects for their kind cooperation. Author gratefully acknowledges Prof. Shashi Khare, Ex-Professor & Head, Department of Obstetrics & Gynecology, and Dean, Netaji Subhash Chandra Bose Medical College and Hospital, Jabalpur for referring the subjects for investigations. Thanks are also due to Mr. VK Kachhi and Mr. P.Patel, Laboratory Technicians for their technical support during the investigations.
Funding

Intra-mural, National Institute for Research in Tribal Health (formerly Regional Medical Research Centre for Tribals), Jabalpur, India.

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