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Evaluation of Morphological Patterns of Anemia in Females of Reproductive Age Group and Correlation with Red Cell Indices

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Abstract: ***Globally anemia is a public health problem that affecting both the developing and the developed countries. Anemia is defined by a reduction in total amount of hemoglobin or red blood cells' number leading to the insufficient supply of oxygen to meet the physiologic needs of the body. The present study designed to identify Morphological patterns of anemia in females of reproductive age group and correlation with Red Cell Indices. A total of 500 anemic female patients of age 18 - 45 years attending OPD & IPD were included. Out of 500 cases, 80 were pregnant and 420 were non - pregnant females. Out of 420 non - pregnant anemic women; 32.6% were of microcytic hypochromic, followed by 31.2% of normocytic hypochromic, 27.1% of normocytic normochromic, 6.7% of dimorphic and 2.4 % of macrocytic normochromic anemia. Out of 80 pregnant anemic women; majority 35.0% were of microcytic hypochromic, followed by 32.5% of normocytic hypochromic, 22.5% of normocytic normochromic and 5.0% of each category of dimorphic and macrocytic anemia***

Keywords: Microcytic Hypochromic Anemia, Normocytic Normochromic Anemia, Dimorphic Anemia, Macrocytic Anemia

1. Introduction

Globally anemia is a public health problem that affecting both the developing and the developed countries. [1] According to the National Family Health Survey (NFHS - 4), about 40% of women are mildly anemic, 12% are moderately anemic and 1% are severely anemic [2]. According to NFHS - 5, the 57% women are anemic, 26% mildly anemic, 29 % moderately anemic, 3% are severely anemic. Anemia prevalence has increased between NFHS - 4 and NFHS - 5 from 53 % in 2015 - 2016 to 57% in 2019 - 2021 among women. [3] Anemia (from the ancient Greek ἀναιμία, anaimia, meaning "lack of blood") is defined by a reduction in total amount of hemoglobin or red blood cells' number leading to the insufficient supply of oxygen to meet the physiologic needs of the body [4, 5] and can be caused by the multiple factors that include nutritional deficiencies. According to World Health Organization (WHO) the lower limit of normal hemoglobin concentration in women is 12.0 g/dl. [6] In pregnancy, the criteria to diagnose anemia is

- Hemoglobin <11gm% in first and third trimester. [7]
- Hemoglobin <10.5 gm% in second trimester. [7]

The classification of anemia is based on various parameters i. e. haemoglobin, mean cell volume, hematocrit, mean corpuscular volume, red cell distribution width and mean corpuscular haemoglobin concentration. [8] The common tests performed for the evaluation of the cause of anemia are: peripheral blood smear, complete blood cell count, reticulocyte count, red cell indices, folic acid, vitamin B - 12, serum iron studies. The microscopic examination of a peripheral blood smear provides a wealth of information to the clinician. Various forms of anemia may actually be diagnosed from abnormal red cell morphology reported on a blood smear examination. [9] The different forms of anemia are to be diagnosed from abnormal morphology of the red cell which is reported on the basis of examination of blood smear. Evaluation of the red cell morphology is done by noting the change in size, shape, distribution and Hb concentration. [10] The obtained values through the automated haematology analyzer are to be displayed as visual image and in the form of the RBC histogram. Red cell indices (MCV, MCH and MCHC) are mean value, which cannot express the small variation of red cells size, which is accompanied in early iron deficiency. Whereas RDW can expresses the small variation of different population of red cell size. In prelatent and latent iron deficiency, Hb% and MCV are normal. In latent iron deficiency, RDW would be expected to increase because a microcytic population of cells appears in the blood. [11] MCV is increased slightly in normal pregnancy, so may lead to misinterpretation of microcytosis. MCH and MCHC are reducing only when anemia is severe or established iron deficient women entered in pregnancy. [12] As anisocytosis is less prominent in pregnancy and also in early iron deficiency anemia, Peripheral Blood Film (PBF) examination gives less information though it needs expert's opinion. [13] Iron profile is expensive and time consuming. So, the RBC histogram and the red cell indices along with the examination of peripheral smear are essential for the proper diagnosis of anemia. [8] The present study was done to assess the morphological patterns of anemia in females of reproductive age group and its correlation with the Red Cell Indices.

2. Material and Method

This Hospital based prospective study was conducted at Department of Pathology, Muzaffarnagar Medical College, Muzzafarnagar, U. P. for January 21 - june 22. It included 500 females of reproductive age group of 18 - 45 yrs of age in which anemia is diagnosed. The females having hematological malignancies and blood transfusions in past 3 years were excluded from the study.

Method of data collection / procedure -
Blood samples were collected by venepuncture in vaccutainer containing EDTA anticoagulant (3ml) - For red cell indices and peripheral smear examination. Complete blood count was done on automated hematology analyzer (Nihon Kohden), based on RBC indices anemia is typed as microcytic hypochromic, normocytic normochromic, normocytic hypochromic, macrocytic, dimorphic. Smears were prepared and stained for general blood picture morphological typing of anemia. Suitable statistical significance test was used along with SPSS 17/20 and statistical software. The p - value 0.05 was considered for statistical analysis.

3. Results

The age of the 500 patients included in this study ranged from 18 - 45 years. Out of them, 132 (26.4%) were between 18 - 25 years, 226 (45.2%) were between 26 - 35 years, 142 (28.4%) were between 36 - 45 years. [table 1] Out of these 500 anemic women, 420 (84.0%) were non - pregnant and 80 (16%) were pregnant. Out of 420 cases of non - pregnant women, majority 259 (61.71%) of them had moderate degree of anemia, 85 (20.2%) of them had milder degree of anemia and 76 (18.1%) of them had severe anemia. [table 2]. Out of 80 cases of pregnant women, majority 39 (48.8%) of them had moderate degree of anemia, 35 (43.7%) of them had milder degree of anemia and 6 (7.5%) of them had severe anemia. [table 3]. Morphological classification of the patients revealed that, out of 420 non - pregnant anemic women; majority 137 (32.6%) were of microcytic hypochromic, 131 (31.2%) of normocytic hypochromic, 114 (27.1%) of normocytic normochromic, 28 (6.7%) of dimorphic and 10 (2.4 %) of macrocytic normochromic anemia. [table 4]. Out of 80 pregnant anemic women; majority 28 (35.0%) were of microcytic hypochromic, 26 (32.5%) of normocytic hypochromic, 18 (22.5%) of normocytic normochromic and 4 (5.0%) of each category of dimorphic and macrocytic anemia [table 4]. Out of 500 anemic subjects, majority of cases 317 (63.4%) showed RDW >14.5%, followed by 178 cases (35.6%) in the range of 11.5 - 14.5 % and remaining 5 cases (1.0%). Out of 500 anemic cases, Maximum cases 303 (60.6%) had MCV < 80 fl, followed by 183 cases (36.6%) with MCV 80 - 100 fl and remaining 14 cases (2.8%) with MCV >100 fl. Maximum cases 346 (69.2%) had MCH < 27pg, followed by 136 cases (27.2%) with MCH 27 - 32 pg and remaining 18 cases (3.6%) with MCH > 32 %. MCHC ranged between 31 - 35% was the most common finding seen in 377 (75.4%) cases, followed by 120 cases (24.0%) with MCHC < 31% and remaining 3 cases (0.6%) with MCHC > 35%.

Table 1: Distribution of study population according to age:

Age groups (in years)	No. of Cases (n)	%
18 - 25	132	26.4
26 - 35	226	45.2
36 - 45	142	28.4
Total	500	100.0

Table 2: Distribution of study population according to Hb levels among non - pregnant women

Hb level (g/dl)	No. of cases (n)	%
Mild (11 - 11.9)	85	20.2
Moderate (8 - 10.9)	259	61.7
Severe (< 8)	76	18.1
Total	420	100.0

Table 3: Distribution of study population according to Hb levels among pregnant women

Hb levels (g/dl)	No. of cases (n)	%
Mild (10 - 10.9)	35	43.7
Moderate (7 - 9.9)	39	48.7
Severe (<6.9)	6	7.5
Total	80	100.0

Table 4: Distribution of study population according to morphological type of anemia among pregnant and non - pregnant women

Type of Anemia	Non – pregnant women		Pregnant women	
	No. of cases (n)		No. of cases (n)	
	No. of cases (n)	%	No. of cases (n)	%
Microcytic Hypochromic	137	32.6	28	35.0
Normocytic Hypochromic	131	31.2	26	32.5
Normocytic Normochromic	114	27.1	18	22.5
Dimorphic (microcytic + macrocytic)	28	6.7	4	5.0
Macrocytic Normochromic	10	2.4	4	5.0
Total	**420**	**100.0**	**80**	**100.0**

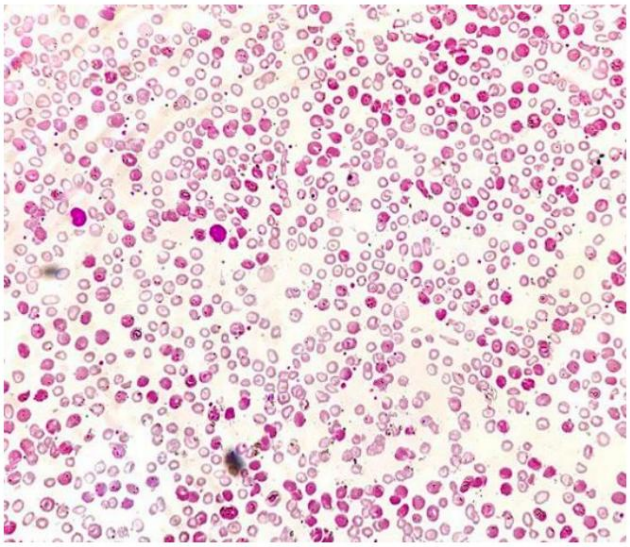

Image 1: Photomicrograph - Microcytic Hypochromic Blood Picture along with Target cells and Pencil cells

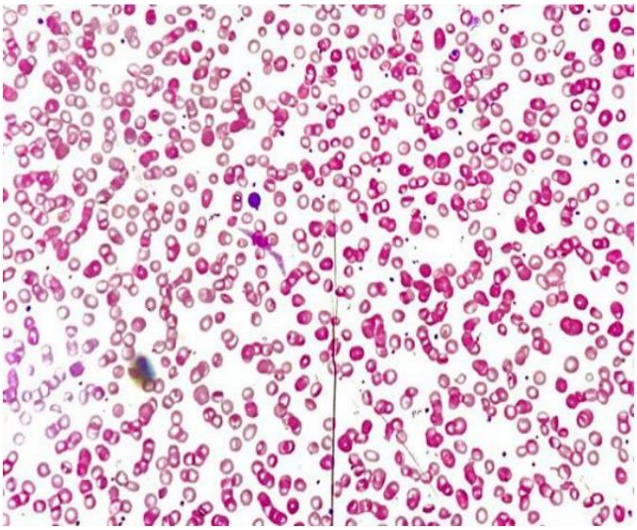

Image 2: Photomicrograph – Normocytic Hypochromic Blood Picture

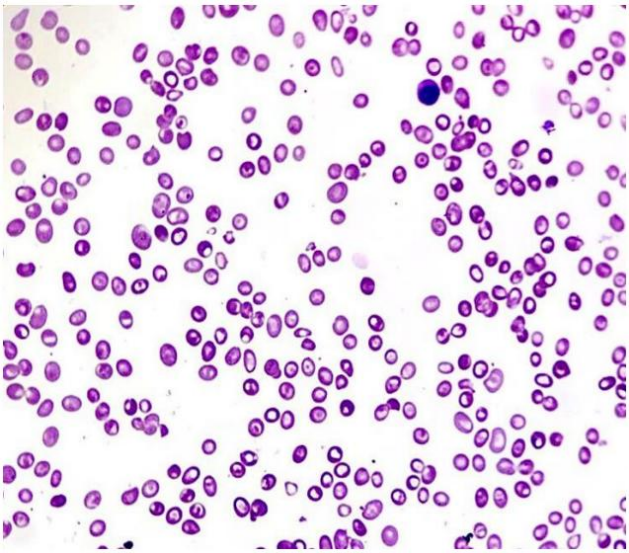

Image 3: Photomicrograph - - Dimorphic Blood Picture (Microcytic and Macrocytic)

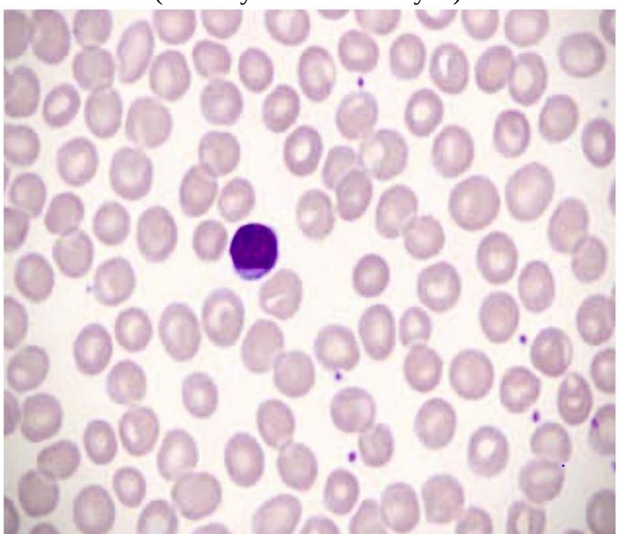

Image 4: Photomicrograph - - Macrocytic Blood Picture

4. Discussion

Anemia is of moderate public health importance among women, and of mild public health importance in men, in rural and urban populations, based on the WHO prevalence - based classification. It is a persistently widespread health issue that affects women of reproductive age and is a major contributor to the high morbidity and death rates that are observed among females. The present study was conducted on 500 anemic women of reproductive age groups who came to the central lab of pathology of Muzaffarnagar Medical College, Muzaffarnagar from January 2021 to June 2022.

Distribution of study population

The present study was conducted on 500 anemic women, out of 420 (84.0%) were non - pregnant and 80 (16%) were pregnant. Among 80 pregnant women 10 (12.5%) were in 1st trimester, 50 (62.5%) were in 2nd trimester and 20 (25.0%) were in 3 rd trimester. Similarly Gautam et al (2000) reported 96.5% prevalence of anemia in antenatal women in 12 - 20 weeks of gestation in a rural area of Delhi, such a high prevalence in second trimester could be because of occurrence of dilution during this phase.

Age wise distribution of cases

In present study, we studied 500 anemic women, ranging from 18 - 45 years of age. It was seen that most of the anemic women 226 (45.2%) were in the age group of 26 - 35 years followed by 142 (28.4%) anemic women in 36 - 45 years of age group and 132 (26.4%) anemic women in 18 – 25 years of age group. Similar to present study; Hafiz et al (2019) who conducted the study on the patients' aged 21 - 86 years, and found that the mean age was 41.3 years and majority 58% were in the age group of 21 - 40 years of age group. This resemblance might result from a connection between this age group's potential higher nutritional requirements and parity. In contrast to our study; Mishra P et al. (2012) conducted his study in women of reproductive age group (15 - 45 years) in PHC Barara of the Ambala district and found that majority (63.9%) of the anemic women were in the age group of 15 - 30 years. According to Bhargava et al. (2021) the most affected age group were 15 - 25 years of age both in females (45.5%) and males (47.3%).

Categorisation of Anemia in non - pregnant and pregnant women

In present study, 500 cases of anemic females were studied and was divided into pregnant and non - pregnant females. Out of 420 cases of non - pregnant women, moderate anemia was found to be the most common category 259 cases (61.71%) it was followed by mild anemia in 85 cases (20.2%) and remaining 76 cases (18.1%) were of severe anemia. Among 80 pregnant anemic women, the most common category was moderate anemia 39 cases (48.7%) which was followed by mild anemia 35 cases (43, 7%) and remaining 6 cases (7.5%) were of severe anemia. Among pregnant women, moderate anemia was found to be the most common. Hb concentrations fluctuate during pregnancy in healthy women who have enough iron reserves to meet the growing maternal blood volume and foetal metabolism. Similar to the present study - Rawat et al. (2016) showed that the hemoglobin concentration ranged from 1.7 - 15.5 gm/dl with a mean of 10.7 gm/dl. The percentages of mild,

moderate and severe anemia were 35.1%, 51.3% and 13.4% respectively. Similar to the present study moderate anemia was found to be more prevalent by Toteja et al: who reported in his study population 60.1% had moderate anemia and 13.1% had severe anemia. The other similar study is Qadir et al. (2022) who conducted his study on women of reproductive age (15 - 49 years), 75% of females were non - anemic, and among anemic women 13% were moderately anemic followed by 10% mildly anemic and 2% severely anemic. They all reported that moderate anemia is the most common anemia among the reproductive age women. In contrast to our study - Faiza Hafiz et al. (2019) found that majority of the patients (45%) had mild degree of anemia with haemoglobin levels more than 9gm/dl. Few other studies which are discordant with our present study - ME Bentley and PL Griffiths et al. (2002) found that predominantly (32.4%) women had mild anemia, (14.19%) had moderate anemia and (2.2%) had severe anemia. They all reported that mild anemia was the most common type and this difference may be due to difference in selection criteria. Haemoglobin concentration falls during pregnancy principally as a result of hemodilution.

Red cell Distribution Width

In our study, out of 500 anemic subjects, majority of cases 317 (63.4%) showed Red cell Distribution Width (RDW) >14.5%, followed by 178 cases (35.6%) in the range of 11.5 - 14.5 % and remaining 5 cases (1.0%) Similarly: Mukesh Chaudhary et al. (2015) advised that the RDW be employed as a powerful instrument for the early detection of iron deficiency anemia in a large number of samples at big hospitals, saving time and labor.

Haematological indices

In our study, out of 500 anemic cases; analysis of red cell indices showed that MCV ranged from 48 – 110 fl. Maximum cases 303 (60.6%) had MCV < 80 fl, followed by 183 cases (36.6%) with MCV 80 - 100 fl and remaining 14 cases (2.8%) with MCV >100 fl. MCH ranged from 13 – 38 pg. Maximum cases 346 (69.2%) had MCH < 27pg, followed by 136 cases (27.2%) with MCH 27 - 32 pg and remaining 18 cases (3.6%) with MCH > 32 %. MCHC ranged between 31 - 35% was the most common finding seen in 377 (75.4%) cases, followed by 120 cases (24.0%) with MCHC < 31% and remaining 3 cases (0.6%) with MCHC > 35%. The other study which is in line, Singla et al. (2017) found that MVC < 80 fl in 49.2% cases, normal in 44.4% cases and in 6.4% MCV is > 100fl. So majority of anemic cases showed < 80 fl MCV. Thus on the basis of automatic analyser, it was found that majority of cases had low MCV, MCH, MCHC favouring Microcytic hypochromia anemia.

Types of Anemia

Some commonly employed strategies to tackle anemia include fortification, diet diversification, iron and folic acid supplementation and nutrition education and counseling Despite of all these strategies, in our study, Out of those 420 non - pregnant females, the maximum cases of anemia was of Microcytic hypochromic 137 cases (32.6%), followed by Normocytic hypochromic 131 cases (31.2%), Normocytic normochromic 141 cases (27.1%), Dimorphic anemia 28 cases (6.7%) and remaining (2.4 %) 10 cases were Macrocytic. In our study, out of 80 pregnant anemic females, the maximum cases of anemia was of Microcytic hypochromic 28 cases (35.0%), followed by Normocytic hypochromic 26 cases (32.5%), 18 cases (22.5%) of Normocytic normochromic and remaining (5.0%) 4 cases each was of Dimorphic and Macrocytic anemia. Majority of the cases were Microcytic hypochromic in both pregnant and nonpregnant women. Almost similar results were obtained by, Bhargava et al. (2021) who observed that the most common morphological typing of anemia in females was Microcytic hypochromic i. e.68.5% The other similar studies were - Singla et al. (2017) stated that the most common morphological type of anemia was Microcytic hypochromic (76.4%) followed by Normocytic normochromic anemia. Chanchal Ashok et al (2019) who studied 100 cases and concluded that the most common anemia was Microcytic hypochromic 62.0%, followed by 18% Normocytic normochromic, 14% Dimorphic and remaining 6.0% were of Macrocytic. Similarly: Ramya et al (2016); concluded that the most common morphological pattern of anemia prevalent in females was Microcytic hypochromic. The high prevalence of microcytic anemia emphasises the necessity for iron supplementation to pregnant and non - pregnant women in reproductive age group. The study which was in contrast to our study is - Hafiz et al. (2019) who concluded that Normocytic normochromic anemia was the most common pattern of anemia seen in 40% patients, this was followed by Microcytic hypochromic anemia seen in 29% cases, Macrocytic anemia in 10 % cases and Dimorphic anemia was seen in 14 % cases. This difference in the study may be due to the diference in the selection criteria and is not statistically significant. According to trimester wise, the typing of anemia: Out of 80 anemic cases maximum cases 50 (62.5%) were in second trimester, followed by 20 cases (20.0%) in third trimester and remaining 10 cases (12.5%) in first trimester. Among 10 anemic cases in first trimester, 4 cases (40%) were found to be Normocytic normochromic, followed by 2 cases (20%) of each Microcytic hypochromic and Normocytic hypochromic and 1 case (10%) was of Dimorphic and remaining 1 case (10%) was Macrocytic. Thus it could be said that Normocytic normochromic was found to be more common in first trimester. Out of 50 cases in second trimester, maximum cases 19 (38%) were found to be Microcytic hypochromic, followed by 17 cases (34%) of Normocytic hypochromic, 10 cases (20%) of Normocytic normochromic, and 2 cases (4.0%) of each type including Dimorphic and Macrocytic anemia. Thus it could be said that Microcytic hypochromic was found to be more common in second trimester because of increase demands. Out of 20 cases in third trimester, maximum cases 7 (35.0%) of each type including Microcytic hypochromic and Normocytic hypochromic, followed by 3 cases (15.0%) of Normocytic normochromic, 2 cases (10.0%) of Macrocytic and remaining 1 case (5.0%) of Dimorphic anemia. p - value = 0.75

5. Conclusions

Our study concluded that anemia is major health problem in reproductive age group females. Typing of anemia based on both morphology on PBS and RBC indices an important step for the management of anemia. Furthermore there is a need for further caqtegorisation to know the cause of anemia, so

that necessary steps can be taken for the management and reducing the prevalence of anemia.

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