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# RED CELL INDICES IN MEN AND WOMEN WITH NORMAL AND LOW PLASMA IRON LEVELS

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# ЕРИТРОЦИТАРНІ ПОКАЗНИКИ У ЧОЛОВІКІВ І ЖІНОК З НОРМАЛЬНИМ ТА НИЗЬКИМ РІВНЕМ ЗАЛІЗА У ПЛАЗМІ КРОВІ

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## ABSTRACT

**The aim** of this study was to analyze changes in morphological blood parameters in women and men with reduced and normal iron levels. In this study, morphological blood parameters such as the count of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were studied in four groups of individuals (women with normal iron levels; women with reduced iron levels; men with normal iron levels; men with reduced iron levels).

**Methodology.** This study was carried out in a group of 203 individuals. The group of women participating in the study consisted of 106 individuals (52.2 %), while the group of men consisted of 97 individuals (47.8 %). After analysis of plasma iron levels, all patients were divided into the following groups: 1) women with normal iron levels (37-145 μg/dl, n = 48); 2) women with reduced iron levels (< 37 μg/dl; n = 58); 3) men with normal iron levels (59-158 μg/dl, n = 41); 4) men with reduced iron levels (< 59 μg/dl, n = 56). In each group of individuals, the number of erythrocytes and erythrocyte parameters was determined. Plasma iron was assessed using a substrate method. Hematological measurements were made in fresh venous blood. Hematology parameters were determined on an ABX Pentra DF120 hematology analyzer (Horiba ABX).

**Scientific novelty.** Erythrocyte indices analyzed in the blood of women with reduced iron levels compared to women with normal iron levels showed lower values of hemoglobin, hematocrit, MCV, MCH, and MCHC in the blood. Increased values of RDW and the count of erythrocytes in the blood of women with reduced iron levels compared to the control group of women were noted. Similarly, when comparing the values of erythrocyte indices obtained in the group of men with reduced iron levels to the control group of men with normal iron levels, reduced values of MCH, MCV, and MCHC were demonstrated. However, the values of the count of erythrocytes, RDW, hematocrit, and hemoglobin levels were elevated compared to the control. The reverse trend in erythrocyte indices such as hemoglobin and hematocrit indices between the group of women and the group of men with reduced iron levels was observed. Comparing the obtained values with the reference values, it was noted that the reduced values of the count of erythrocytes, and the level of hemoglobin and hematocrit were obtained in all study groups. An increased MCV value compared to the reference values was noted in the group of women and men with normal iron levels. Men with normal iron levels had elevated MCH values. In all studied groups, an increased level of RDW was noted compared to reference values.

**Conclusions.** Erythrocyte count, hemoglobin concentration, and certain erythrocyte indices (MCV, MCH, MCHC, and RDW) can be additional indices in the diagnosis of iron deficiency state both in men and women. It should be emphasized that even in non-anemic patients with erythrocyte count, hemoglobin concentration, and MCV, MCH, and MCHC above the lower limit of normal, the concentration of iron in the plasma could be lower than the reference values.


**Key words:** iron concentration, erythrocyte count, hematocrit, hemoglobin, erythrocyte indices


## АНОТАЦІЯ

**Метою** цього дослідження був аналіз змін морфологічних показників крові у жінок і чоловіків зі зниженим і нормальним рівнем заліза. У цьому дослідженні морфологічні показники крові, такі як кількість еритроцитів (RBC), концентрація гемоглобіну (HGB), гематокрит (HCT), середній об'єм еритроцита (MCV), середній вміст гемоглобіну в еритроцитах (MCH), середня концентрація гемоглобіну в еритроцитах (MCHC) і відносна ширина

розподілу еритроцитів по об'єму (RDW) вивчали в чотирьох групах осіб (жінки з рівнем заліза в нормі в плазмі; жінки з рівнем заліза нижче норми; чоловіки з рівнем заліза в нормі; чоловіки з рівнем заліза нижче норми).

**Методологія.** Це дослідження проводилося в групі 203 осіб. Група жінок, які брали участь у дослідженні, склала 106 осіб (52,2 %), а група чоловіків – 97 осіб (47,8 %). Після аналізу рівня заліза в плазмі всі пацієнти були розподілені на такі групи: 1) жінки з рівнем заліза в нормі (37-145 мкг/дл, n = 48); 2) жінки з рівнем заліза нижче норми (< 37 мкг/дл; n = 58); 3) чоловіки з рівнем заліза в нормі (59-158 мкг/дл, n = 41); 4) чоловіки з рівнем заліза нижче норми (< 59 мкг/дл, n = 56). У кожній групі визначали кількість еритроцитів і еритроцитарні показники. Залізо в плазмі оцінювали субстратним методом. Вимірювання гематологічних показників визначали на гематологічному аналізаторі ABX Pentra DF120 (Horiba ABX) у свіжій венозній крові з етилендіамінтетраоцтовою кислотою ($K_3$-EDTA).

**Наукова новизна.** Аналіз еритроцитарних показників крові жінок із дефіцитом заліза порівняно з жінками з рівнем заліза в нормі показав нижчі значення рівня гемоглобіну, гематокриту, MCV, MCH та MCHC у крові. У жінок з дефіцитом заліза відмічено підвищення значень RDW та кількості еритроцитів у крові порівняно з контрольною групою жінок. Так само при порівнянні значень еритроцитарних показників, отриманих у групі чоловіків із дефіцитом заліза, з контрольною групою чоловіків з рівнем заліза в нормі було відмічено знижені значення MCH, MCV та MCHC. Проте кількость еритроцитів, значення RDW, гематокриту та рівня гемоглобіну були підвищені порівняно з контролем. Спостерігалася зворотна динаміка еритроцитарних показників, таких як рівень гемоглобіну і гематокрит, між групою жінок і групою чоловіків із дефіцитом заліза. Порівнюючи отримані значення з показниками норми, відзначено, що знижені значення кількості еритроцитів, рівня гемоглобіну та гематокриту отримані в усіх досліджуваних групах. У групі жінок і чоловіків з нормальним рівнем заліза відзначено підвищення значення MCV порівняно з референтними значеннями. Чоловіки з рівнем заліза в нормі мали підвищені значення MCH. В усіх досліджуваних групах відзначено підвищення рівня RDW порівняно з референтними значеннями.

**Висновки.** Додатковими показниками в діагностиці залізодефіцитного стану як у чоловіків, так і у жінок можуть бути кількість еритроцитів, концентрація гемоглобіну та деякі еритроцитарні індекси (MCV, MCH, MCHC, RDW). Слід підкреслити, що навіть у пацієнтів без анемії з кількістю еритроцитів, концентрацією гемоглобіну та показниками MCV, MCH і MCHC, які знаходяться вище нижньої межі норми концентрація заліза в плазмі може бути нижчою за референтні значення.

**Ключові слова:** концентрація заліза, кількість еритроцитів, гематокрит, гемоглобін, еритроцитарні показники

## Introduction

Iron deficiency is the leading cause of anemia and is a serious public health problem worldwide (Pasricha et al., 2021). Since approximately two-thirds of the total iron in the body is used in the synthesis of hemoglobin, its deficiency affects the production of red blood cells (Koury and Ponka, 2004). Approximately three billion people worldwide suffer from iron deficiency anemia, which is caused by iron intake being less than required (Ning and Zeller, 2019). Iron deficiency can also result from blood loss, gastrointestinal bleeding, blood donation, or pregnancy. Iron deficiency anemia can also be caused by cancer (of the esophagus, stomach, or colon) (Pasricha et al., 2021). Children and women are much more likely to be iron deficient (Percy et al., 2017; Means, 2020). Iron deficiency can also result from premature birth, poor growth, and cognitive development, and affects the nervous system. Patients presenting to physicians may experience symptoms related to anemia, which include chronic fatigue, poor exercise tolerance, headaches, and difficulty concentrating (Andrews, 2008; Chifman et al., 2014).

Untreated iron deficiency anemia can lead to serious complications such as irregular heartbeat, angina pectoris, heart attack, low birth weight, increased risk of infection, and delayed growth (Andrews, 1999). Changes in diet and iron supplementation can help treat mild iron deficiencies, while severe cases of iron deficiency may require red blood cell transfusions, intravenous iron, or iron injections (Pasricha et al., 2021).

Iron-resistant iron deficiency anemia is caused by a rare mutation in the TMPRSS6 gene, which encodes Matriptase-2 and is expressed in the liver. This mutation leads to a decrease in TMPRSS6 activity and, consequently, to an increase in hepcidin concentration. As a result, the absorption of iron from the intestine and the release of iron from macrophages is inhibited, causing severe iron deficiency (Ganz, 2011; Hentze et al., 2014). High levels of hepcidin block intestinal iron absorption and iron recycling by macrophages, causing iron-restricted erythropoiesis and anemia. Low levels of hepcidin help supply iron to the bone marrow for hemoglobin synthesis and red blood cell production. Extended erythropoiesis after hemorrhage or treatment with erythropoietin

blocks hepcidin by sharply reducing transferrin saturation and releasing the erythroblast hormone and the hepcidin inhibitor erythroferrone (Pagani et al., 2019).

Anemia due to chronic inflammation, also called chronic anemic disease (ACD), is a systemic iron disorder and occurs in association with malignancies, chronic infections, trauma, inflammatory disorders, and organ failure (Andrews, 2004). Iron stores in chronic anemic disease are not depleted, but iron is cumulated in macrophages. In addition, reduced iron absorption inhibits hemoglobin synthesis. Serum iron deficiency is a consequence of an increase in hepcidin in response to inflammation, which may be an attempt to limit the availability of iron to invasive microorganisms and tumor cells (Nemeth et al., 2003). Hepcidin production is induced by the inflammatory cytokine interleukin-6 (IL-6), bacterial pathogens, and lipopolysaccharides (Nemeth et al., 2004). Chronic anemia is considered mild to moderate anemia, and its treatment usually focuses on iron supplementation (Chifman et al., 2014).

Paying attention to the current issue of iron deficiency and the occurrence of iron deficiency anemia among different age groups, the aim of this study was to analyze changes in morphological blood parameters in women and men with reduced and normal iron levels. In this study, morphological blood parameters such as the count of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were studied in four groups of individuals (women with normal iron levels; women with iron levels below the norm; men with normal iron levels; men with iron levels below normal).

## Materials and methods

***Participants.*** The participants of the study were recruited among patients of non-public Health Care Center U & O Zdrowie – Home-based long-term care (Lębork, Poland). A detailed medical history was taken, and a physical examination was performed on all participants. The Research Ethics Committee of the Regional Medical Commission in Gdańsk (Poland) approved the study (KB-21/19). All patients provided written informed consent before the start of the study procedures. This study was carried out in a group of 203 individuals. The group of women participating in the study consisted of 106 individuals (52.2 %), while the group of men consisted of 97 individuals (47.8 %). The mean age of individuals who participated in the study was (44.85 ± 6.74) years for women, and (47.13 ± 5.92) years for men.

After analysis of plasma iron levels, all patients were divided into the following groups: 1) women with normal iron levels (37-145 μg/dl, n = 48); 2) women with iron levels below the norm (< 37 μg/dl; n = 58); 3) men with normal iron levels (59-158 μg/dl, n = 41); 4) men with iron levels below normal (< 59 μg/dl, n = 56). In each group of patients, the number of erythrocytes and erythrocyte parameters was determined.

***Blood samples.*** Blood samples were collected into commercial tubes after overnight fasting for the analysis of laboratory parameters. Venous blood samples (5 ml) were obtained from the capital vein of each participant using sterile disposable plastic syringes. Specimens were collected at the same standardized time to minimize any effect of diurnal variation. Hematological measurements were made in fresh venous blood with ethylenediaminetetraacetic potassium ($K_3$-EDTA). The analytical material for the determination of the iron level was plasma collected on heparin.

***Determination of the iron level.*** Plasma iron was assessed using a substrate method. Iron levels were determined on a Roche/Hitachi cobas® Integra 8000 analyzer. The method used to determine the iron concentration is the Guanidine/FerroZine® method, in which iron ($Fe^{3+}$) after being released from transferrin by guanidine tetrachloride, is then reduced to $Fe^{2+}$ by ascorbate and hydroxylamine. Iron ions $Fe^{2+}$ then form a chelating complex with FerroZine®. This complex is red in color and, in order to avoid interference with copper, $Ca^{2+}$ ions are then bound by thiourea. The color intensity developed during the reaction is directly proportional to the concentration of iron in the tested sample. The cobas® Integra 8000 analyzer automatically dispenses both reagent and test material and measures absorbance.

***Hematological indices.*** Hematology parameters were determined on an ABX Pentra DF120 hematology analyzer (Horiba ABX). The

essence of the quantitative determination of morphotic elements of peripheral blood is based on the conductometric method. This method is based on the change in impedance caused by the passage of blood cells through calibrated microapertures. The blood sample is diluted with a fluid with electrolytic properties. The electrical resistance of the diluent is much lower than that of blood cells. The solution is vacuum sucked through the micro diaphragm. Two electrodes are placed on either side of the diaphragm. Current flows between the two electrodes continuously. When a blood cell appears in the aperture of the diaphragm, the electrical resistance between the electrodes increases in proportion to the volume of the blood cell. The generated pulses of very low voltage are amplified and shaped by the electronic system. This system eliminates interference pulses with a value lower than the set switching threshold. The analyzer uses two measuring chambers: one for counting leukocytes, and the other for counting erythrocytes and platelets. Reagents used: ABX Diluent, ABX Basolyse, ABX Cleaner, ABX Alphalyse, ABX Leucodiff, ABX Lysebio. Well-mixed samples with blood were inserted into the analyzer to determine the morphological parameters. The morphological study of peripheral blood included the following parameters: count of red blood cells (RBC); hemoglobin concentration (HGB); hematocrit (HCT); mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); red cell distribution width (RDW).

***Statistical analysis.*** The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the erythrocyte parameters (significance level, $p < 0.05$) was examined using the Mann-Whitney U test and the Kruskal-Wallis test by rank (Zar, 1999). All statistical calculations were performed on separate data from each individual with STATISTICA 13.3 software (TIBCO Software, Poland).

## Results and discussion

The level of iron in the plasma of women and men with normal and reduced iron levels is shown in Fig. 1.

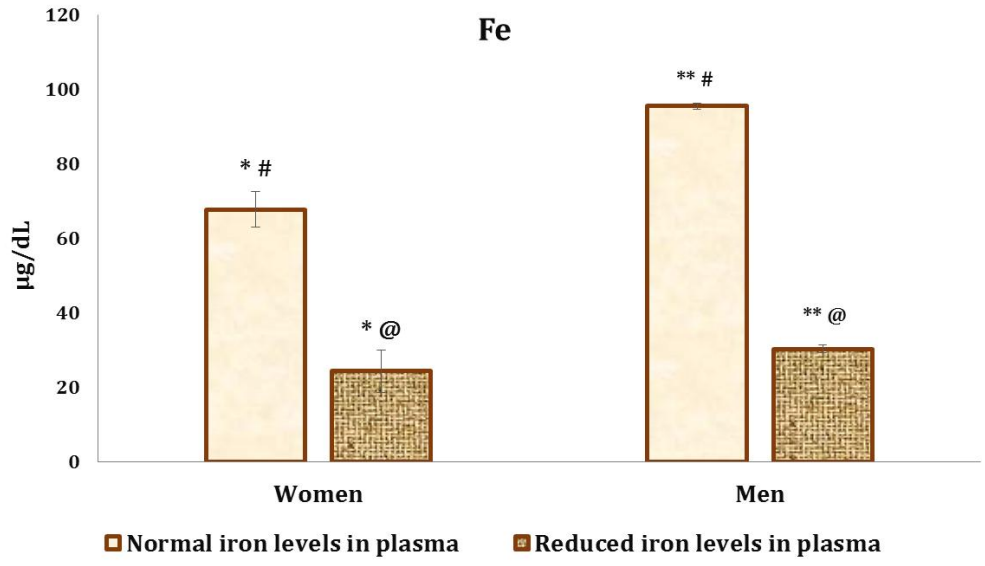


**Fig. 1. The level of iron in the plasma of women and men with normal and reduced iron levels.**

*** – statistically significant changes between the means in the groups of women with normal and reduced iron levels;**
**** – statistically significant changes between the means in the groups of men with normal and reduced iron levels;**
**# – statistically significant changes between the means in the groups of women and men with reduced and normal iron levels;**
**@ – statistically significant changes between the means in the groups of women and men with reduced and normal iron levels**

According to the reference values, the normal plasma iron level in women is 37-145 µg/dl, and in men 59-158 µg/dl. According to the results obtained in our study, it was noted that the highest plasma iron level was obtained by a group of men with normal iron levels (95.46 ± 5.74 µg/dl). Slightly lower iron levels were noted in the group of women with normal iron levels (67.69 ± 4.70 µg/dl). Iron-deficient men had plasma iron values of (30.24 ± 1.05 µg/dl), while iron-deficient women had iron levels at (24.33 ± 0.83 µg/dl). Analyzing the groups of women among themselves, a significantly higher level of iron was recorded in the plasma of women with normal iron levels and amounted to 178% ($p < 0.05$) compared to those who possessed reduced iron levels. Similarly, men with normal iron levels obtained three times higher plasma iron levels than men with its deficiency, and these changes are statistically significant ($p < 0.05$). Comparing the group of women with the group of men with normal iron levels, a higher value of iron in the blood was recorded in the group of men (by 41%, $p < 0.05$). However, the analysis of the group of women compared to the group of men with iron deficiency showed that the level of iron in the plasma was higher by 24% in the group of men and these changes were statistically significant ($p < 0.05$) (Fig. 1).

Erythrocytes, otherwise known as red blood cells, are specialized blood cells whose goal is to transport oxygen from the lungs to the rest of the body's tissues. Too high values of erythrocytes most often indicate dehydration of the patient, i.e. a situation in which the amount of water in the blood decreases, which increases the amount of morphotic elements. An increase in the number of erythrocytes can also lead to chronic hypoxia of the body, most often in people who smoke cigarettes, habitat in areas located at significant geographical heights, as well as in people struggling with heart defects or lung diseases that lead to disorders of gas exchange processes in the blood. Rare problems contributing to a high number of erythrocytes are cancers that produce compounds that increase the intensity of hematopoietic processes or treatment with the use of glucocorticoids (www.diag.pl).

The number of erythrocytes in the blood of men and women with normal and reduced iron levels is shown in the figure 2.

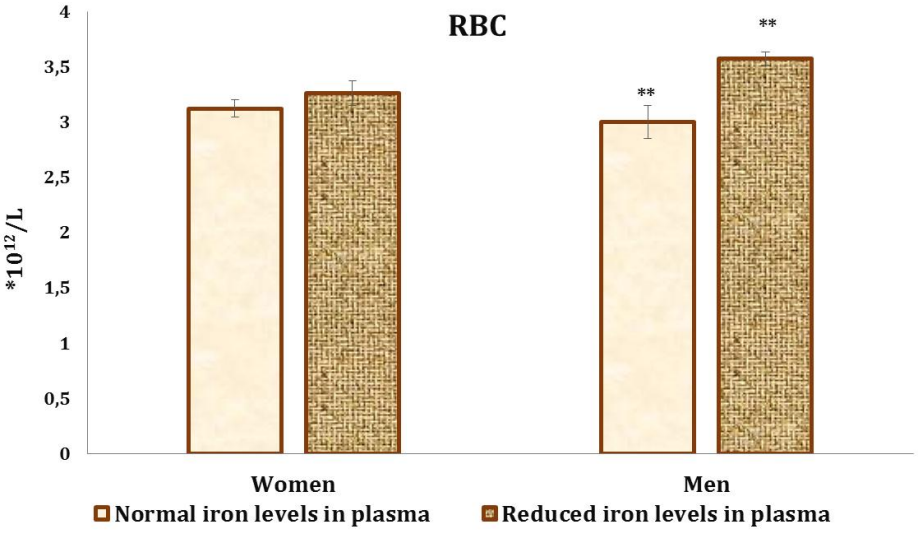


**Fig. 2. The number of erythrocytes in the blood of men and women with normal and reduced iron levels.**

**** – statistically significant changes between the mean in the groups of men with normal and reduced iron levels**

According to the results of our study, the highest number of erythrocytes was recorded in the blood of women (3.26 ± 0.15 M/ml) and men with reduced iron levels (3.37 ± 0.06 M/ml) compared to the number of erythrocytes obtained in the blood of women (3.12 ± 0.08 M/ml) and men with normal iron levels (3.00 ± 0.11 M/ml). In the blood of women with reduced iron levels, a

higher number of erythrocytes was noted (by 4.5 %, $p > 0.05$) compared to the values in the group of women with normal iron levels. Similarly, in the group of men with reduced iron levels, a statistically significantly higher number of erythrocytes was found (by 12.3 %, $p < 0.05$) compared to the values obtained in men with normal iron levels. Comparing the values of the number of erythrocytes in the blood of women and men with normal iron levels, a higher number of erythrocytes in the blood of women [(3.12 ± 0.08 M/ml) *vs.* (3.00 ± 0.11 M/ml)], which was 4 % ($p > 0.05$). Analyzing the number of erythrocytes in the blood in the groups of women and men with reduced iron levels, a reverse trend was observed: a higher number of erythrocytes was found in the blood of men (3.37 ± 0.06 M/ml) compared to the group of women (3.26 ± 0.15 M/ml), which was 3 % ($p > 0.05$) (Fig. 2). The reference values of the number of erythrocytes in the blood of women are 3.5 – 5.2 million/mm$^3$ and for men are 4.2 – 5.4 million/mm$^3$. The results of our study revealed that both in the blood of women and men, the number of erythrocytes was below the reference values.

The World Health Organization defines anemia as a blood hemoglobin concentration below 7.7 mmol/L (13 g/dL) in men and 7.4 mmol/L (12 g/dL) in women. Typically, evaluation of the cause of anemia includes a complete blood count, peripheral smear, reticulocyte count, and serum iron indices. The severity of anemia depends on the patient's hemoglobin/hematocrit level. Iron deficiency anemia is characterized by microcytic, hypochromic erythrocytes and low iron stores. Mean blood cell volume is a measure of the average volume of red blood cells, and mean corpuscular hemoglobin concentration is a measure of the hemoglobin concentration in a given volume of red blood cells. The normal reference ranges for mean cell volume are 80-100 fL and the mean cell hemoglobin concentration is 320-360 g/L. A patient's cells are said to be microcytic and hypochromic, respectively, when these values are less than the normal reference range. It is worth noting that up to 40 % of patients with true iron deficiency anemia will have normocytic erythrocytes (i.e. normal mean cell volume does not rule out iron deficiency anemia) (Bermejo and Garcia-Lopez, 2009).

Red blood cell distribution width is a measure of red cell width variability and is used in conjunction with mean red cell volume to distinguish mixed-cause anemia from single-cause anemia. The normal reference range is 11–14 %; an increased value of the width of the distribution of red blood cells indicates a change in the size of the red blood cells, which is known as anisocytosis. The width of red blood cell distribution may be increased in the early stages of iron deficiency anemia or when the patient has both iron deficiency anemia and folic acid deficiency with or without vitamin $B_{12}$ deficiency, both of which cause macrocytic anemia. It is common for the platelet count to be greater than 450,000/µl in the presence of iron deficiency anemia. When examining the peripheral smear of a patient with chronic iron deficiency anemia, hypochromic, microcytic erythrocytes can usually be seen. Thrombocytosis may also be seen. It should be noted that microcytosis seen in a peripheral smear may be seen before abnormalities in a complete blood count. If the patient has concomitant folic acid or $B_{12}$ deficiency, the peripheral smear will be a mix of hypochromic macrocytic and microcytic erythrocytes, along with normalization of mean corpuscular volume (Johnson-Wimbley and Graham, 2011).

One of the basic blood tests is the determination of the level of hemoglobin, which provides a lot of information about the patient's health. Hemoglobin molecules contain iron in their structure, thanks to which they are able to effectively fulfill their role, which is the transport of oxygen and carbon dioxide. The measurement of hemoglobin in the complete blood count is referred to as Hb or HGB. Most cases where there is an increase in hemoglobin in the blood are not a cause for concern, and its most common cause is dehydration of the body. High hemoglobin concentrations may also occur in the situation of prolonged, slight hypoxia, or in people intensively practicing endurance sports. It should also be taken into account that a high concentration of hemoglobin may indicate the development of diseases associated with impaired production of erythrocytes in the bone marrow, as well as certain genetic and oncological diseases (www.diag.pl).

The figure 3 shows the level of hemoglobin in the blood of women and men with normal and reduced iron levels.

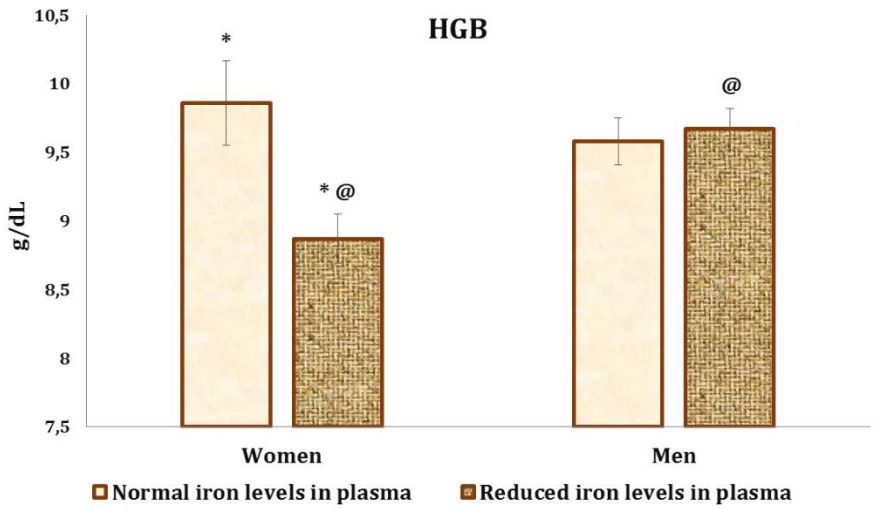


**Fig. 3. The level of hemoglobin in the blood of women and men with normal and reduced iron levels.**

*** – statistically significant changes between the means in the groups of women with normal and reduced iron levels;**
**@ – statistically significant changes between the means in the groups of women a nd men with reduced iron levels**

Our results showed that the highest level of hemoglobin is found in women with normal blood iron levels (9.86 ± 0.31 g/dl), while both men with normal iron levels (9.58 ± 0.18 g/dl), and those with reduced iron levels (9.67 ± 0.15 g/dl) have approximate values. The lowest hemoglobin level was noted in women with reduced iron levels (8.87 ± 0.17 g/dl). Comparing the results obtained between groups of women with normal iron levels and women with reduced iron levels [(9.86 ± 0.31 g/dl) *vs.* (8.87 ± 0.17 g/dl)], we notice statistically significant changes (by 11%, $p < 0.05$). Similarly, in the group of men with reduced iron levels compared to men with normal levels [(9.67 ± 0.15 g/dl) *vs.* (9.58 ± 0.18 g/dl)] we notice similar results. Comparing the results in women and men with normal iron levels, we notice a difference in the results obtained of 2% ($p > 0.05$), while in women and men with reduced iron levels, this difference is 9% and is a statistically significant ($p < 0.05$) (Fig. 3). The reference values of the hemoglobin level in women are 11.5-16.5 g/dl, while in men 13.0-18.0 g/dl. The results obtained in our study indicate that all groups are deficient in hemoglobin.

The hematocrit is a measure of the volume ratio of red blood cells (erythrocytes) to whole blood. Based on the percentage, it allows you to determine the proportion of erythrocytes compared to other blood cells, such as leukocytes and platelets. Both increased and decreased hematocrit indicate a disorder in the functioning of the body (www.medonet.pl). Its elevated level can be the result of diarrhea, vomiting, excessive sweating, cancer, or pregnancy. On the other hand, a reduced hematocrit may indicate anemia caused by iron deficiency, gastrointestinal bleeding, bone marrow disorders, or kidney problems (www.medonet.pl).

Figure 4 presents blood hematocrit values in women and men with normal and reduced iron levels.

According to the results obtained in our study, the highest level of hematocrit was achieved by men with reduced iron levels (30.04 ± 0.45 %) compared to the other groups. Women with normal iron levels ranked second (29.03 ± 0.55 %), followed by men with normal iron levels (28.77 ± 0.49 %) and women with reduced iron levels (27.83 ± 0.48 %). Analyzing the results between women with normal iron levels and those with reduced iron levels [(29.03 ± 0.55 %) *vs.* (27.83 ± 0.48 %)] we note that women with normal iron levels have 4 % higher hematocrit levels ($p > 0.05$). When checking the results between men with normal and reduced iron levels [(28.77 ± 0.49 %) *vs.* (30.04 ± 0.45 %)], we can observe that men with reduced iron levels have higher hematocrit levels (by 4 %, $p > 0.05$).

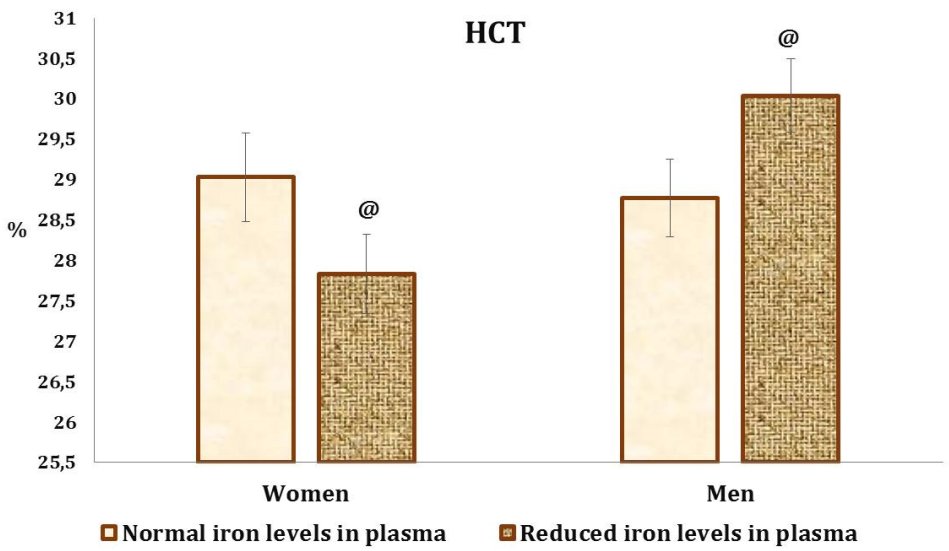


**Fig. 4. Blood hematocrit values in women and men with normal and low iron levels.**

**@ – statistically significant changes between the means in the groups of women and men with reduced iron levels**

Comparing the hematocrit levels obtained in the group of women and men with normal iron levels, higher results were noted in women (by 0.9 %, $p > 0.05$). On the other hand, in the group of men with reduced iron levels, a statistically significant difference was found in the values obtained in women with reduced iron levels (by 7 %, $p < 0.05$) (Fig. 4). The hematocrit reference values for women and men are 41-55 %. The normal hematocrit for an adult woman is in the range of 37 to 47 %, while for an adult man – 40 to 50 %. The results obtained in all of the studied groups are below the reference values.

The figure 5 shows the values of the mean red blood cell volume (MCV) in the groups of men and women with normal and low iron levels.

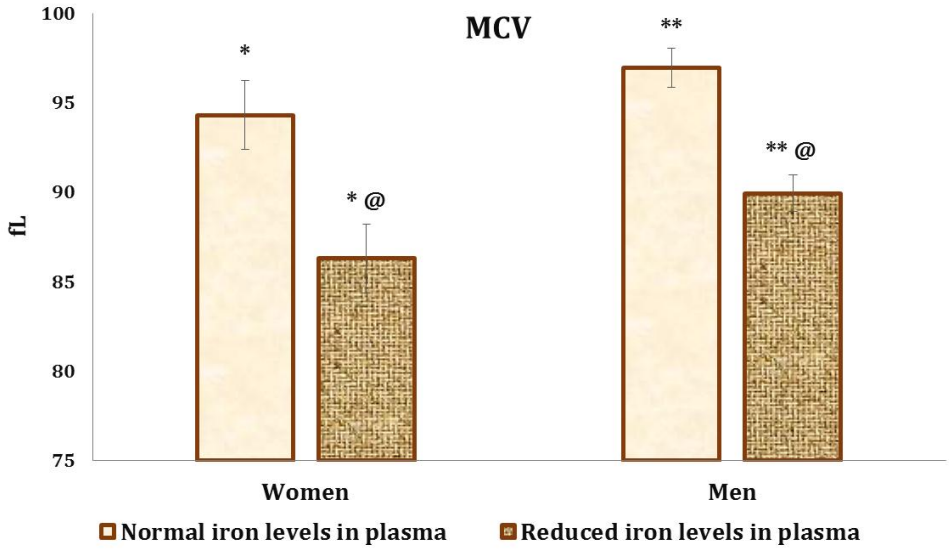


**Fig. 5. Mean corpuscular volume (MCV) of erythrocytes in women and men with normal and low iron levels.**

*** – statistically significant changes between the means in the groups of women with normal and reduced iron levels;**

**** – statistically significant changes between the means in the groups of men with normal and reduced iron levels;**

**@ – statistically significant changes between the means in the groups of women and men with reduced iron levels**

The mean red blood cell volume (MCV) data obtained in our studies, starting with the highest value, are as follows: men with normal iron levels (96.95 ± 1.91 fL), women with normal iron levels (94.30 ± 1.42 fL), men with reduced iron levels (89.94 ± 1.04 fL), women with reduced iron levels (86.29 ± 1.10 fL). When analyzing a group of women, we can suggest that women with normal iron levels have higher MCV values compared to the group of women with reduced iron levels (by 9 %), and these changes are statistically significant between the mean in these groups ($p < 0.05$). In the case of groups of men, men with normal iron levels showed higher MCV values than men with reduced iron levels (by 7 %) and these changes are statistically significant ($p < 0.05$). Comparing the mean values of MCV among groups of women and men with normal iron levels [(94.30 ± 1.42 fL) *vs.* (96.95 ± 1.91 fL)], we noted a difference in the results of 2 % ($p > 0.05$). However, the situation is slightly different when comparing groups of men and women with reduced iron levels [(89.94 ± 1.04 fL) *vs.* (86.29 ± 1.10 fL)] because the difference in results is 4 % ($p < 0.05$) in men with the reduced iron levels (Fig. 5). Reference MCV values are in the range of 82-92 fL. Our research has shown that the MCV values in the groups of women and men with reduced iron levels are within the norm, while in the group of women and men with normal iron levels, the MCV values are above the norm.

MCH is the average mass of hemoglobin in an erythrocyte and is not dependent on age and gender, while the test result may be affected by pregnancy or menstruation. Both elevated and decreased MCH results most often indicate anemia. Changes in MCH values may be indicative of serious pathological conditions. Elevated levels of MCH can be associated with many comorbidities. It mainly occurs when there is hyperchromic or megaloblastic anemia (macrocytic anemia resulting from abnormal DNA synthesis, vitamin $B_{12}$, or folic acid deficiency) and in the case of liver cirrhosis. A reduced MCH value is called microcytosis and occurs in the case of water and electrolyte disorders, hypochromic anemia due to iron deficiency, cancer-induced anemia, anemia in chronic disease states, and as a result of a large of blood loss (Yamaguchi et al., 2022).

The mean corpuscular hemoglobin (MCH) in the groups of women and men with normal and reduced iron levels is presented in Figure 6.

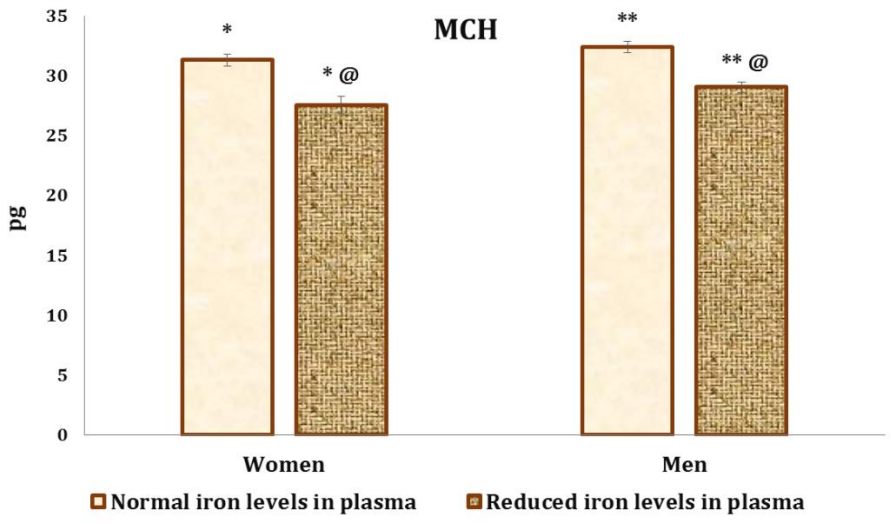


**Fig. 6. The mean corpuscular hemoglobin (MCH) in the groups of women and men with normal and reduced iron levels.**

*** – statistically significant changes between the means in the groups of women with normal and reduced iron levels;**

**** – statistically significant changes between the means in the groups of men with normal and reduced iron levels;**

**@ – statistically significant changes between the means in the groups of women and men with reduced iron levels**

The results of our research showed that the highest MCH value was recorded in the group of men with normal iron levels (32.36 ± 0.71 pg). A high value of MCH was noted in the group of women with normal iron levels (31.29 ± 0.51 pg), and slightly lower values were obtained in men with reduced iron levels (29.03 ± 0.42 pg). The lower MCH value was observed in the women with reduced iron levels (27.53 ± 0.46 pg). In the blood of women with normal iron levels, the MCH was higher than in women with reduced iron levels (by 13 %, $p < 0.05$). Similarly, in the group of men with normal iron levels, the MCH value was higher than in men with reduced iron levels (by 11 %, $p < 0.05$). Taking into account the group of women and the group of men with normal iron levels, we observe a higher level of MCH in the group of men (by 3 %, $p > 0.05$). Analyzing the MCH values in the groups of women and men with reduced iron levels [(27.53 ± 0.46 pg) *vs.* (29.03 ± 0.42 pg)], we observe a higher MCH value in men (by 5 %, $p < 0.05$) (Fig. 6). Reference values of MCH are in the range of 27-32 pg in both sexes. The MCH within the reference values was recorded in the groups of women with normal iron levels and in women with reduced iron levels, as well as in men with reduced iron levels. Only the group of men with normal iron levels had the MCH more than the reference values.

The mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin in the erythrocyte and is one of the indicators of the red blood cell system. Its reduced value in erythrocytes is often found in patients with anemia, mainly the one caused by iron deficiency. Elevated MCHC value, on the other hand, accompanies the abnormal structure of red blood cells in congenital spherocytosis (Cascio and DeLoughery, 2017).

The MCHC value in the groups of women and men with normal and reduced iron levels is presented in Figure 7.

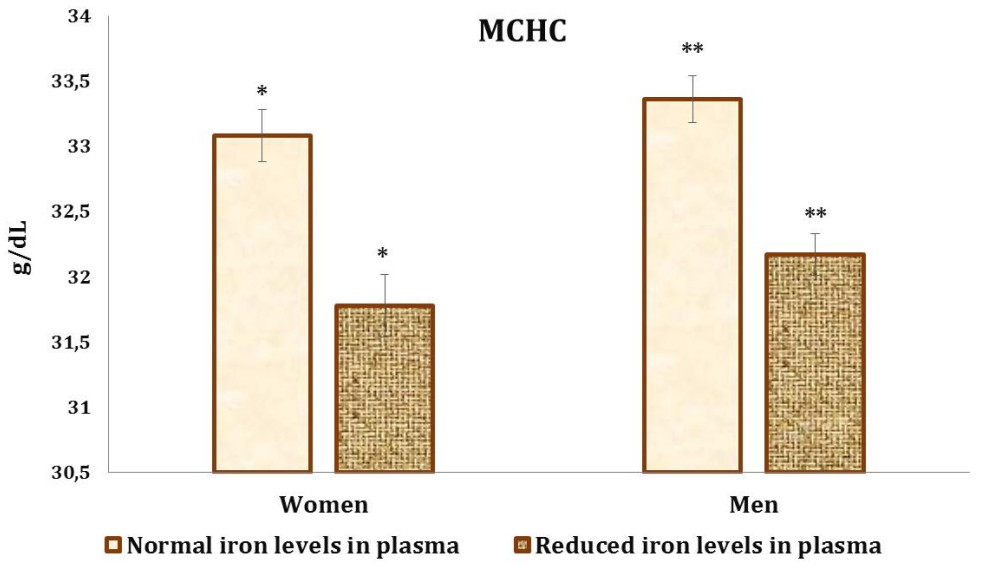


**Fig. 7. The mean corpuscular hemoglobin concentration (MCHC) in the groups of women and men with normal and reduced iron levels.**

*** – statistically significant changes between the means in the groups of women with normal and reduced iron levels;**

**** – statistically significant changes between the mean in the groups of men with normal and reduced iron levels**

As shown by the results of studies conducted in groups of women and men with normal and reduced iron levels, the highest MCHC value was recorded in men with normal iron levels (33.36 ± 0.24 g/dl), a slightly lower value was recorded in women with normal iron levels (33.08 ± 0.20 g/dl). Men with reduced iron levels had a mean corpuscular hemoglobin concentration of (32.17 ± 0.16 g/dl), and the lowest value was recorded in women with reduced iron levels (31.78 ± 0.18 g/dl). Analyzing the MCHC values in the groups of women with normal iron levels and women with reduced iron levels, it was noticed that

women with normal iron achieved a 4 % ($p < 0.05$) higher MCHC value. Similarly, in the group of men, a higher MCHC value was recorded in the group of men with normal iron levels compared to men with reduced iron levels, and these changes were statistically significant (by 3 % $p < 0.05$). Comparing the groups with normal iron levels, there was a similar level of MCHC values in the groups of women and men. The MCHC values recorded in the groups of women and men with reduced iron levels were higher in the group of men by 1 % ($p > 0.05$) compared to the group of women with reduced iron levels (Fig. 7). The MCHC reference values in an adult should be between 31 and 38 g/dL. The mean corpuscular hemoglobin concentration in the groups of women and men with normal and reduced iron levels was in reference levels in each group.

The red cell distribution width (RDW) test measures variation in red blood cell size or red blood cell volume as a part of a complete blood count (CBC). It is used along with other erythrocyte indices, especially mean corpuscular volume (MCV), to help determine the causes of anemia. RDW is an indicator expressed as a percentage. It is a coefficient of variability of the volume distribution of erythrocytes present in the blood. RDW is elevated in accordance with variation in red cell size (anisocytosis); that is, when elevated RDW is reported on complete blood count, marked anisocytosis (increased variation in red cell size) is expected on peripheral blood smear review. Increased RDW values are observed in anemias with a change in the size of erythrocytes, mainly in microcytic anemia caused by iron deficiency and macrocytic anemia associated with vitamin $B_{12}$ deficiency. An elevated RDW may also indicate poikilocytosis, i.e. the presence of deformed blood cells (sickle-shaped, disc-shaped, spherocytes, acanthocytes, etc.) (Salvagno et al., 2015).

Over the past few decades, RDW with mean corpuscular volume (MCV) has been used to identify quite a few hematological system diseases including iron-deficiency anemia and bone marrow dysfunction. In recent years, many clinical studies have proved that the alterations of RDW levels may be associated with the incidence and prognosis of many cardiovascular and cerebrovascular diseases (CVDs). Therefore, early detection and intervention in time for these vascular diseases are critical for delaying their progression. RDW as a new predictive marker and an independent risk factor plays a significant role in assessing the severity and progression of CVDs. However, the mechanisms of the association between RDW and the prognosis of CVDs remain unclear (Li et al., 2017).

Figure 8 presents the red cell distribution width (RDW) in groups of women and men with normal and reduced iron levels.

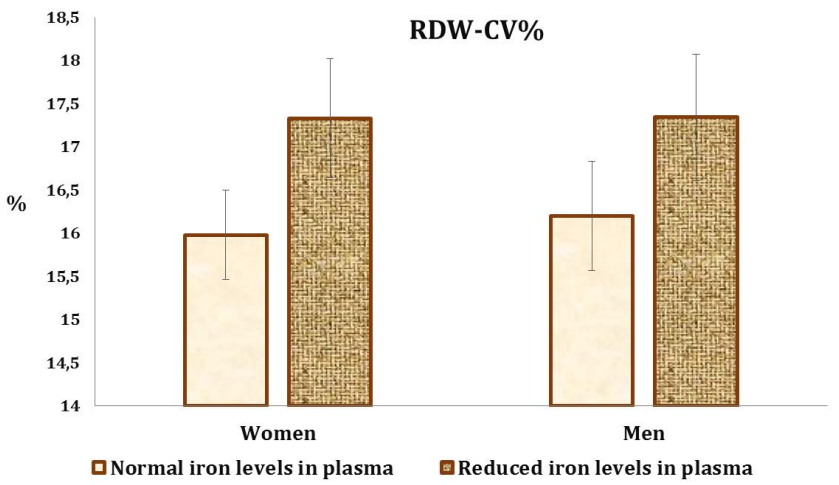


**Fig. 8. The red cell distribution width (RDW) in groups of women and men with normal and reduced iron levels**

As our results showed, the highest percentage of RDW was found in the group of men with reduced iron levels (17.34 ± 0.73 %) and the group of women with reduced iron levels (17.33 ± 0.63 %). Slightly lower values were noted in the group of men with normal iron levels (16.20 ± 0.69 %) and in the group of women with normal iron levels (15.98 ± 0.52 %). When analyzing the RDW values in the groups of women with normal iron levels and women with reduced iron levels, we noted a higher percentage of RDW in the group of women with reduced iron levels (by 8 %, $p > 0.05$). Similarly, in the group of men, men with reduced iron levels have a higher red cell distribution width than men with normal iron levels (by 7 %, $p > 0.05$). Analyzing the situation between the groups of women and men with normal iron levels, a higher RDW value can be observed in the group of men (by 1 %, $p > 0.05$), while in the groups of women and men with reduced iron levels, the obtained values were almost identical (the difference in the results was higher in men by 0.05 %, $p > 0.05$) (Fig. 8). The reference range for RDW is 11.5-14.5 % in adults. As the results of our research show, in each group, RDW values were higher than the reference values.

Low MCV, MCH, and MCHC reflect advanced iron-limited erythropoiesis in the bone marrow, and the pattern of microcytic and hypochromic anemia is typical of the laboratory findings of iron deficient anemia (IDA) (Camaschella and Pagani, 2011). An increase in RDW is another indicator of IDA, but this parameter is also elevated in megaloblastic anemia (Briggs, 2009; Tkaczyszyn et al., 2018). In the study of Tkaczyszyn and co-workers (2018), including a large international cohort of patients with heart failure, these researchers have shown that although hemoglobin concentration and certain erythrocyte indices (MCV, MCH, MCHC, CHR, and RDW) are closely correlated with various iron parameters (presence of iron deficiency, serum iron, serum ferritin, and transferrin saturation) independently of other clinical and laboratory variables (including the etiology and severity of heart failure and important comorbidities), iron deficiency is also common comorbidity in patients without any hematological abnormalities. It should be emphasized that even in non-anemic [as defined by the World Health Organization (WHO)] patients with MCV, MCH, and MCHC above the lower limit of normal, the prevalence of iron deficiency reached 36 % (Tkaczyszyn et al., 2018).

Erythrocyte mean cell volume (MCV) is used clinically to classify anemia, and normal values may be used to exclude iron deficiency. Åsberg and co-workers (2014) have studied the diagnostic accuracy of mean cell volume (MCV) and the related measures of mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) in diagnosing empty iron stores in children and young adults. The diagnostic accuracy of MCV, MCH, and MCHC was studied by ROC curve analysis in 6443 ambulant patients aged 0.5-25 years, of which 476 were anemic. In all patients, blood hemoglobin, MCV, MCH, and serum ferritin were measured in specimens sampled at the same time. MCHC was calculated as MCH divided by MCV. The gold standard of empty iron stores was s-ferritin <10, 15, or 20 μg/L. The cutoff limit of MCV giving 90 % sensitivity in diagnosing serum ferritin <15 μg/L was constructed using quantile regression. Generally, MCH was slightly more accurate than MCV and MCHC. In the whole study population, the area under the ROC curve was 0.68–0.93 for MCV, 0.73–0.96 for MCH, 0.68–0.87 for MCHC; and 0.70–0.86, 0.71–0.89, and 0.68-0.88, respectively, in the anemic subpopulation. At the cutoff limits of MCV giving a sensitivity of 90 % at all ages in anemic patients, the specificity was about 50 %. Mean cell hemoglobin, MCH, and MCHC are only moderately accurate in diagnosing empty iron stores in children and young adults, and normal values of these tests do not exclude empty iron stores in anemic patients (Åsberg et al., 2014).

The sensitivity and nonspecificity of parameters for the detection of iron deficiency: mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); serum iron (SI); total iron binding capacity (TIBC); transferrin saturation (TS); free erythrocyte protoporphyrin (Epp) and serum ferritin (SF) were studied by Piedras and co-workers (1981) in 78 children from 0.2-3.9 years old and in 165 mothers. MCH seems to be a sensible and specific screening test for detecting possible cases of iron deficiency. The best tests to establish the deficiency appear to be transferrin saturation of free erythrocyte protoporphyrin tests in children, and transferrin saturation plus serum ferritin in mothers (Piedras et al., 1981). Abnormal values of MCH + MCV or MCV alone established a high probability of identifying

infants with normal iron stores. Simultaneous alteration of MCH and MCV had similar specificity and predictive positive values in the study of iron storage in infants (Baptista-González et al., 1993). Zhan and co-workers (2020) have explored the predictive values of routine blood test results for iron deficiency (ID) screening in children. These researchers demonstrated that MCV, RDW, and MCHC can be used to screen ID in primary healthcare settings.

The diagnostic usefulness in iron deficiency anemia of serum ferritin, red cell protoporphyrin (Epp), mean corpuscular volume, mean corpuscular hemoglobin (MCH), and transferrin saturation measurements has been studied by Hershko and co-workers (1981) in a population of 294 children aged 1 to 6 years. The Pearson correlation coefficient for hemoglobin was highest with MCH, followed in decreasing order of magnitude by MCV, Epp, transferrin saturation, and finally by ferritin. Sensitivity and specificity were highest for MCH and lowest for ferritin. Of anemic, iron deficient individuals 97 to 100 % could be identified by low MCH, 88 to 100 % by transferrin saturation, 66 to 83 % by ferritin, and 61 to 74 % by Epp. In contrast, only 0 to 6 % of normal, nonanemic individuals had low MCH, 0 to 4 % had high Epp, but 21 to 39 % had low transferrin saturation and 25 to 39 % had low ferritin. Although reduced serum ferritin in anemic individuals is good evidence of iron deficiency, a significant proportion of anemic iron-deficient patients is missed by this procedure rendering it less useful than other, less expensive laboratory methods (Hershko et al., 1981).

Kai and co-workers (2021) compared the diagnostic values of red blood cell distribution width-coefficient of variation (RDW-CV) and red blood cell distribution width-standard deviation (RDW-SD) in mid-pregnancy women with iron deficiency anemia (IDA). To obtain the results, 115 mid-pregnancy women with IDA, defined as the IDA group, and 142 healthy mid-pregnancy women, selected as the control group, were enrolled in this study. Hematological parameters and ferritin concentrations in the serum were analyzed. The efficiency of RDW-CV and RDW-SD to distinguish IDA from mid-pregnancy women was evaluated using receiver operating characteristic (ROC) curves. The RDW-SD value in the IDA group was significantly higher than that in the control group ($p < 0.05$), while the RDW-CV value did not differ between them ($p = 0.84$). Significantly negative correlations were found between RDW-CV ($r = -0.297$, $p = 0.001$), RDW-SD ($r = -0.404$, $p = 0.000$), and serum ferritin in the IDA group but not in the control group. For the diagnosis of IDA, RDW-CV and RDW-SD produced areas under the ROC curves of 0.58 and 0.84. To conclude, our results suggest that RDW-SD, but not RDW-CV, can be used as a diagnostic index of IDA for mid-pregnancy women (Kai et al., 2021).

In the diagnosis of mild and moderate iron deficiency anemia, RDW had a higher sensitivity than peripheral smear (PS). Red cell morphology, Hb, PCV, and RDW showed significant improvement after iron therapy. In the study of Viswanath and co-workers (2001), children in the age group of six months to five years with microcytic (MCV < 80fl) anemia (Hemoglobin <11 g%) were evaluated. Those who had received a blood transfusion and /or were already on iron therapy were excluded. The evaluation included clinical examination, complete blood count (CBC), RDW estimation microscopic examination of peripheral smear, measurement of serum iron, and transferrin saturation. Children with IDA were treated with oral iron for 8 weeks and PS, CBC including RDW were repeated. Of the 100 children evaluated, 89 had IDA. 48 % had mild, 42 % had moderate and 10 % had severe anemia. Transferrin saturation correlated with the severity of anemia. Peripheral smear showed microcytosis and hypochromia in all cases with severe anemia, 61.5 % and 22.5 % of those with moderate and mild anemia respectively. RDW was suggestive of iron deficiency in 100 %, 82.05 %, and 100 % of patients with mild, moderate, and severe anemia respectively (Viswanath et al., 2001).

Red cell volume distribution width (RDW-CV) was examined in the study of Uchida (1989) as a means of diagnosing iron deficiency. Iron deficiency was classified as iron deficiency anemia, prelatent or latent iron deficiency in 1648 students. MCV and RDW-CV (mean ± ISD) in each group were (89 ± 4) fl, 12.7 ± 0.7 % in normal individuals, (89 ± 4) fl, (13.2 ± 0.8) % in prelatent deficiency, (86 ± 6) fl, (14.0 ± 1.5) % in latent deficiency, and (79 ± 7) fl, (15.6 ± 1.7) % in iron deficiency anemia, respectively. Although microcytosis was evident only in iron deficiency anaemia, RDW-CV showed larger values concomitant with the development of

iron deficiency. The sensitivity of RDW-CV for the diagnosis of iron deficiency anemia was 77.1 %, and for iron deficiency anemia and latent deficiency 49.2 %, the specificity is 90.6 %. In countries with a high prevalence of iron deficiency and low thalassaemia, iron deficiency should be screened by RDW-CV determination without serum iron or ferritin measurements (Uchida, 1989).

RDW has limited specificity for the diagnosis of IDA among children with microcytic hypochromic anemia. Aulakh and co-workers (2009) studied the utility of red cell distribution width (RDW) in the diagnosis of iron deficiency among children with microcytic hypochromic anemia. 151 children (6 months – 12 years) with microcytic (MCV < 75 fl) anemia were classified into iron deficient (IDA) and non-iron deficient anemia (non-IDA) on the basis of serum ferritin and total iron binding capacity (TIBC). RDW values were obtained on an automated hematology analyzer. Receiver operator curves (ROC) were constructed and the utility of RDW in the diagnosis of iron deficiency was studied. The mean RDW value was (18.37 ± 2.22) % in IDA group (97 children) compared to (16.55 ± 1.51) % in the non-IDA group (54 children) ($p < 0.0001$, unpaired t test). In IDA group, the mean RDW value was (16.60 ± 1.78) %, (17.95 ± 1.91)% and (20.55 ± 1.32) % among mild, moderate and severely anemic children ($p < 0.0001$, ANOVA test). The corresponding values in non-IDA group were (16.03 ± 1.25) %, (16.76 ± 1.20) % and (16.77 ± 2.68) % respectively ($p = 0.269$, ANOVA test). At a cut-off value of 17.4 %, as obtained from the ROC curve, the sensitivity and specificity of RDW in the diagnosis of IDA were 81.0 % and 53.4 %, and a positive and negative predictive value of 63.0 % and 72.2 % respectively (Aulakh et al., 2009).

Red cell distribution width (RDW) is an automated laboratory determination of red cell anisocytosis. Buch and co-workers (2011) analyzed the role of RDW in differentiating iron deficiency anemia (IDA) from thalassaemia traits. There were 500 patients who were screened for the study. The selection criteria of microcytic anemia were Hb < 13 g/dl in males, Hb < 12 g/dl in females with mean corpuscular volume (MCV) < 80 fl. These cases were subjected to complete iron profile and hemoglobin chromatography for a definite diagnosis. The values of RDW were analyzed in all these cases to see the utility of RDW in classifying microcytic anemia; especially differentiating iron deficiency anemia from thalassaemia minor cases. There were 133 out of 500 cases of anemia; 105/133 cases had microcytic anemia, of which 53 had iron deficiency anemia, 39 were thalassaemia traits, 6 were thalassaemia major, and 7 had other hemoglobinopathies. Thirty-six cases (67.92 %) out of 53 iron deficiency anemia had increased RDW, 32.08 % (n = 17) had normal RDW; 71.79 % (n = 28) of thalassaemia trait had increased RDW, 28.21 % (n = 11) had normal RDW. Evaluation of RDW as a screening test to detect microcytic anemia had a sensitivity of 71.42 % and specificity of 40 %, Evaluation of RDW as a screening test for IDA had a sensitivity of 67.9 % and specificity of 25 %. It was found uniform increase in RDW in all cases of microcytosis. It is concluded that RDW adds useful but limited information in classifying microcytic anaemia (Buch et al., 2011).

Early detection of iron deficiency (ID) and iron deficiency anemia (IDA) in young children is important to prevent impaired neurodevelopment. Unfortunately, many biomarkers of ID are influenced by infection, thus limiting their usefulness. Akkermans and co-workers (2015) investigated the value of red blood cell distribution width (RDW) and the platelet count for detecting IDA among otherwise healthy children. A multicenter prospective observational study was conducted in the Netherlands to investigate the prevalence of IDA in 400 healthy children aged 0.5-3 years. ID was defined as serum ferritin (SF) <12 μg/L in the absence of infection (C-reactive protein [CRP] <5 mg/L) and IDA as hemoglobin <110 g/L combined with ID. RDW (%) and the platelet count were determined in the complete blood cell count. RDW was inversely correlated with SF and not associated with CRP. Calculated cutoff values for RDW to detect ID and IDA gave a relatively low sensitivity (53.1 % and 57.1 %, respectively) and specificity (64.7 % and 69.9 %, respectively). Anemic children with an RDW >14.3% had a 2.7 higher odds (95 % confidence interval [CI]: 1.2-6.3) to be iron deficient, compared with anemic children with an RDW <14.3 %. The platelet count showed a large range in both ID and non-ID children. In conclusion, RDW can be helpful for identifying ID as the cause of anemia in 0.5- to 3-year-old children, but not as a primary biomarker of ID(A). RDW values are

not influenced by the presence of infection. There appears to be no role in the platelet count in diagnosing ID(A) in this group of children (Akkermans et al., 2015).

**Conclusions**

Erythrocyte indices analyzed in the blood of women with reduced iron levels compared to women with normal iron levels constituting the control group showed reduced values of hemoglobin, hematocrit, MCV, MCH, and MCHC. Increased values of RDW and the count of erythrocytes in the blood of women with reduced iron levels compared to the control group of women were noted. Similarly, when comparing the values of erythrocyte indices obtained in the group of men with reduced iron levels to the control group of men with normal iron levels, reduced values of MCH, MCV, and MCHC were demonstrated. However, the values of the count of erythrocytes, RDW, hematocrit, and hemoglobin levels were elevated compared to the control group.

The reverse trend in erythrocyte indices such as hemoglobin and hematocrit indices between the group of women and the group of men with reduced iron levels was observed. Comparing the obtained values with the reference values, it was noted that the reduced values of the count of erythrocytes, and the level of hemoglobin and hematocrit were obtained in all study groups. An increased MCV value compared to the reference values was noted in the group of women and men with normal iron levels. Men with normal iron levels had elevated MCH values. In all studied groups, an increased level of RDW was noted compared to reference values.

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