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Patterns Of Anemia In Antenatal Women Attending A Tertiary Care Hospital In Northwest India.

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ABSTRACT

Anemia, a prevalent health issue, particularly affects pregnant women in developing countries like India**.** Caused by factors such as nutritional deficiencies, increased demands during pregnancy, and socioeconomic conditions, it poses significant risks to both mother and child. This study aims to identify specific anemia types and patterns among pregnant women to enable timely diagnosis and treatment. This observational study analyzed blood samples from 220 pregnant women with anemia at a tertiary care hospital. Hematological parameters, including complete blood count, RBC indices, and peripheral blood smear, were assessed. Iron studies and HPLC were conducted for further evaluation. Anemia types (normocytic, microcytic, macrocytic, dimorphic) and severity (mild, moderate, severe) were determined. Statistical analysis was performed to identify patterns and associations. The study included 220 pregnant women with a mean age of 26.01 years. Most women (79.1%) were aged 21-30 years, with a majority (50.9%) being multiparous. Anemia prevalence was high, with 61.8% of women experiencing moderate anemia and 8.6% severe anemia. Anemia in pregnancy is complex, with iron deficiency being a primary cause. However, diverse anemia types necessitate comprehensive assessment. Individualized care and a multidisciplinary approach are crucial for effective management.

Keywords: Antenatal women, Anemia, Pregnancy, Patterns of anemia

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INTRODUCTION

Anemia is the most common nutritional deficiency worldwide, especially affecting developing countries. Despite being preventable and treatable, it remains a significant health concern, particularly among pregnant women. This is troubling because anemia during pregnancy can have serious consequences for both the mother and the developing baby. Anemia, a condition with low red blood cells, is a frequent complication of pregnancy, especially in developing countries and the main reasons are: nutritional deficiencies, infections, blood loss, genetic conditions, and close pregnancies [1].

During pregnancy, three main factors can lead to vitamin and mineral deficiencies in expecting mothers: Increased demands both the developing baby and the mother's body require a greater number of vitamins and minerals, nutritional deficits, and reduced absorption. Furthermore, poverty, limited access to quality healthcare, and lack of awareness about proper nutrition during pregnancy can indirectly contribute to anemia, a common complication arising from vitamin and mineral deficiencies [2].

Anemia in Pregnant Women in India is a serious health concern and the following reasons could be attributable.

Studies reveal unequal food distribution within families, with women receiving less nutritious food compared to their needs. This, coupled with heavy workloads, leads to deficiencies like iron, making them susceptible to anemia. Low income further restricts access to iron-rich sources like meat and vitamin C (ascorbic acid), hindering iron absorption [3-6].

While malnutrition affects all segments of the population, women are disproportionately impacted, often starting in childhood and continuing throughout life. The World Health Organization (WHO) estimates a significantly higher prevalence of anemia in pregnant women in India (65-75%) compared to developed (14%) and other developing countries (51%). WHO recommends maintaining haemoglobin levels above 11.0 g/dl ideally, and not letting it fall below 10.5 g/dl during the second trimester [7].

The purpose of this study is to identify the type and specific morphological patterns of anemia attending our hospital that may affect the pregnancy. Their detailed analysis can ensure the prompt diagnosis so that an early and accurate treatment can be timely instituted.

MATERIALS AND METHODS

The present research was a hospital based observational study conducted in Hematology laboratory, Department of Pathology. Faculty of Medicine and Health Sciences, SGT University Budhera, Gurugram. The study was conducted on blood samples drawn from all 220 consecutive antenatal patients visiting the hospital for the first time with clinical symptoms of anaemia, during the time period October 6, 2022 to April 6, 2024. The patients who had received blood transfusion, with positive tests for occult blood in stool and complications of third stage of labor were excluded from the study.

Under all aseptic conditions blood sample for the following test are collected in Ethylene Diamine Tetraacetate (EDTA) vacutainer and analyzed within one hour of sample collection. The test is done using hematology autoanalyzer (Mindray BC-6200) with standard calibration used in pathology laboratory.

The following parameters were obtained

- Hemoglobin (Hb)
- Complete blood count (CBC), Total leukocyte count (TLC), Differential leukocyte counts (DLC), RBC and platelets.
- RBC indices (Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Cell Distribution Width-Coefficient of Variation (RDW-CV))

Peripheral blood smears (PBS) were prepared and stained by Leishman stain and examined for abnormalities in size and shape of red blood cells. The morphology of white blood cells and platelets were also analyzed for any abmormalities [8].

Blood sample were also obtained for the following iron studies in plain vacutainer and analyzed in the Biochemistry lab.

- Serum iron was performed using fully automated EM 360 chemistry analyzer.
- Serum ferritin was measured by chemiluminescence immunoassay (CLIA)technique based fully automated instrument.
- Transferrin saturation was calculated as serum iron/ total iron binding capacity (TIBC).

HPLC was done if clinical history and hematological profile was suggestive of thalassemia syndrome and iron profile was not conclusive of IDA.

After charting the data on excel and SPSS statistical software version 20.00 Anemia was classified as: Normocytic normochromic, microcytic hypochromic, macrocytic and dimorphic. The severity of anemia was graded as mild (Hb 09-10.9 g/dL), moderate (Hb7-8.9 g/dL), or severe (Hb less than 7 g/dL) [9].

The data was statistically analysed using the following: Continuous variables were presented as mean ± standard deviation (SD), categorical variables were expressed as frequencies and percentages and a $p < 0.05$ was considered statistically significant.

RESULTS

The present study included 220 pregnant females who attended the antenatal clinic for the first time. The mean maternal age was 26.01 years with a SD of 4.052 years. The range of maternal ages spanned from 18.0 years to 36.0 years. Among all the patients, 17 individuals (7.7%) fell into the age group of ≤20 years, while the majority, constituting 86 patients (39.1%), belonged to the age range of 21-25 years. Additionally, 88 individuals (40.0%) were aged between 26 and 30 years, and 29 patients (13.2%) were above 30 years of age. Among the patients, 112 individuals (50.9%) were multiparas. Additionally, 95 participants (43.2%) were primiparas. Furthermore, 13 patients (5.9%) were classified as grand multiparas. One hundred and fifty-three individuals (69.5%) were in the first trimester of pregnancy and 67 patients (30.5%) were in the second trimester.

Table 1 shows the distribution of CBC parameters among the study participants. The TLC demonstrated a mean of 7210.332 with an SD of 1643.0063, the Platelet count presented a mean of 2.6370 with an SD of 0.59724, the RBC count displayed a mean of 4.0157 and SD of 4.88114, the Hemoglobin levels exhibited a mean of 8.846 with an SD of 1.3019. Additionally, parameters like MCV, MCH, and MCHC also show diverse distributions within the study population, as reflected by their respective means and SDs. Red Cell Distribution Width-Coefficient of Variation (RDW-CV) demonstrated a mean of 17.8114 with an SD of 3.49716, Reticulocyte Count (Retic) presented a mean of 2.5873 and an SD of 1.51166.

Table 1: Distribution of CBC among the study participants

	Mean	Median	SD	Minimum	Maximum
TLC	7210.332	6790.000	1643.0063	4300.0	11000.0
Platelet count	2.6370	2.6000	.59724	1.50	4.30
RBC count	4.0157	3.7000	4.88114	1.73	75.60
Haemoglobin	8.846	9.000	1.3019	3.3	10.5
MCV	82.885	78.900	11.7559	58.2	115.7
MCH	25.750	26.300	3.9405	15.4	37.2
MCHC	30.371	30.600	2.1017	21.7	33.9
RDW-CV	17.8114	17.1000	3.49716	12.30	29.90
Retic	2.5873	2.2000	1.51166	.30	10.80

The distribution of study participants according to the severity of anemia revealed varying frequencies across different categories. Among the participants, 65 patients, constituting 29.5% of the total, were classified as having mild anemia. A larger proportion of patients, accounted for 136 individuals or 61.8%, fell under the category of moderate anemia. In contrast, a smaller subset of the patients, comprising 19 individuals or 8.6%, were categorized as having severe anemia.

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The distribution of study participants based on PBS characteristics revealed diverse patterns within the population. Among the patients, 122 (55.4%) of the total, exhibited microcytic hypochromic characteristics. A notable proportion of participants, accounting for 49 individuals (22.3%), displayed normocytic normochromic features. Additionally, 27 patients, (12.3%), demonstrated macrocytic characteristics. A smaller subset of the participants, comprising 22 (10.0%), exhibited dimorphic features (figure 1).

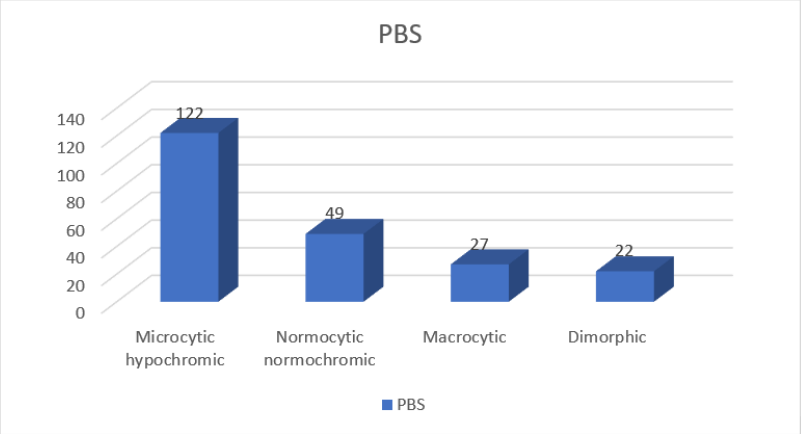

Figure 1: Distribution of study participants according to PBS

Additional findings from peripheral blood smear (PBS) analysis revealed various abnormalities within the population. Among the observed findings, elliptocyte cells were present in 36 (16.4%) participants. Additionally, tear drop cells were observed in 42 participants, representing 19.1% of the population. Target cells were detected in 15 individuals, accounting for 6.8%. Pencil cells were found in 23 participants, making up 10.5% of the population. Moreover, hyper-segmented cells were identified in 5 individuals, comprising 2.3% of participants. Other abnormalities, such as ovalocytes and macro-ovalocytes, were observed in 11 participants, representing 5.0% of the population (Figure 2).

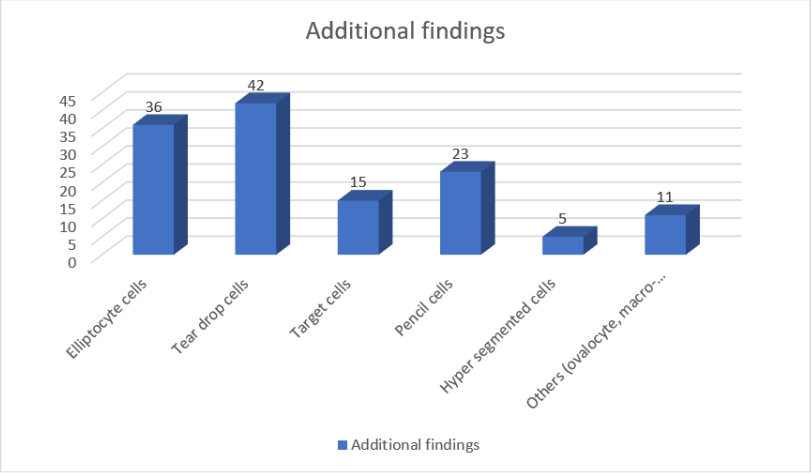

Figure 2: Distribution of study participants according to additional findings

Table 2 depicts the distribution of other laboratory investigations among the study participants including various parameters with their respective mean, median, SD, minimum, and maximum values. Serum iron levels (S. Iron) were measured in 79 participants, with a mean of 34.552 and an SD of 35.5628. The percentage of transferrin saturation (%Transferrin) had a mean of 14.9897 among the same group. Serum transferrin levels (S. transferrin) had a mean of 268.249, while TIBC had a mean of 383.1087. Vitamin B12 levels were measured in 19 participants, with a mean of 165.95, while folate studies had a mean of 1.126 in the same group. Hemoglobin A2 (HbA2) levels were measured in 88 participants, with a mean of 3.033, and Hemoglobin A1c (HbA1c) levels were measured in 89 participants, with a mean of 5.010. The distribution of study participants according to etiology, showcased the frequency and percentage of each category. Among the participants, 98 individuals (44.5%) were attributed to nutritional deficiency as

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the primary etiological factor. Additionally, there were singular cases representing other etiological factors, including Beta thalassemia trait, Hb D Iran, and Hb D Punjab, each accounting for 0.5% of the total participants. The patients in category others included those with normal HPLC studies and the ones whose serum iron profile/serum B12 and folate studies were not available.

Table 2: Distribution of other laboratory investigation among the study participants

Parameters	N	Mean	Median	SD	Minimum	Maximum
S. Iron	79	34.552	22.900	35.5628	3.2	221.2
%Transferrin	79	14.9897	6.4000	40.45025	1.00	351.90
S. transferrin	79	268.249	251.500	119.6072	49.7	599.7
TIBC	79	383.1087	368.1000	155.21327	27.40	803.40
Vit B12	19	165.95	133.00	135.006	108	719
Folate studies	19	1.126	1.000	.1939	.9	1.5
HbA2	89	3.033	3.000	.7214	2.0	7.7
HbA_1c	89	5.010	4.900	.5719	3.7	7.0
A_0	89	85.584	86.200	4.2547	49.0	88.7

Figure 3 presents a comparison of age distribution among different types of morphological patterns of anemia. In the ≤ 20 years age group, the distribution across different types of anemia was as follows: Dimorphic (2, 9.1%), Macrocytic (3, 11.1%), Microcytic hypochromic (8, 6.6%), and Normocytic (4, 8.2%). Similar distributions were observed across the other age groups. In the 21-25 years age group, the distribution was: Dimorphic (4, 18.2%), Macrocytic (11, 40.7%), Microcytic hypochromic (51, 41.8%), and Normocytic (20, 40.8%). For the 26-30 years age group, the distribution was Dimorphic (14, 63.6%), Macrocytic (11, 40.7%), Microcytic hypochromic (43, 35.2%), and Normocytic (20, 40.8%). Lastly, in the >30 years age group, the distribution was Dimorphic (2, 9.1%), Macrocytic (2, 7.4%), Microcytic hypochromic (20, 16.4%), and Normocytic (5, 10.2%). The p-value for the comparison among these groups was 0.379, suggesting no statistically significant difference in the distribution of anemia types in this age group.

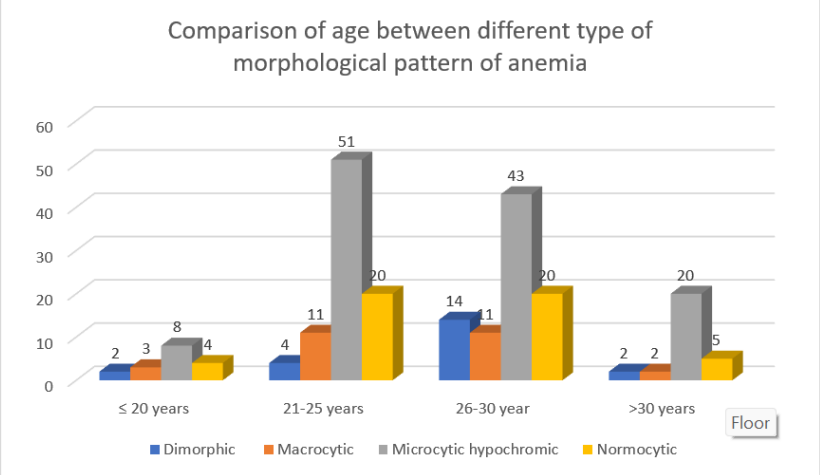

Figure 3: Comparison of gravida between different type of morphological pattern of anemia

Among grand multipara women, the distribution of anemia types was as follows: Dimorphic (2, 9.1%), Macrocytic (0, 0.0%), Microcytic hypochromic (10, 8.2%), and Normocytic (1, 2.0%). In the multipara group, the distribution was Dimorphic (8, 36.4%), Macrocytic (16, 59.3%), Microcytic hypochromic (69, 56.6%), and Normocytic (19, 38.8%). For primipara women, the distribution was Dimorphic (12, 54.5%), Macrocytic (11, 40.7%), Microcytic hypochromic (43, 35.2%), and Normocytic (29, 59.2%). The p-value for the comparison among these groups was 0.041, indicating a statistically significant difference in the distribution of anemia types among different gravida categories (figure 4).

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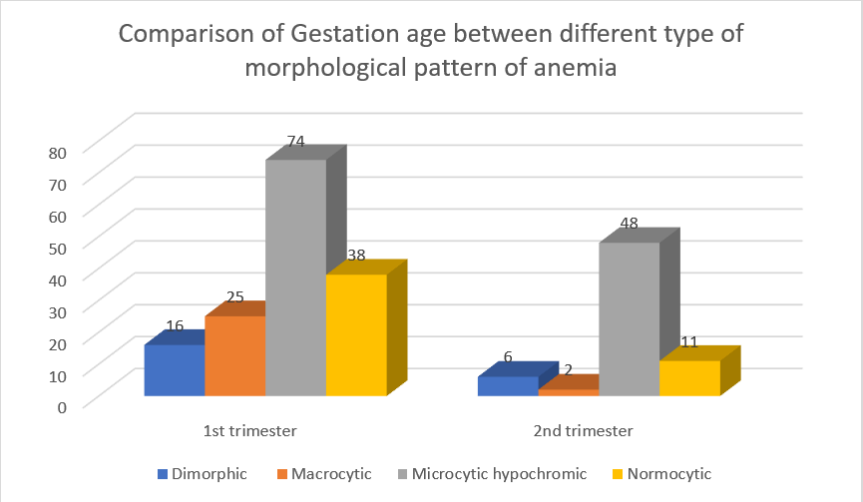

Figure 4: Comparison of gestation between different type of morphological pattern of anemia

On comparing the severity of anemia with different morphological patterns we observed that in the mild category, the distribution across anemia types was Dimorphic (5, 22.7%), Macrocytic (8, 29.6%), Microcytic hypochromic (15, 12.3%), and Normocytic (37, 75.5%). For moderate severity, the distribution was Dimorphic (12, 54.5%), Macrocytic (19, 70.4%), Microcytic hypochromic (93, 76.2%), and Normocytic (12, 24.5%). In the severe category, the distribution was Dimorphic (5, 22.7%), Microcytic hypochromic (14, 11.5%), and there were no cases of severe anemia in the Macrocytic and Normocytic categories. The p-value for the comparison among these groups was 0.001, indicating a statistically significant difference in the severity of anemia among different morphological patterns.

DISCUSSION

Anemia is a significant public health concern, particularly among pregnant women, where it can have serious consequences for both the mother and the developing fetus. Understanding the patterns and etiologies of anemia in this population is crucial for effective prevention and management strategies. This study aimed to study the morphological pattern of anemia in antenatal women using peripheral blood smear analysis, to study the correlation of RBC indices with peripheral blood smear findings and to determine the predominant causes of anemia among the antenatal patients.

Maternal age averaged 26.01 years (SD: 4.052), ranging 18-36 years. Most women (79.1%) were 21-30 years old, typical for childbearing. These findings align with other studies (Abusharib et al [2], Mathur et al [10]). The young age reflects the typical antenatal patient population.

The study cohort comprised 50.9% multiparous, 43.2% primiparous, and 5.9% grand multiparous women. This parity distribution aligns with typical antenatal populations, which include a mix of women with varying pregnancy experiences. Similar findings were reported by Abusharib et al. [2] and Nirmala et al. [11], indicating that the current study's parity distribution is representative of the broader antenatal landscape.

The majority of participants (69.5%) were in their first trimester, while 30.5% were in their second trimester. This gestational age distribution reflects common antenatal care-seeking practices, where initial prenatal visits often occur early in pregnancy. While comparable to studies by Abusharib et al. [2] and Rawat et al. [12], the higher proportion of first-trimester participants in the current study might be attributed to local healthcare-seeking behaviors or study design differences.

The study cohort exhibited a substantial anaemia burden, with 29.5% mild, 61.8% moderate, and 8.6% severe cases. These findings align with those of Rawat et al [12] and Sinha et al [13], indicating a prevalent issue among antenatal women.

Microcytic hypochromic anaemia was the most common morphological pattern (55.5%), followed by normocytic normochromic, macrocytic, and dimorphic types. This distribution is consistent with studies

by Abusharib et al. [2], Nirmala et al. [11], and Ramya et al. [14]. The predominance of microcytic hypochromic anaemia reflects the increased iron requirements of pregnancy [15]. However, the presence of other patterns suggests potential deficiencies in folate and vitamin B12 (Figure 5). Variations in morphological pattern distribution across studies (e.g., Nkwabong et al. [16]) highlight the influence of geographic, socioeconomic, and dietary factors. This emphasizes the need for tailored anaemia management strategies based on specific population characteristics.

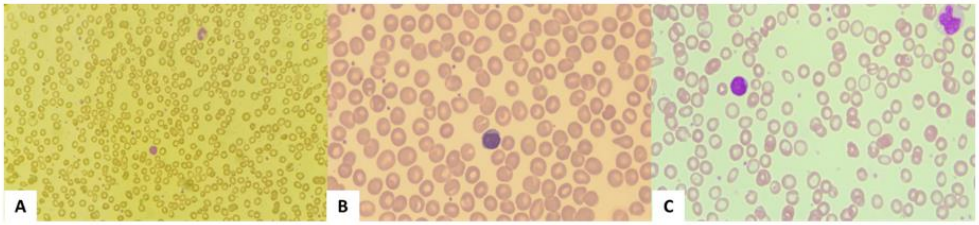

Figure 5: Leishman stained PBFs at 400x magnification showing Microcytic hypochromic blood picture (A, C) and Macrocytic blood picture (B).

Peripheral blood smears revealed diverse red blood cell (RBC) abnormalities. Elliptocytes (16.4%), tear drop cells (19.1%), target cells (6.8%), pencil cells (10.5%), and hyper segmented cells (2.3%) were prevalent. Additionally, 5.0% exhibited ovalocytes or macro-ovalocytes. These findings shed light on the underlying mechanisms of anaemia. The presence of elliptocytes and tear drop cells, often linked to iron deficiency anaemia, supports the study's predominant microcytic hypochromic anaemia. Target cells and hyper segmented cells suggest potential folate or vitamin B12 deficiencies [2, 17, 18]. This approach aligns with previous research. Abusharib et al. and Nirmala et al. also reported similar RBC abnormalities, emphasizing the value of blood smears in assessing anaemia during pregnancy [2, 11].

Iron-related parameters included mean serum iron of 34.552 µg/dL, transferrin saturation of 14.9897%, serum transferrin of 268.249 mg/dL, and TIBC of 383.1087 µg/dL. These indicate iron deficiency, a common cause of microcytic hypochromic anaemia. Similar findings were reported by Hasan et al. and Bushra et al., highlighting the significance of iron deficiency in pregnancy-related anaemia [19, 20]. Vitamin B12 levels averaged 165.95 pg/mL (n=19), while folate levels were 1.126 ng/mL in the same group. Low folate levels suggest potential folate deficiency, especially linked to the dimorphic anaemia pattern. Nkwabong et al. and Abusharib et al. also reported the association of folate deficiency with anaemia [2, 21]. While the sample size for vitamin analysis was limited, the findings emphasize the importance of assessing both iron and vitamin status in understanding anaemia's complexity during pregnancy.

Haemoglobin A2, A1c, and parameter A0 were measured in nearly 90 participants to assess potential genetic or haematological contributors to anaemia (Figure 6). While most results indicated normal haemoglobin profiles, rare instances of beta-thalassemia trait, Hb D Iran, and Hb D Punjab were detected. These conditions, as documented by Petrakas et al [22], and Singh et al. [23], can lead to hereditary anaemias. However, such genetic abnormalities were not the primary drivers of anaemia in this cohort. Instead, nutritional deficiencies emerged as the predominant cause, affecting 64.5% of tested participants. This aligns with extensive research highlighting iron, folate, and vitamin B12 deficiencies as primary culprits in pregnancy-related anaemia. The prevalence of these deficiencies as the root cause underscores the importance of targeted nutritional interventions for anaemia management in this population.

Anaemia in pregnancy is multifaceted. While nutritional deficiencies, primarily iron, folate, and vitamin B12, were the dominant causes in this study, aligning with findings by Nkwabong et al. [16], Rukuni et al. [24], Garzon et al. [25], and Agarwal et al. [26], the complex nature of anaemia was evident. Morphological patterns varied by trimester and anaemia severity. Microcytic hypochromic anaemia, often linked to iron deficiency, was prevalent in severe cases, while dimorphic patterns, suggesting combined deficiencies, were more common in mild anaemia. These findings, similar to Abusharib et al [2]. and Nirmala et al. [11], underscore the importance of comprehensive assessments to guide management. The dynamic changes in maternal physiology throughout pregnancy influence nutrient requirements and anaemia development. Consequently, tailored interventions based on individual needs are crucial for optimal maternal and fetal health.

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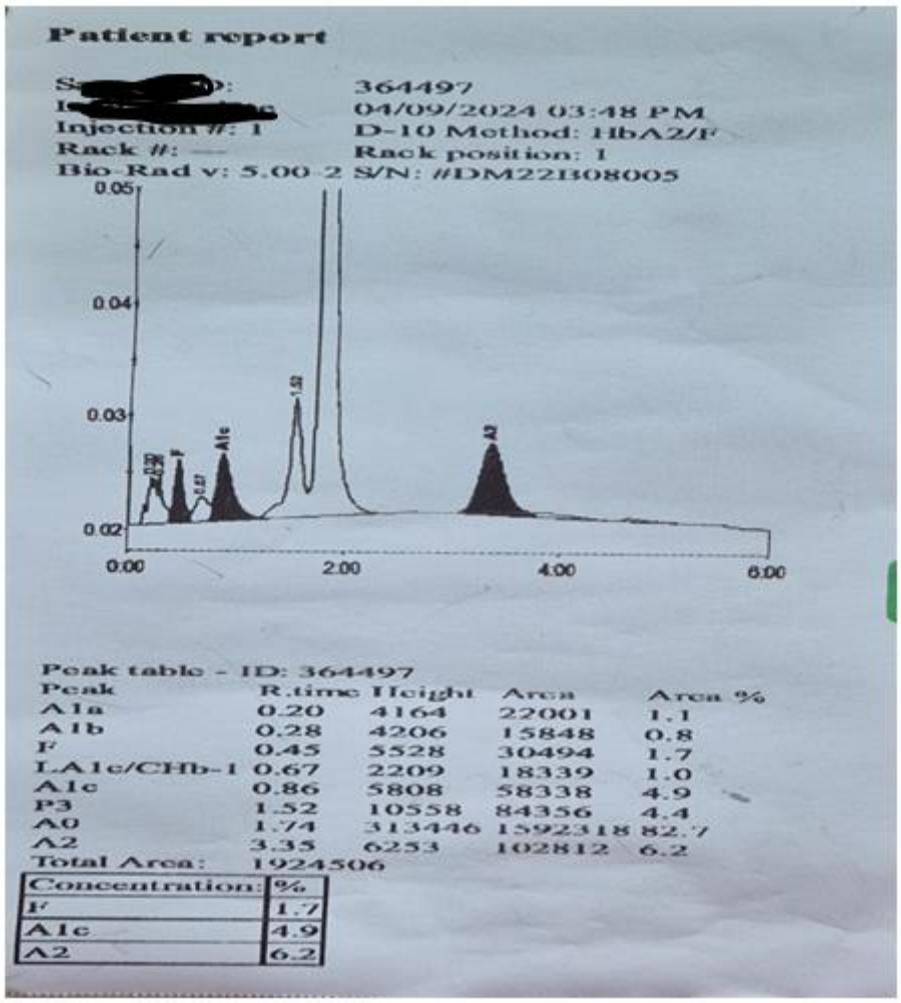

Figure 1: HPLC chart showing raised HBA2 levels.

While nutritional deficiencies, primarily iron, folate, and vitamin B12, remain significant contributors as highlighted by Nkwabong et al. [16], Rukuni et al. [24], Garzon et al. [25], and Agarwal et al. [26], the complex interplay of genetic, physiological, and environmental factors underscores the need for a comprehensive approach and highlights the need for continued research to refine anaemia management strategies. However, the challenge lies in differentiating physiological changes from pathological conditions, as discussed by Agarwal et al. [26]. Comprehensive assessments, including peripheral blood smear analysis and genetic testing, are essential for accurate diagnosis and management. The presence of diverse anaemia patterns, including microcytic hypochromic, macrocytic, and dimorphic types, as reported by Abusharib et al. [2] and Nirmala et al. [11], highlights the importance of comprehensive assessments. Factors like gestational age, anaemia severity, and underlying haematological conditions, such as thalassemia (Petrakas et al. [22]), influence these patterns.

Addressing anaemia requires a multipronged approach. Early identification and management of iron deficiency anaemia, as emphasized by Garzon et al. [25], are crucial. Overall, the current study's findings are largely aligned with the existing literature, reinforcing the understanding of anaemia patterns in antenatal women. The study provides additional insights into the associations between morphological patterns, anaemia severity, and gestational age, which can inform the development of targeted strategies for the prevention and management of anaemia in pregnancy.

CONCLUSION

This study elucidates anemia patterns and etiologies in pregnant women, with microcytic hypochromic anemia predominating due to iron deficiency. However, diverse morphological patterns necessitate a comprehensive assessment approach, including peripheral blood smear analysis and RBC

indices, to identify underlying causes like folate or vitamin B12 deficiencies. Anemia's dynamic nature, varying across trimesters and severity, underscores the need for individualized care and ongoing monitoring. Addressing nutritional deficiencies, hematological conditions, and broader determinants through a multidisciplinary approach is essential for optimal maternal and fetal outcomes. While the study's comprehensive assessment and relatively large sample size contribute to its strengths, limitations include its single-site design, limited data on certain nutrients, and cross-sectional nature. Expanding the study to multiple sites, incorporating longitudinal design, and broadening nutrient assessment are recommended, along with developing multidisciplinary anemia management programs.

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