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Key Words

Anemia, pregnancy, peripheral smear, clinical findings, types, severity

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Received: 20 December 2023

Accepted: 23 January 2024

Published: 25 January 2024

Citation: Priyanka Sachdeva, Rekha Sapkal, Vaishali Chourasia and Abha Chaudhary, 2024. A Cross-Sectional Study on Prevalence of Different Type of Anemia in Pregnancy Diagnosed by Peripheral Blood Smear. Res. J. Med. Sci., 18: 196-201, doi: 10.59218/makrjms.2024.4.196.201

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A Cross-Sectional Study on Prevalence of Different Type of Anemia in Pregnancy Diagnosed by Peripheral Blood Smear

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ABSTRACT

One of the most significant issues affecting public health is the presence of anemia in pregnant women. The peripheral blood smear examination is an essential approach for diagnosing the kind of anemia that is present in pregnant women and it is a supplement to the full blood picture. This research was carried out with the purpose of determining the prevalence of various kinds of anemia in pregnant women by utilizing peripheral blood smears and analyzing their clinical and laboratory profiles. This study was carried out as a cross-sectional study on pregnant women who visited the antenatal outpatient department (OPD) at the tertiary care center in Bhopal over the course of the study period, which lasted for a total of 18 months. A comprehensive clinical history was gathered, and haematological examinations, including a complete blood count and a peripheral smear, were carried out. The prenatal women who participated in this study had a mean age of 25.96 ± 4.285 years. The total number of participants in this study was 392. With a hemoglobin level of less than 11 gm%, there were 313 patients (79.8%) that were found to have anemia. Of these instances, 207 (52.8%) had mild anemia, 99 (25.2%) cases had moderate anemia and 7 (1.8%) cases had severe anemia. The statistical significance of the observed correlation between the type of anemia and the peripheral smear finding was determined to be $p < 0.05$ on the statistical scale. It may be concluded that iron deficiency anemia is the most prevalent form of anemia that occurs during pregnancy. The detection of several types of anemia during pregnancy, such as sickle cells, as well as the diagnosis of sickle cell anemia and sickle cell trait, can be accomplished with the assistance of peripheral smears. Palor, exhaustion, weakness and giddiness are just few of the nonspecific symptoms that are typically seen in females who are suffering from anemia. Different kinds of anemia, on the other hand, may be characterized by a number of distinct characteristics.

INTRODUCTION

The decrease in the amounts of hemoglobin or the quantity of red blood cells that occurs during pregnancy is referred to as anemia. This condition is considered to be one of the most significant public health issues. Anemia in pregnancy is defined as a hemoglobin concentration that is lower than 10 gm L^{-1} , as stated by the World Health Organization^[1]. The cause of anemia in pregnant women might be either physiological or pathological. The physiological increase in blood and plasma volume that occurs during pregnancy is the cause of physiological anemia that has been observed throughout pregnancy. When a pregnant woman has a hemoglobin level that is lower than 10 g L^{-1} , this is an indication of an underlying disease, most typically deficiencies in nutrition^[2,3]. According to the World Health Organization, the percentage of pregnant women who suffered from anemia in 2019 was 36.5%.⁴ According to the data from the National Family Health Survey 5 (2019-2021), the prevalence of anemia in pregnant women in India was 52.2%, with 45.7% of pregnant women living in urban areas and 54.3% living in rural areas^[5]. NFHS-5 State factsheet found that the prevalence of anemia in pregnant women in Madhya Pradesh was 52.9%. The prevalence was greater in rural areas (54.9%) compared to urban areas (45.1%), with rural areas having a higher prevalence than urban areas^[6].

Even though nutritional deficiencies (iron, folic acid, vitamin B12 and vitamin A) are the most common cause of anemia in pregnancy in low-and middle-income countries, anemia can also be caused by other factors. These factors include parasitic infections (malaria, hookworm infestation, Giardiasis, and Schistosomiasis), chronic infections (tuberculosis, HIV), hemoglobinopathies (Glucose 6-phosphate dehydrogenase deficiency, Sickle cell disease and Thalassemia), hemorrhoids and antepartum hemorrhage, among other things^[7]. Through the administration of folic acid supplements during the first trimester of pregnancy and iron folic acid supplements during the second and third trimesters of pregnancy, maternal and child health programs primarily concentrate on the prevention of nutritional deficiency anemia during pregnancy^[8]. When a woman has several nutritional deficiencies, such as iron deficiency, folate deficit, vitamin B12 insufficiency and so on the underlying anemia may not respond to iron therapy alone. This is because the female may have multiple nutritional deficiencies^[7]. When it comes to the evaluation of hematological disorders the peripheral smear examination is a key component. It assists in displaying the size of the red blood cells as well as the amount of hemoglobin they contain. Iron deficit on peripheral smear reveals microcytic micro chromic, whereas folate or B12 deficient RBCs are macrocytic^[9]. However, during pregnancy, females

may present with a blood picture that is dimorphic or polymorphic. This is because iron shortage causes microcytic micro chromic^[7]. Blood smears facilitate the identification of specific morphological abnormalities of red blood cells (RBCs), including but not limited to spherocytes, sickle cells and target cells, amongst others. In sickle cell anemia, sickle cells and target cells are detected, whereas spherocytes are present in autoimmune hemolytic anemia, hereditary spherocytosis, hemolytic transfusion responses and other conditions. Microangiopathic hemoglobinopathy, which is associated with severe preclampsia and HELLP syndrome, has been found to contain schistocytes^[9]. Therefore, the peripheral blood smear test is an essential approach for diagnosing the type of anemia that manifests itself during pregnancy and it serves as a supplement to the comprehensive blood picture. For this reason, the research was carried out at a tertiary care center in order to investigate the prevalence of various types of anemia in pregnant women through the utilization of peripheral blood smears, along with their clinical and laboratory profiles.

MATERIALS AND METHODS

The current study was carried out as an observational cross-sectional study on pregnant women who visited the antenatal outpatient department (OPD) the Department of Obstetrics and Gynaecology at People's College of Medical Sciences and Research Centre and the associated People's Hospital in Bhopal. The study lasted for a total of 18 months, beginning on December 1, 2020 and ending on May 31, 2022. During the course of the study, all pregnant women who were registered at PCMS and RC and who attended the antenatal outpatient clinic were included. This was the case regardless of the trimester or gravida they were in. However, pregnant women who had other complications related to pregnancy, such as obesity, diabetes, or multiple pregnancies, as well as those who were unwilling to give consent, were not participating.

Using the formula, sample size was estimated to be 392. After obtaining ethical clearance from Institute's ethical committee (ECR/519/inst/MP/2014/RR-20), all the pregnant women fulfilling the inclusion criteria and willing to participate in study were enrolled. A comprehensive clinical history was acquired and documented in a proforma. This history included sociodemographic characteristics, presenting problems, obstetrics history, and relevant previous history. Following that, a comprehensive clinical evaluation was performed on each and every participant in the study. In order to conduct preliminary haematological studies, two milliliters of venous blood were taken in the EDTA vacutainer while adhering to all of the necessary aseptic precautions. The complete blood count (CBC)

was carried out immediately on an automated haematology analyzer that was calibrated according to established procedures and at the same time, a blood sample was submitted for peripheral smear testing. According to the World Health Organization's (WHO) guidelines, anemia was delineated by a hematocrit (HB) content of less than 11 g dL^{-1} . The levels of hemoglobin (HB) concentration that were judged to be mild, moderate and severe anemia were $10\text{-}10.9 \text{ g dL}^{-1}$, $7\text{-}9.9 \text{ g dL}^{-1}$ and less than 7 g dL^{-1} , respectively.

Statistical Analysis: Data was compiled using MsExcel and analysis was done with the help of IBM SPSS software version (SPSS Inc., Chicago, IL, USA). The HB measurements of the pregnant woman were measured using HemoCue analyzer (HemoCue Hb 301). Categorical variables were expressed as frequency and percentage whereas continuous variables were expressed as mean and standard deviation. Association of type of anemia with peripheral smear finding was done using chi square test. We applied univariate and multivariate logistic regression analyses to determine the predictors of anemia. $p > 0.05$ was considered statistically significant.

RESULTS

This study was conducted on a total of 392 antenatal women with mean age of 25.96 ± 4.285 years. Majority of cases belonged to age range of less than 25 years (51.5%) and about 75.3% cases belonged to lower socioeconomic status. Majority of females were primigravida (42.1%) (Table 1). Anemia was observed in 79.8% cases with hemoglobin level below 11 gm\% , of them, 52.8% cases had mild anemia, 25.2% cases had moderate anemia and 1.8% cases had severe anemia. Most common type of anemia was iron deficiency anemia (73.7%) (Table 2). Peripheral smear revealed normocytic normochromic blood picture in 53.3% cases and microcytic hypochromic picture was observed in 42.9% cases (Fig. 1).

About 64.6-88.7% cases with normocytic normochromic blood picture and microcytic hypochromic blood picture had iron deficiency anemia respectively. About 75% cases with sickling on peripheral smear had sickle cell anemia. The observed association of type of anemia with peripheral smear finding was found to be statistically significant ($p < 0.05$) (Table 3).

Bald/pale tongue was the most common mode of presentation observed in 100% cases, followed by weakness, fatigue and giddiness in 98.7%, 93.9% and 92.1% cases respectively. Fatigue and pale tongue were documented in 100% cases with thalassemia. In patients with sickle cell anemia, giddiness and pale/bald tongue was reported in 100% cases. All the cases with megaloblastic anemia had giddiness, fatigue, weakness and bald/pale tongue. We reported

bald or pale tongue in 100% cases with iron deficiency anemia. Weakness, fatigue and giddiness were reported in 99%, 93.1-92.7% cases of IDA respectively. We reported giddiness, fatigue, weakness and pale tongue in 100% cases with hemolytic anemia and 66.7% cases had hepatosplenomegaly (Table 4).

DISCUSSIONS

The present study was carried out on a total of 392 pregnant women who presented themselves at our research center during the first, second, or third trimester of their pregnancies. The purpose of the study was to classify the many types of anemia that can be seen in pregnant women by utilizing peripheral blood smears and to investigate clinical symptoms that are associated with anemia. During the course of our research, we found that 79.8 percent of pregnant women suffered from anemia. Although nutritional anemia, and iron deficiency anemia in particular, are the most common causes of anemia in pregnancy, other types of anemia, such as hemolytic anemia, sickle cell anemia, and thalassemia, may also be detected in pregnant women^[7]. In our research, we found that iron deficiency anemia was the most prevalent kind of anemia, accounting for 73.7% of all cases. Our second objective was to determine the extent to which sickle cell anemia, thalassemia, and other haemoglobinopathies are prevalent among pregnant women. In the study that we conducted the prevalence of sickle cell anemia was 2.6% the prevalence of thalassemia was 2% and the prevalence of hemolytic anemia was 0.8%.

An investigation conducted by Agravat and colleagues revealed that hemoglobinopathies, sickle cell trait, β -thalassemia trait, and sickle cell illness were observed in 12.26%, 7.45-2.89%, 0.24% and 1.68% of the individuals, respectively. Based on the findings of the Gebreweld *et al.*^[10] also identified iron deficiency anemia in the majority of pregnant women, which was 51.5% of the whole pregnancy population. The majority of these females, which accounted for 73.3% of the total, had additional deficiencies in vitamin B12 and folate Kaushal *et al.*^[11] reported that iron deficiency anemia was the most common kind of

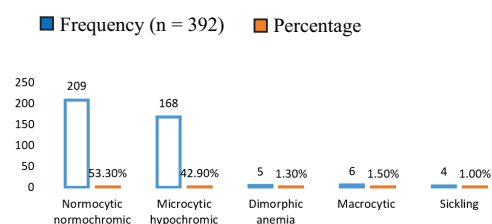


Fig. 1: Distribution of cases according to the finding of peripheral smear

Table 1: Distribution according to baseline variables

Baseline variables	Frequency (n = 392)	Percentage
Age (years)		
<25	202	51.5
26-30	132	33.7
>30	58	14.8
Socioeconomic status		
Upper class	2	0.5
Upper middle class	20	5.1
Middle class	66	16.8
Lower middle class	9	2.3
Lower class	295	75.3
Gravida		
1	165	42.1
2	140	35.7
3	57	14.5
4	29	7.4
>5	1	0.3

Table 2: Distribution according to characteristics of anemia

Characteristics of anemia	Frequency (n = 392)	Percentage
Anemia		
Present	313	79.8
Absent	79	20.2
Severity		
Mild (10-10.9 gm%)	207	52.8
Moderate (7-9.9 gm%)	99	25.2
Severe (<7 gm%)	7	1.8
Type		
Hemolytic anemia	3	0.75
Iron deficiency anemia	289	73.7
Megaloblastic anemia	3	0.75
Sickle cell anemia	10	2.6
Thalassemia	8	2.0
Normal	79	20.2

Table 3: Association between type of anemia and peripheral smear findings

Type of anemia	Normocytic normochromic	Microcytic hypochromic	Dimorphic anemia	Macrocytic	Sickling
Hemolytic anemia	1(0.5%)	2 (1.2%)	0 (0%)	0 (0%)	0 (0%)
Iron deficiency anemia	135(64.6%)	149 (88.7%)	1 (20%)	3 (50.0%)	1 (25%)
Megaloblastic anemia	0 (0%)	0 (0%)	3 (60%)	0 (0%)	0 (0%)
Sickle cell anemia	0 (0%)	4 (2.4%)	1(20%)	2 (33.3%)	3 (75%)
Thalassemia	0 (0%)	8 (4.8%)	0 (0%)	0 (0%)	0 (0%)
Normal	73(34.9%)	5 (3%)	0 (0%)	1 (16.7%)	0 (0%)
Total	209	168	6	5	4
p-value	0.001				

Table 4: Clinical features in study participants

Clinical features	Total (n = 392)	Thalassemia (n = 8)	SCA (n = 10)	Megaloblastic (n = 3)	IDA (n = 289)	Hemolytic anemia n = 3(%)
Giddiness	361 (92.1%)	7 (87.5%)	10 (100%)	3 (100%)	268 (92.7%)	3 (100)
Fatigue	368 (93.9%)	8 (100%)	9 (90%)	3 (100%)	269 (93.1%)	3 (100)
Weakness	387 (98.7%)	7 (87.5%)	9 (90%)	3 (100%)	286 (99%)	3 (100)
Angular stomatitis	170 (43.4%)	6 (75%)	4 (40%)	1 (33.3%)	119 (41.2%)	1 (33.3)
Bald/Pale tongue	392 (100%)	8 (100%)	10 (100%)	3 (100%)	289 (100%)	3 (100)
Koilonychia	41 (10.5%)	0 (0%)	1(10%)	0 (0%)	3 (1%)	0 (0)
Brittle hair/ Nails	25 (6.4%)	0 (0%)	0(0%)	1(33.3%)	12 (4.2%)	0 (0)
Pearly white sclera	18 (4.6%)	1 (12.5%)	0(0%)	0 (0%)	17 (5.9%)	0 (0)
Abdominal swelling	1 (0.3%)	0 (0%)	0(0%)	0 (0%)	0 (0%)	1 (33.3)
Dark urine	1 (0.3%)	0 (0%)	0(0%)	0 (0%)	0 (0%)	1 (33.3)
Skin pigmentation	3 (0.8%)	0 (0%)	0(0%)	0 (0%)	1 (0.3%)	1 (33.3)
Hemorrhagic patches	1 (0.3%)	1 (12.5%)	0(0%)	0 (0%)	0 (0%)	0 (0)
Tingling numbness	3 (0.8%)	0 (0%)	0(0%)	0 (0%)	1 (0.3%)	0 (0)
Jaundice	4 (1%)	0 (0%)	0(0%)	0 (0%)	1 (0.3%)	1 (33.3)
Hepatomegaly	41 (10.5%)	3 (37.5%)	4(40%)	0 (0%)	23 (8%)	2 (66.7)
Splenomegaly	23 (5.9%)	1 (12.5%)	3(30%)	0 (0%)	13 (4.5%)	2 (66.7)
H/O Pica	126 (32.1%)	2 (25%)	5 (50%)	2 (66.7%)	116 (40.1%)	1 (33.3)
H/O Antiepileptic drug	18 (4.6%)	1 (12.5%)	0 (0%)	0 (0%)	17 (5.9%)	0 (0)

anemia, accounting for 100% of all cases^[12]. In each and every one of the instances, a peripheral smear test was carried out in order to ascertain the morphological characteristics of the red blood cells. After that, we reported a microcytic hypochromic blood image in 42.9% of the cases, which was followed by a normocytic normochromic blood picture in the

majority of instances, which was 53.3%. Sickling was observed in one percent of the cases, whereas macrocytic and dimorphic blood pictures were seen in one percent of the cases and one third of the cases, respectively. Anemia with microcytic and hypochromic characteristics was observed in 47.5% of pregnant women, while a blood picture with normocytic and

hypochromic characteristics was observed in 32.5% of the pregnant women^[13]. Following the microcytic hypochromic blood image in the majority of cases (51.5%) the normocytic hypochromic blood picture (27.3%) and the normocytic normochromic blood picture (18.2%) were the next most common types of blood pictures^[11]. In the majority of cases (50%) in a study conducted by Okia *et al.*^[14] a peripheral smear indicated a microcytic hypochromic blood picture. This was followed by a macrocytic anemia representation (41.7%). We presented the results of a peripheral smear examination used to diagnose several kinds of anemia. In a total of 289 patients, we found evidence of iron deficiency anemia. The majority of these instances were microcytic hypochromic (n = 149), while 135 cases were normocytic normochromic. Three patients were found to have hemolytic anemia, two of them had microcytic hypochromic anemia and one of them had normocytic normochromic anemia on the peripheral smear. Every single one of the three cases of megaloblastic anemia exhibited a blood picture that was dimorphic. A total of ten patients were reported to have sickle cell anemia the majority of which were microcytic hypochromic. There were three cases in which sickling was observed in the peripheral smear. In every single one of the eight cases of thalassemia, the peripheral smear revealed a microcytic hypochromic blood pattern. In the study conducted by Warner *et al.*^[15] microcytic hypochromic anemia was shown on peripheral blood smears in one hundred percent of the cases of iron deficiency anemia. These findings provided support for the findings of the present investigation. Microcytic hypochromic blood image was also described by Johnson *et al.*^[16] as the major finding on peripheral smear in iron deficiency anemia. Additionally, macrocytic and microcytic hypochromic blood picture were found in patients who had both folate and vitamin B12 deficiency occurring at the same time. Schistocytes, spherocytes and bite cells were observed on peripheral smears conducted by Philips *et al.*^[17] in patients who were diagnosed with hemolytic anemia.

The clinical characteristics of a number of different kinds of anemia were evaluated by us. The presence of angular stomatitis was observed in 75% of the instances with thalassemia, while splenomegaly was observed in 37.5% of the cases. 40 percent of sickle cell anemia patients had hepatomegaly and 30 percent of sickle cell anemia patients had splenomegaly. The presence of angular stomatitis and brittle nails or hairs was seen in approximately 33.3% of each instance with megaloblastic anemia. Cases of iron deficiency anemia were found to have angular stomatitis in approximately 41.2% of cases, koilonychia in 1% of cases, hepatomegaly in 8% of cases and splenomegaly in 4.5% of cases. We found that 66.7% of the cases had hemolytic anemia and 33.3% of the cases had jaundice, angular stomatitis, pearly

white sclera, abdominal edema and black urine. Hepatosplenomegaly was not seen in any of the instances. Both the findings of the present investigation and the findings of Goonewardene *et al.*^[7] in which the authors identified weariness, weakness, tiredness, and lethargy as the most common and non-specific clinical characteristics of anemia, provided support for the findings of the present study. The findings of the present study were also supported by the findings of Warner *et al.*^[15] who reported that pallor was the most common clinical symptom of anemia with non-specific features such as fatigue, shortness of breath, decreased work capacity, and so on. The findings of the present study were also supported by these findings. There is a possibility that the decreased oxygen carrying capacity of hemoglobin is the cause of the shortness of breath and the ease with which anemia causes fatigue^[18]. jaundice is a specific feature of hemolytic anemia, glossitis and cheilitis are associated with iron and folic acid deficiency anemia, splenomegaly is documented in a higher proportion of cases with hemolytic anemia and koilonychia is documented to be a specific feature of iron deficiency anemia. These findings were reported by Turner *et al.*^[19]. However, Bhasin *et al.*^[20] observed that 74% of the patients were characterized by exhaustion, while anemia and pallor were noted in 59% of the cases, edema in 20% of the cases, and glossitis in 1% of the cases.

CONCLUSION

Iron deficiency is responsible for the majority of cases of anemia that occur during pregnancy. It is estimated that approximately five percent of pregnant women are affected by hemoglobinopathies such as thalassemia, sickle cell disease, and hemolytic anemias. When it comes to diagnosing sickle cell anemia and sickle cell trait during pregnancy, peripheral smears are an efficient method for identifying a variety of anemias, including sickle cells, as well as sickle cell patients. A pallor, tiredness, weakness and dizziness are some of the non-specific symptoms that are frequently seen by females who suffer from anemia. However, there are a few distinguishing traits that can be noted for each of the several types of anemia.

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