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Overview of Immature Reticulocyte Fraction (IRF) and Red Cell Distribution Width-Coefficient Variation (RDW-CV) in Patients with Hemoglobin Levels Below 8

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ABSTRACT

Background & Objective: Anemia is a condition in which the number or volume of erythrocytes or red blood cells and hemoglobin (Hb) molecules in the body are reduced. This condition occurs when hemoglobin levels or hematocrit (Ht) values decrease. Anemia can occur due to several factors, such as iron deficiency, vitamin B12 deficiency, folic acid deficiency, genetic factors, chronic diseases, intestinal worms, and bleeding. This study aims to determine the profile of immature reticulocyte fraction (IRF) and red cell distribution width-coefficient variation (RDW-CV) in patients with hemoglobin levels below 8. **Method:** This type of research is descriptive research, using purposive sampling with 30 respondents. **Result:** The results of this study obtained IRF RDW values -CV of 15 with a percentage of (50%), normal IRF RDW-CV of 10 with a percentage of (33%), normal IRF RDW-CV of 2 with a percentage of (7%), high IRF RDW-CV of 2 with a percentage of (7%), and low IRF RDW-CV of 1 with a percentage of (3%). **Conclusion:** The results showed that high IRF and high RDW-CV 15 with a percentage of 50% indicated a more active bone marrow response in producing new red blood cells in response to anemia and the presence of cell size differences known as anisocytosis.

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Introduction

Anemia is a condition in which the number or volume of erythrocytes or red blood cells and hemoglobin (Hb) molecules in the body are reduced. This condition occurs when hemoglobin levels or hematocrit (Ht) values decrease. Anemia can occur due to several factors, such as iron deficiency, vitamin B12 deficiency, folic acid

deficiency, genetic factors, chronic diseases, parasitic infections, and bleeding ((Eva Ayu Maharani, 2023)(Ministry of Health, 2018)

According to the World Health Organization (WHO), the prevalence of anemia in Indonesia among women is 23.9%, which is divided into a prevalence of anemia in women aged 5-14 years of 26.4% and aged 15-25 years of 18.4%. Adolescent girls are ten times more likely to suffer from anemia than adolescent boys (WHO 2018, 2018).

Anemia is diagnosed through clinical symptoms and laboratory analysis. The most common clinical symptom of anemia is pale skin, especially around the conjunctiva of the eyes. This is caused by a decrease in the number of erythrocytes, hemoglobin concentration, and vasoconstriction as the body's response to optimize oxygen delivery to vital organs. However, pale skin is not the main indicator of anemia because it can be influenced by various factors such as skin pigmentation, body temperature, and the depth and distribution of capillaries. In clinical laboratory tests, there are several supporting tests for anemia, including hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), peripheral blood smear, and reticulocyte count (Eva Ayu Maharani, 2023). (Purnamasari, 2024).

Immature Reticulocyte Fraction (IRF) is the percentage of High Fluorescence Ratio (HFR) + Middle Fluorescence Ratio (MFR) divided by total reticulocytes and multiplied by 100% in peripheral blood, and measured using flow cytometry. The principle of flow cytometry is to measure reticulocytes based on the size of stained cells. The dyes used are acridine orange or polymethanine, which are mixed with the blood sample to stain the remaining RNA in the reticulocytes. The stained blood cells are then exposed to a laser beam, causing fluorescence that can be detected by the instrument's detector. The detector can distinguish reticulocytes based on the level of light emitted by the fluorescence, namely reticulocytes with low, medium, and high fluorescence. Reticulocytes with high fluorescence indicate a high RNA content and are referred to as immature reticulocytes (Eva Ayu Maharani, 2023) (Chung-Che Chang, MD, PhD, Lawrence Kass, 1997).

Red cell distribution width (RDW) is the difference in size abnormalities of red blood cells. Anisocytosis is a condition where red blood cells are not the same size as normal, while poikilocytosis is a shape abnormality in red blood cells. There are two methods for measuring RDW values, namely RDW-SD (red cell distribution width-standard deviation) and RDW-CV (red cell distribution width-coefficient variation) (Putra & U. Y. Bintoro, 2019). RDW-CV is most effective in detecting red blood cell size abnormalities (anisocytosis), but RDW-CV is highly influenced by MCV values (Hanggara, 2019).

Immature Reticulocyte Fraction (IRF) testing aims to observe the bone marrow's response in producing red blood cells (RBC). A high IRF indicates that the bone marrow is active, while a low IRF may indicate that bone marrow activity is insufficient. In addition, RDW-CV testing aims to observe the variability in size or volume of red blood cells. A high RDW-CV value indicates greater cell size variability,

while a lower value indicates uniform cell size (Putra & U. Y. Bintoro, 2019)(Anovadia et al., 2023).

Based on the background description above, the researchers were interested in conducting a study entitled "Description of Immature Reticulocyte Fraction (IRF) and Red Cell Distribution Width-Coefficient Variation (RDW-CV) in Patients with Hemoglobin Levels Below 8". This study is rarely conducted because IFR and RDW-CV are used as separate parameters when performing a complete blood count test.

Objective

This study aims to determine the characteristics of immature reticulocyte fraction (IRF) and red cell distribution width-coefficient variation (RDW-CV) in patients with hemoglobin levels below 8.

Method

The type of research used was descriptive research, which was conducted to determine the description of the Immature Reticulocyte Fraction (IRF) and Red Cell Distribution Width-Coefficient Variation (RDW-CV) in patients with hemoglobin levels below 8. The research was conducted from March to May 2025. The population in this study consisted of 44 patients with hemoglobin levels below 8 at QIM Hospital in Batang Regency. The margin of error used was 0.1 (10%), resulting in a sample size of 30 samples using the Slovin formula and purposive sampling method. The examination was conducted at the QIM Hospital clinical laboratory. Data Analysis Technique: the data obtained from the research results were compiled in tables and analyzed descriptively.

Results

After conducting research on the description of Immature Reticulocyte Fraction (IRF) and Red Cell Distribution Width-Coefficient Variation (RDW-CV) in patients with hemoglobin levels below 8 with a sample size of 30 samples obtained in March 2025, the results in Table 1 are as follows:

TABLE 1. IRF and RDW-CV Examination Results in Patients with Hemoglobin Levels below 8

Sample Code	Hemoglobin g/dl	IRF (%)	Description	RDW-CV (%)	Description
S1	7,7	5,0	Normal	12,6	Normal
S2	6,8	19,5	High	20,2	High
S3	7,5	10,2	Normal	15,5	High
S4	2,4	19,8	High	22,5	High
S5	6,5	20,4	High	19,5	High
S6	7,8	22,4	High	23,5	High
S7	6,8	4,9	Normal	15,5	High
S8	2,5	42,0	High	22,9	High
S9	6,8	8,7	Normal	16,3	High
S10	7,0	5,3	Normal	15,8	High
S11	7,7	11,6	Normal	14,1	Normal
S12	7,8	25,3	High	14,5	Normal

Sample Code	Hemoglobin g/dl	IRF (%)	Description	RDW-CV (%)	Description
S13	7,3	15,6	Normal	14,8	High
S14	4,8	31,8	High	16,4	High
S15	6,8	35,2	High	20,1	High
S16	4,9	29,5	High	18,0	High
S17	6,8	16,5	High	34,4	High
S18	5,3	9,8	Normal	16,4	High
S19	7,9	35,5	High	16,2	High
S20	7,8	37,6	High	14,6	High
S21	7,5	18,4	High	16,3	High
S22	6,8	5,0	Normal	15,0	High
S23	6,7	1,2	Low	14,2	Normal
S24	6,8	6,7	Normal	16,2	High
S25	7,4	9,4	Normal	34,2	High
S26	6,7	17,6	High	34,4	High
S27	6,1	6,3	Normal	15,3	High
S28	5,2	29,5	High	15,7	High
S29	5,8	31,4	High	20,9	High
S30	7,4	19,4	High	13,4	Normal

TABLE 2. IRF and RDW-CV percentages in patients with hemoglobin levels below 8.

No	IRF	RDW-CV	Total	Persentage (%)
1.	High	High	15	50%
2.	Normal	High	10	33%
3.	Normal	Normal	2	7%
4.	High	Normal	2	7%
5.	Low	Normal	1	3%
	Total		**30**	**100%**

Discussion

Based on Table 2, the percentage results of IRF and RDW-CV examination in patients with hemoglobin levels below 8 who were hospitalized at QIM Hospital, Batang Regency, showed the following: patients with high IRF and high RDW-CV were 15 (50%), normal IRF and high RDW-CV were 10 (33%), normal IRF and normal RDW-CV were 2 (7%), high IRF and normal RDW-CV were 2 (7%), while low IRF and normal RDW-CV were 1 (3%).

In Table 2 of this study, 50% of patients had high IRF and high RDW-CV. This occurred because a high IRF indicates a more active bone marrow response in producing new red blood cells in response to anemia. An increased IRF also signifies the release of younger or immature reticulocytes into the blood circulation (Eva Ayu Maharani, 2023; Adane T, Asrie F, 2021). Meanwhile, a high RDW-CV occurs because of low MCV values, resulting in unequal red blood cell sizes, known as anisocytosis. This condition is suspected to be caused by chronic disease or iron deficiency (Putra & U. Y. Bintoro, 2019).

The 33% result with normal IRF and high RDW-CV indicates that a normal IRF reflects the bone marrow response in producing reticulocytes at a level appropriate to the body's needs, without a significant increase in production even in severe anemia

(Roes et al., 2024). However, the high RDW-CV occurs because of low MCV values, resulting in anisocytosis (Putra & U. Y. Bintoro, 2019).

The 7% result with normal IRF and normal RDW-CV suggests that both values reflect a bone marrow response in producing reticulocytes at a level appropriate to the body's needs, without a significant increase in production even in anemia (Roes et al., 2024). Meanwhile, a normal RDW-CV indicates that red blood cell sizes are relatively uniform with little variation, which signifies proper red blood cell maturation in the bone marrow (dr. Dian Sukma Hanggara, SpPK, 2019).

The 7% result with high IRF and normal RDW-CV occurred because a high IRF indicates an active bone marrow response in producing new red blood cells in anemia. An increased IRF also signifies the release of younger or immature reticulocytes into circulation (Eva Ayu Maharani, 2023; Adane T, Asrie F, 2021). Meanwhile, a normal RDW-CV shows that red blood cell sizes are relatively uniform, which reflects proper red blood cell maturation in the bone marrow (dr. Dian Sukma Hanggara, SpPK, 2019).

The 3% result with low IRF and normal RDW-CV occurred because a low IRF indicates that the bone marrow is not producing or releasing many new reticulocytes into the blood circulation. This happens due to reduced or less active red blood cell production in the bone marrow in response to the body's needs (Anovadia et al., 2023). Meanwhile, a normal RDW-CV shows that the sizes of red blood cells are relatively uniform (dr. Dian Sukma Hanggara, SpPK, 2019).

Conclusion

Based on the results of the study, it can be concluded that from 30 samples, 15 (50%) showed high IRF and high RDW-CV, indicating a more active bone marrow response in producing new red blood cells in anemia and the presence of cell size variation known as anisocytosis. Meanwhile, 10 samples (33%) had normal IRF and high RDW-CV, 2 samples (7%) had normal IRF and normal RDW-CV, 2 samples (7%) had high IRF and normal RDW-CV, and 1 sample (3%) had low IRF and normal RDW-CV.

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