# PERIPHERAL BLOOD SMEAR AS A PROGNOSIS OF NORMOCYTIC ANAEMIA PREGNANT WOMEN IN MAKASSAR CITY

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## Abstract

Normocytic anaemia is one of the classifications of anaemia according to the morphology of the cells and the haemoglobin they contain. This study aimed to analyse the degree of normocytic anaemia in pregnant women based on peripheral blood smears. This study used a quantitative, descriptive, and analytic observational design with a cross-sectional method. The sampling technique used a purposive side based on criteria: third-trimester pregnant women who checked their pregnancies at the mother and child hospital and health centre in Makassar City. The population in this study was 92 third-trimester pregnant women; of the 92 third-trimester pregnant women, there were 50 pregnant women with normocytic anaemia (haemoglobin < 11 g/dL) with an MCV of 80-100 fL. The analysis used the SPSS version 23 application, and the analysis was carried out using the chi-square test and the risk factor test to see the risk factor (OR). Most of the anaemia was normocytic, mild anaemia was 92%, followed by moderate anaemia, 8% and no severe anaemia was found. Five features of peripheral blood smears were found in normocytic anaemia, namely a description of the cause of chronic disease accompanied by signs of infection 52%, a description of the cause of infection 24%, a description of decreased kidney function 14%, description of peripheral blood analysis within normal limits 8% and description of the cause of chronic disease accompanied by impaired liver function 2%. Examination of peripheral blood smears produces diagnostic clues for normocytic anaemia even though it only describes the results in terms of the cause, so additional investigations are needed to determine the cause and true diagnosis of normocytic anaemia in third-trimester pregnant women.

***Keywords:*** Normocytic Anaemia, Peripheral Blood Smear, Pregnant Women

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## 1. Introduction

Anaemia can be classified based on its morphology: microcytic, macrocytic and normocytic anaemia. Normochromic normocytic anaemia is one of the classifications of anaemia according to the morphology of the cell size and the haemoglobin it contains. Normocytic normochromic anaemia is a type in which circulating red blood cells are the same size (normocytic) and have a normal red colour (normochromic). Normocytic anaemia has the normal size and shape of red blood cells and contains normal amounts of haemoglobin, but the individual is anaemic. In normocytic anaemia, the mean corpuscular volume (MCV) is within the normal range (80-100 fL). The most common cause of normochromic normocytic anaemia is a chronic disease; the estimated prevalence of the underlying cause of anaemia is a chronic disease (infection 18%-95%), cancer 30%-77%, autoimmune 8%-71%, (1)(Bukhari, et al., 2020). Anaemia in pregnancy was determined using the WHO classification of haemoglobin (Hb) level <11 g/dl. The classification of anaemia according to WHO is divided into 3, namely the degree of anaemia is defined as mild anaemia (Hb level 9.0-10.9 g / dL), moderate anaemia (Hb level 7.0-8.9 g / dL), and severe anaemia (Hb level less than 7.0 g / dL)(2). In a cohort study conducted by Judistiani et al., it was shown that anaemia has a 4-fold increase in prevalence in the third trimester(3).

Anaemia is not a separate disease but a symptom of various underlying diseases (4). Therefore, in the diagnosis of anaemia, it is not enough just to label anaemia but to be able to determine the basic disease that causes anaemia. This is important because the underlying disease is often hidden, so if this can be uncovered, it will lead clinicians to hidden dangerous diseases. Determining the underlying disease is also important in managing anaemia cases because knowing the underlying cause is necessary for complete therapy to be given to the anaemia case. An approach to anaemia patients requires understanding the pathogenesis and pathophysiology of anaemia and skills in selecting, analysing and summarising the results of anamnesis, physical examination, laboratory tests and other supporting examinations. (3,5).

General examinations needed to diagnose normocytic anaemia include a complete blood count (CBC) by looking at the average corpuscular volume (MCV), after which the reticulocyte count must be obtained to determine the pathophysiological mechanism of anaemia, whether the reticulocyte count is increased or decreased/normal. This approach often allows a functioning diagnosis and many disorders to be eliminated. Most published algorithms for diagnosing normocytic anaemia begin with a corrected reticulocyte index and a peripheral blood smear. (6–8).

Examination of the peripheral blood smear often yields diagnostic clues or confirmatory evidence. Easily identifiable RBC findings associated with normocytic anaemia include large polychromatic "shifting cells", which represent reticulocytosis; target cells, which may be found in liver disease; basophilic spots, which may be present in a hemolytic anaemia; and mixed large and small RBCs, which may indicate a mixed microcytic and macrocytic disease process (a finding that should be demonstrated by increased red cell distribution width)(1,9,10).

According to the World Health Organization (WHO), anaemia is estimated to affect half a billion women aged 15-49 years and 269 million children aged 6-59 months worldwide. Globally, it is estimated that 40% of all children aged 6–59 months, 37% of pregnant women and 30% of women aged 15–49 years are affected by anaemia. In 2019, 30% (539 million) of non-pregnant women and 37% (32 million) of pregnant women aged 15–49 years were affected by anaemia(11). In Indonesia, the causes of anaemia during pregnancy are multifactorial. Still, iron deficiency is generally considered the main cause because the diagnosis of anaemia is usually based on measuring haemoglobin levels. Based on the 2018 Basic Health Research (Riskesdas) data, the prevalence of anaemia in pregnant women in Indonesia increased from 37.1% in 2013 to 48.9% in 2018. This proves that the incidence of anaemia in pregnant women increases yearly. Hence, this health problem is one of the main priorities to be addressed immediately (12).

One of the government programs in Indonesia to prevent anaemia is the administration of 90 iron tablets during pregnancy. However, the incidence of anaemia is still quite high, where the incidence of anaemia in Indonesia based on Basic Health Research 2018 data, is 48.9% above the average incidence of anaemia

worldwide, which is 40%.(11,12). From this phenomenon, the researchers conducted a study to analyse the degree of normocytic anaemia in pregnant women based on the peripheral blood smear.

## 2. Method

This study used a quantitative, descriptive, observational analytic design with a cross-sectional method. The sampling technique used a purposive side based on certain criteria, namely third-trimester pregnant women. The population consisted of third-trimester pregnant women who checked their pregnancies at Mother and Child Hospital, Masyita, Siti Fatimah, Sitti Khadija I and the Pampang, Antang, Tamalate, Makkasau, Dahlia Health Centers in Makassar City. The inclusion criteria in this study were haemoglobin <11 gr/dL and Mean Corpuscular Volume (MCV) 80-100 fL. This research was conducted from October 2021-June 2022. Pregnant women in their third trimester were then taken to collect 3 mL of blood samples by laboratory personnel. They carried out examinations in the clinical pathology laboratory at Dr Central General Hospital. Wahidin Sudirohusodo Makassar. Of the 92 blood samples of third-trimester pregnant women who underwent a complete blood count (CBC), 42 women did not have anaemia (Hemoglobin > 11 g/dL) and 50 pregnant women who had anaemia (Hemoglobin < 11 gr/dL) with an MCV value of 80-100 fL (Normocytic). Fifty samples of third-trimester pregnant women with normocytic anaemia were then subjected to peripheral blood tests.

Analysis used the SPSS version 23 application, and bivariate analysis was carried out using the chi-square and risk factor tests to see the risk factor (OR). The Faculty of Medicine has approved the research ethics, of Hasanuddin University's health research ethics committee, with the ethical approval recommendation number 638/UN4.6.4.5.31/PP36/2021.

## 3. Results

Pregnancy is a physiological process, so a mother must plan a pregnancy to know things that can affect the health of the mother and fetus. Risk factors for third-trimester pregnant women, such as parity, age, and degree of anaemia, need to be known to assess the health status of the mother.

Table 1. Analysis of the relationship between anaemia status of third-trimester pregnant women and risk factors

| Variable | Haemoglobin levels | | p-value | OR (95% CI) |
|---|---|---|---|---|
| | Anaemia (Hgb < 11 g/dL) | Not Anaemia (Hgb > 11 gr/dL) | | |
| Parity | | | | |
| ≥ 4 times | 11 (84.6%) | 2 (15.4%) | 0.018 | 5.64 (1.17-27.1) |
| < 4 times | 39 (49.4%) | 40 (50.6%) | | |
| Birth Intervals | | | | |
| < 2 years | 5 (55.6%) | 4 (44.4%) | 1,000 | 1.05 (0.26-4.21) |
| ≥ 2 years / first-time pregnant | 45 (54.2%) | 38 (45.8%) | | |
| Age | | | | |
| < 20 years and > 35 years | 18 (85.7%) | 3 (14.3%) | 0.001 | 7.31 (1.97-27.06) |
| 20-35 years | 32 (45.1%) | 39 (54.9%) | | |
| Mid Upper Arm Circumference (MUAC) | | | | |
| < 23.5 cm | 11 (100%) | 0 (0.0%) | 0.001 | 2.07 (1.65-2.60) |
| 23.5cm | 39 (48.0%) | 42 (51.9%) | | |

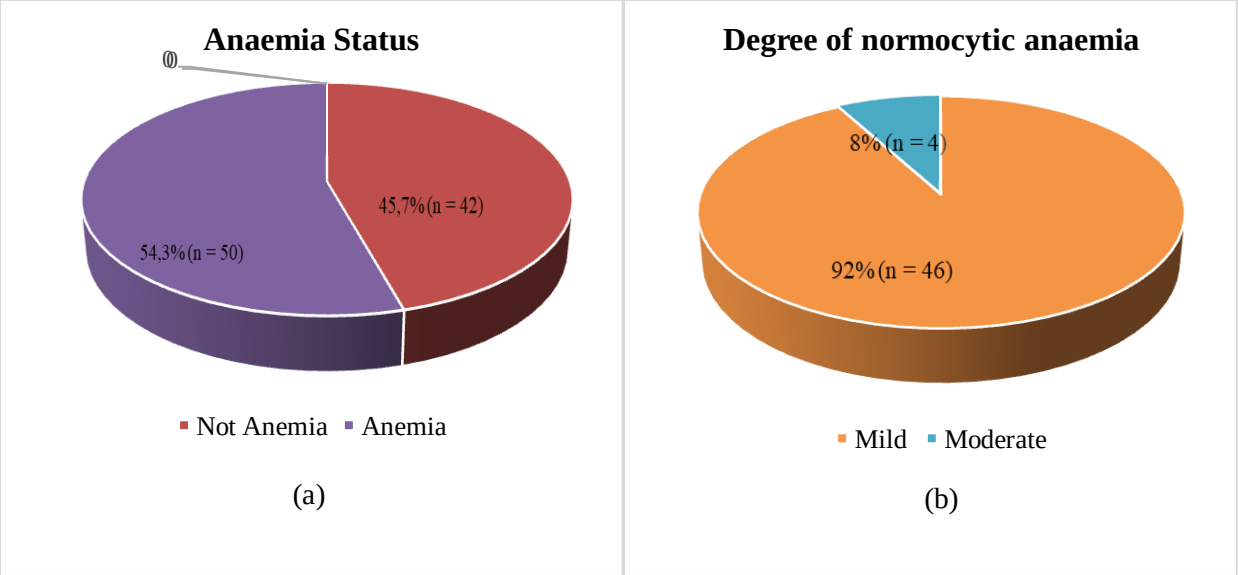


Figure 1: Anaemia status of third-trimester pregnant women, not anaemic if haemoglobin level > 11 gr/dL and anaemia if haemoglobin level < 11 gr/dL (a) and degree of normocytic anaemia (80-100 fL) third-trimester pregnant women (b) mild anaemia (Hemoglobin level 9.0-10.9 g/dL), moderate anaemia (Hemoglobin level 7.0-8.9 g/dL).

Table 2. Overview of peripheral blood smears in third-trimester pregnant women with normocytic anaemia

| | | **Peripheral Blood Smear** | | | | | |
|---|---|---|---|---|---|---|---|
| | | Peripheral blood analysis picture within normal limits | Description of the cause of infection | The causative picture of chronic disease is accompanied by signs of infection | Features of decreased kidney function | Description of the causation of chronic disease accompanied by impaired liver function | n |
| **Degree of normocytic anaemia** | Mild Anaemia (9.0-10.9 g/dL) | 4 (8.7%) | 9 (19.6%) | 25 (54.3%) | 7 (15.2%) | 1 (2.2%) | 46 (92.0%) |
| | Moderate Anaemia (7.0-8.9 g/dL) | 0 (0.0%) | 3 (75%) | 1 (25%) | 0 (0.0%) | 0 (0.0%) | 4 (8.0%) |
| | **Total** | **4 (8%)** | **12 (24%)** | **26 (52%)** | **7 (14%)** | **1 (2%)** | **50 (100)** |

Based on Table 2, it was found that from a total sample of 50 third-trimester pregnant women with normocytic anaemia with a mild degree of anaemia, 4 (8.7%) of the results of peripheral blood analysis were within normal limits, 9 (19.6%) described the cause of infection, 25 (54.3%) described the cause of chronic disease accompanied by signs of infection, 7 (15.2%) peripheral blood smears with a picture of decreased kidney function and 1 (2.2%) picture of the cause of chronic disease accompanied by impaired liver function. For women with moderate anaemia, 3 (75%) peripheral blood smears were found to be the

cause of infection, and 1 (25%) was described as the cause of chronic disease accompanied by signs of infection.

## 4. Discussion

### Degree of Anaemia

Based on the WHO cutoff value regarding anaemia, this study showed that most third-trimester pregnant women had a mild degree of normocytic anaemia. This study aligns with that conducted in Africa with a normocytic type of anaemia with a mild degree of anaemia (71.2%)(5). Likewise, the findings of refugee pregnant women in Sudan (89.2%) with mild anaemia(13). Physiologically the lowest decrease in haemoglobin levels in pregnant women occurs in the second trimester of pregnancy, where Hb is 10.5 g/dL; for trimesters I and III, haemoglobin levels are 11 g/dL, this is associated with normocytic anaemia, where HB levels are low but MCV is normal, with normal MCV levels and in trimester III pregnancy Hb levels in pregnant women increase again after a decrease in the second trimester, this allows women with normocytic anaemia to have more mild anaemia compared to moderate and severe levels(5). The level of anaemia can also be related to the cause of normocytic anaemia. Anaemia caused by an acute blood disorder shows the picture of normocytic anaemia because blood loss occurs massively. It should also cause a large amount of body iron loss. Therefore, in acute large blood loss, it gives a picture of normocytic anaemia on examination of the erythrocyte index (MCV and MCHC values) and provides an interpretation of normocytic and normochromic results on blood morphology examination with peripheral blood smear preparations. The presence of acute blood loss can worsen the level of anaemia(14). This study differs from that conducted on pregnant women in Sudan (100%) with mild anaemia. The small sample of women with normocytic anaemia in this study causes no variation in the degree of anaemia in pregnant women (15).

### Parity

Parity in this study showed a significant relationship between parity and the incidence of anaemia. High-risk parity has 5.64 times the possible risk of experiencing anaemia compared to mothers with low-risk parity. This study aligns with research in Rajasthan West India in fourth pregnant women (100%) experiencing anaemia (16). Likewise, with the findings of Tanziha et al., (2016) (62.5%) tendency of anaemia in pregnant women with high-risk parity, there was no significant relationship between pregnant women with high-risk parity and low-risk pregnant women with anaemia and not anaemia. This study does not align with refugee pregnant women in Sudan (62.1%) multiparous women do not experience anaemia(13). Iron reserves will decrease during pregnancy for mothers getting pregnant more often. The higher the frequency of pregnancies, most women will experience iron loss. In addition, high parity can also increase the risk of bleeding both before, during and after delivery. This is also associated with many children, indicating a high sharing of available food with other families, which interferes with food intake in pregnant women.

### Birth Intervals

The birth interval in this study showed no significant relationship between parity and the incidence of anaemia. This study is the same as the findings of pregnant women in Nigeria (64.0%) tendency to anaemia in pregnant women with high-risk birth intervals (<2 years). Still, there is no significant relationship between the birth intervals of high-risk pregnant women and low-risk pregnant women with anaemia and not anaemic (18). In contrast to the results of a study of pregnant women in Menoufia, Egypt, it was found (57.0%) that birth intervals < 2 years occurred in pregnant women with anaemia with a p-value of 0.001 or there was a significant relationship between pregnant women with high-risk birth intervals (< 2 years) in pregnant women with anaemia and women who were not anaemic (4). The interval of births or consecutive births was reported as a predictor of anaemia in many studies but not in this study. It is possible that the birth interval was relatively longer for this study's respondents than in previous studies. The birth interval, also known as the pregnancy interval, is the interval between two consecutive births of a woman. The distance between pregnancies is a consideration to determine the current pregnancy with subsequent pregnancies. The ideal birth spacing is > 2 years because conditions >

2 years are a recovery period for the reproductive organs, so it would be better if a mother gets pregnant again after > 2 years.

**Age**

The age of pregnant women in this study showed a significant relationship between age and the incidence of anaemia. High-risk age (< 20 and > 35 years) have a 7.31 times more likely risk of experiencing anaemia compared to mothers with low-risk age (20-35 years). In contrast to studies in Nigeria, in this study, there was a tendency for anaemia in pregnant women at high risk (<20 and > 35 years) 55.1%, but there was no significant relationship between pregnant women at high risk and low risk in pregnant women with anaemia and not anaemia(18), as well as the findings at King Abdul Asis Hospital, Saudi Arabia, the tendency of anaemia in pregnant women with high-risk age (57%) but there is no significant relationship between the age of pregnant women with high risk and low-risk age in pregnant women with anaemia and not anaemia where a p-value of 0.26 is obtained (2). The younger and older the pregnant woman will likely affect her nutritional needs. Pregnant women aged <20 years can experience competition for consumption between the fetus and the mother, who is growing and unable to meet the needs of iron for her fetus because it is still in the growth stage. Pregnant women aged > 35 years have entered the early stages of the degenerative phase, where the body's function is less than optimal, and various health problems can arise at this time field (19).

**Mid Upper Arm Circumference (MUAC)**

There was a significant relationship between nutritional status and the incidence of anaemia in this study with an OR value of 2.07, meaning that women with a chronic energy deficiency nutritional status of MUAC < 23.5 cm had 2.07 times the risk of experiencing anaemia compared to mothers with normal nutritional status MUAC > 23.5 cm. The cause of the lack of adequacy of energy is due to an imbalance of sources of nutrients such as macro and micronutrients; as a supporting source of energy, energy sources are produced from protein, fat, and carbohydrates, assisted by micronutrients such as calcium, zinc, and Fe which are present in daily food. The level of energy needs is less than the nutritional adequacy of pregnant women, caused by insufficient protein care as an energy source. Lack of protein consumption as an energy source can cause anaemia because the breakdown of protein is no longer intended for the formation of red blood cells, but red blood cells are reduced. The formation of haemoglobin could be better. This study is the same as that conducted in Indonesia (61.9%) of chronic energy deficiency mothers who experienced anaemia compared to non-anaemic mothers (17). The same thing was found in pregnant women at the Dustrik Dera Ethiopia Health Center (54.4%); women with chronic energy deficiency experienced compared to mothers who were not anaemic. (20)

**Peripheral Blood Smear Overview**

Examination of the peripheral blood smear often yields diagnostic clues or confirmatory evidence. Easily identifiable Red Blood Cell (RBC) findings associated with normocytic anaemia include large polychromatic shift cells representing reticulocytosis: target cells which may be found in liver disease, basophilic spots which may be present in hemolytic anaemia and mixed large and small RBC, which may indicate a mixed microcytic and macrocytic disease process (10,19,21). Other findings include uremia in renal disease and nucleated red blood cells (hemolytic anaemia, acute blood loss). The corrected reticulocyte index and the white blood cell and platelet count indicate whether the bone marrow is functioning properly. The corrected reticulocyte index should be increased in patients with acute anaemia but in competent bone marrow (22,23).

The picture of the peripheral blood in women with normocytic anaemia mostly shows the cause of chronic disease accompanied by signs of infection 26 (52%), followed by the picture of the cause of infection 12 (24%). On the results of Peripheral Blood Smear with Normocytic normochrome, the suspected cause of chronic disease accompanied by signs of infection characterized by leukocytes with toxic granulation (+). Toxic granulation is a sign that there is an infection that occurs. Normal normochromic with peripheral blood analysis of decreased kidney function marked erythrocytes with burr cells (+). Peripheral blood smears of patients with chronic kidney disease will find burr cells. Burr cell is a deformity of the erythrocyte where this cell has 10-30 short spines, which usually fill the surface of the

erythrocyte and have different sizes. Erythrocytes become rough and spiny, sometimes shaped like a star. This change in shape may be due to free radicals' peroxidation of the lipid membrane so that the erythrocyte membrane changes and will affect the shape of the erythrocyte. Overview Normochromic normocytic anaemia caused by chronic disease accompanied by impaired liver function was found to have erythrocytes with acanthocytes (+). Acanthocytes are a form of erythrocyte disorder in which the shape of the erythrocytes is irregular around the membrane and varies in size; these cells have 5-10 spines, which is usually found in cirrhosis of the liver associated with hemolytic anaemia, or rare inherited diseases. Overview Normochromic normocytic anaemia caused by chronic disease accompanied by impaired liver function was found to have erythrocytes with acanthocytes (+). Acanthocytes are a form of erythrocyte disorder in which the shape of the erythrocytes is irregular around the membrane and varies in size. These cells have 5-10 spines, usually found in liver cirrhosis associated with hemolytic anaemia or rare inherited diseases. Overview Normochromic normocytic anaemia caused by chronic disease accompanied by impaired liver function was found to have erythrocytes with acanthocytes (+). Acanthocytes are a form of erythrocyte disorder in which the shape of the erythrocytes is irregular around the membrane and varies in size. These cells have 5-10 spines, usually found in liver cirrhosis associated with hemolytic anaemia or rare inherited diseases (10,19,22,24).

Anaemia of chronic disease is the second most common anaemia after iron deficiency anaemia. The causes of Anaemia of chronic disease include chronic inflammation, chronic infections (viral, bacterial, parasitic and fungal infections) 19-95%, and malignancy (hematologic, tumour) 30-70%. Additional diagnostic laboratory tests for pregnant women with normocytic anaemia, such as creatinine and blood urea nitrogen for chronic kidney disease, prothrombin time with International Normalized Ratio (INR) and liver function tests for patients with liver disease. For patients with infections causing hemolysis eg sepsis due to β-hemolytic streptococcal infection, blood cultures should be obtained (1).

Limitations in this study namelyThe cause is only based on data from the results of a peripheral blood smear examination. There are no other follow-up tests, such as C-Reactive Protein (CRP), to assess chronic diseases and infections experienced by pregnant women.

## 5. Conclusion

Examination of peripheral blood smears produces diagnostic clues even though they only describe the results in the form of causes, so additional investigations are needed to determine the cause and true diagnosis of normocytic anaemia in third-trimester pregnant women. The picture of the peripheral blood in women with normocytic anaemia mostly shows the cause of chronic disease accompanied by signs of infection 26 (52%), followed by the picture of the cause of infection 12 (24%). On the results of Peripheral Blood Smear with Normocytic normochrome, the suspected cause of chronic disease accompanied by signs of infection characterized by leukocytes with toxic granulation (+). Toxic granulation is a sign that there is an infection that occurs.

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