

## Which Blood Group is More Anemic: Five Years of Retrospective Experience

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### Abstract

**Aim:** The relationship between blood groups and diseases has been a subject of interest for many researchers. This study aims to investigate the susceptibility of each blood group to anemia based on the relationship between the erythrocyte indices and the biochemical parameters used to diagnose anemia.

**Materials and Methods:** This is a retrospective study in which blood groups, according to the ABO grouping system, were examined in 1162 male individuals aged 18-65 within five years from 2018 to 2022. They were categorized as anemic or healthy based on their hemoglobin concentrations. Individuals with a hemoglobin (HGB) value below 12 g/dL were considered anemic.

**Findings:** Erythrocyte indices, including HGB (Hemoglobin), RBC (Red Blood Cells), HCT (Hematocrit), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), and parameters related to anemia such as iron, total iron-binding capacity (TIBC), vitamin B12, and ferritin were statistically analyzed. Among 1162 male individuals, 2% (n=23) were found to be anemic.

The most common blood group among the 1162 male individuals was A Rh (+) with 41% (n=465), followed by O Rh (+) with 26% (n=296), while the least common blood group was AB Rh (-) with 0.5% (n=6). The average HGB value of anemic individuals was 10.4 g/dL, and the average iron value was 70.6 µg/dL. Among the blood groups, the blood group with the lowest HGB and iron values was A Rh (-).

When comparing the blood groups and erythrocyte indices of anemic and healthy individuals, significant differences were found between the two groups (p<0.001), except for the MCHC parameter, where there was no significant difference (p=0.565). A significant difference was found in iron levels when comparing the blood groups and biochemical parameters

of anemic and healthy individuals. Still, no significant differences were observed in TIBC ( $p=0.885$ ), ferritin ( $p=0.318$ ), and vitamin B12 ( $p=0.108$ ).

**Conclusion:** In light of the study findings, it can be concluded that the parameters MCHC, ferritin, TIBC, and vitamin B12 are not as crucial as iron and HGB in diagnosing anemia, and the blood group most susceptible to anemia is A Rh (-).

**Keywords:** Blood Groups; Anemia, Nutritional Anemia; Erythrocyte Indices; Iron Deficiency; Red Blood Cells; Cobalamin; Folate Deficiency

## Introduction

The relationship between blood groups and diseases is among the subjects that attract the attention of researchers. In our study, we planned our study with the hypothesis that blood groups may be an indicator in determining susceptibility to anemia. In the study, we aimed to investigate the relationship between blood groups and anemia by statistically analyzing hematological and biochemical markers used in the diagnosis of anemia.

Blood is a vital tissue that spans approximately 100 kilometers of vessels within the human body, delivering nutrients and oxygen to every cell, thus ensuring the survival of all organisms. This vital tissue, consisting of approximately 90% water, comprises two main components: 55% plasma, which contains organic and inorganic substances, and 45% blood cells, which are special cells that flow within the bloodstream, reaching all organs. At the forefront of these cells are the erythrocytes, which do not carry a nucleus but instead, thanks to the hemoglobin, they transport for binding more oxygen and iron, giving blood its red color. In addition to these cells, other components in the blood tissue include platelets, also known as thrombocytes, which activate the crucial clotting mechanism necessary for homeostasis, and leukocytes, such as neutrophils, eosinophils, basophils, and monocytes, which play roles in the immune system [1].

Anemia is a condition where the production of blood cells in the bone marrow and hemoglobin, which binds oxygen and iron in red blood cells, can decrease for assorted reasons [2]. Anemia is defined by the World Health Organization (WHO) as a hemoglobin level below 13 g/dL for males over 15 years old and below 12 g/dL for non-pregnant females over 15 years old [3]. Even if there is an average amount of red blood cells, the blood's capacity to transport oxygen to the body tissues decreases with insufficient hemoglobin. This can lead to fatigue, weakness, dizziness, and shortness of breath. The optimum hemoglobin concentration required to meet physiological needs varies according to age, gender, altitude of residence, smoking habits, and pregnancy status.

Among the most common causes of anemia are nutritional deficiencies, especially iron, folate, vitamin B12, and vitamin A. In addition to these deficiencies, anemia can be caused by conditions like hemoglobinopathies, malaria, and tuberculosis. However, iron deficiency is the most prevalent cause of anemia, which occurs most frequently in women and children, irrespective of socio-economic status or geography. Anemia is a severe global public health issue mainly affecting young children and pregnant women. WHO estimates that 42% of children under five globally and 36.5% of pregnant women are anemic [4]. Anemia is also an important indicator that can reveal the socio-economic status of countries. According to WHO data from 2019, anemia affects 570.8 million people worldwide [4].

Identifying the causative factors of anemia is essential for establishing a diagnosis and initiating the correct treatment. Several parameters, known as erythrocyte indices, are used to diagnose anemia and can help determine its cause and type. In addition to parameters like HGB (hemoglobin), RBC (red blood cell count), HCT (hematocrit), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration), various biochemical parameters such as iron, TIBC (total iron-binding capacity), ferritin, and vitamin B12 serve as markers used in the diagnosis of anemia [5].

In addition to laboratory testing methods for diagnosing anemia, preventive measures can be taken in the earlier stages based on individuals' susceptibility to anemia. Therefore, various research studies have been conducted to identify individuals at risk of anemia. Studies correlating anemia with blood groups have drawn attention [6].

In humans, there are four blood groups based on the ABO blood grouping system, and when the Rhesus factor is considered, there are eight different blood groups. A Rh (+), B Rh (+), O Rh (+), AB Rh (+), A Rh (-), B Rh (-), O Rh (-), and AB Rh (-) are among the eight blood groups. In many studies comparing these blood groups, it has been observed that some have lower erythrocyte indices than others. This difference has led to the idea that certain blood groups may be more susceptible to anemia than others [7].

In addition to anemia, when many diseases are categorized based on blood groups, the incidence of certain diseases is higher in specific blood groups. Particularly in studies investigating the relationship between cancers and blood groups, it has been revealed that some blood groups are more predisposed to certain types of cancer than others [8]. Therefore, the risk of developing various diseases can be calculated by examining the distribution of blood groups within a specific population. This analysis of blood groups, particularly in the pre-diagnosis and initial treatment stages, can provide insights into an individual's susceptibility to diseases, enabling preventive measures to reduce the risk of disease. In this study, the susceptibility to anemia of each blood group was investigated retrospectively based on the relationship between blood groups and diseases, focusing on erythrocyte indices and biochemical parameters used to diagnose anemia.

## Materials and Methods

### Study Group

The research comprises 1162 healthy male individuals aged between 18 and 65 (mean age: 33). The study investigates the relationship between blood groups and anemia susceptibility, focusing on hematological parameters that are used as markers for anemia diagnosis, including erythrocyte indices, as well as biochemical parameters such as iron, ferritin, and vitamin B12.

### Excluded Group

Since the prevalence of anemia related to iron, ferritin, and vitamin B12 deficiency is higher in female individuals, and many factors can cause low hemoglobin, only male individuals were included in the study. Male individuals with chronic illnesses, infectious diseases, hemophilia, coagulation disorders, and similar conditions were excluded from the study.

### Data Collection Method

This is a retrospective study, and blood groups were determined using the gel centrifugation method in 1162 male individuals from hospitals affiliated with the Nevşehir Provincial Health Directorate for five years from 2018 to 2022. The results of erythrocyte indices, which include parameters such as HGB, HCT, RBC, MCV, MCH, MCHC, and the biochemical tests related to anemia, including iron, ferritin, TIBC, and vitamin B12, were obtained from the hospital automation system. In order to prevent cross-linking and false positive results in the determination of blood groups, gel centrifugation method with higher sensitivity and specificity was used. All individuals included in the study had their erythrocyte indices and biochemical test results evaluated according to the reference values specified in the automation system. Based on these values, individuals with hemoglobin levels below 12 g/dL were considered anemic, while those above this value were considered healthy.

## Statistical Analysis

The relationship between blood groups and the specified parameters was statistically analyzed using the Mann-Whitney U and Kruskal-Wallis tests in GraphPad Prism 9 software.

## Results

### Distribution of Blood Groups among Groups

When the blood groups of the 1162 male individuals included in the study were examined, the distribution of blood groups from most common to least common is as follows:

A Rh (+) (n=465 / 41%), O Rh (+) (n=296 / 26%), B Rh (+) (n=145 / 12.5%), AB Rh (+) (n=86 / 7%), A Rh (-) (n=64 / 6%), O Rh (-) (n=53 / 5%), B Rh (-) (n=24 / 2%), and AB Rh (-) (n=6 / 0.5%), as shown in Figure 1.

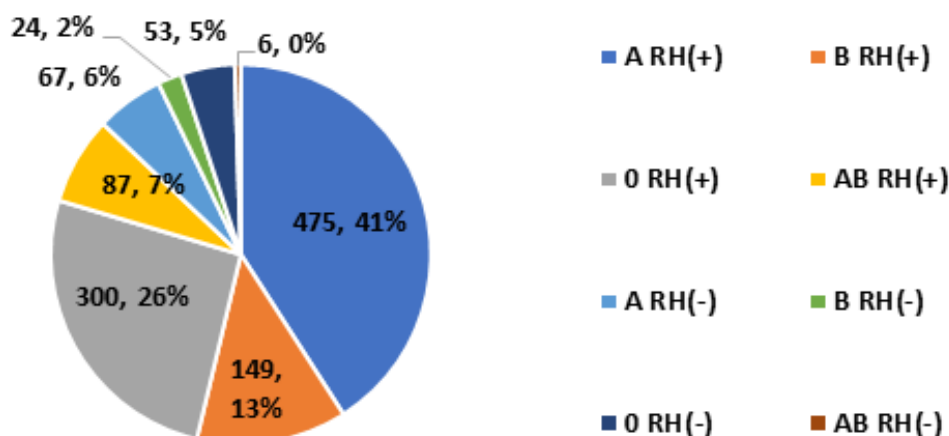


Figure 1: General Distribution of All Individuals Among Blood Groups (n; %)

Parameter	Reference Value
Hemoglobin (HGB)	12- 16 g/dL
Red Blood Cell Count (RBC)	3.8- 6.1. 10 <sup>12</sup> /L
Hematocrit (HCT)	43.5- 53.7 %
Mean Corpuscular Volume (MCV)	80-97 fL
Mean Corpuscular Hemoglobin (MCH)	27- 31.2 pg
Mean Corpuscular Hemoglobin Concentration (MCHC)	31.8- 35.4 g/dL
Iron (Fe)	65- 175 µg/dL
Total Iron-Binding Capacity (UIBC)	120- 370 µg/dL
Ferritin	22- 275 ng/mL
Vitamin B12	185- 890 pg/ mL

Table 1: Test Parameters and Reference Values

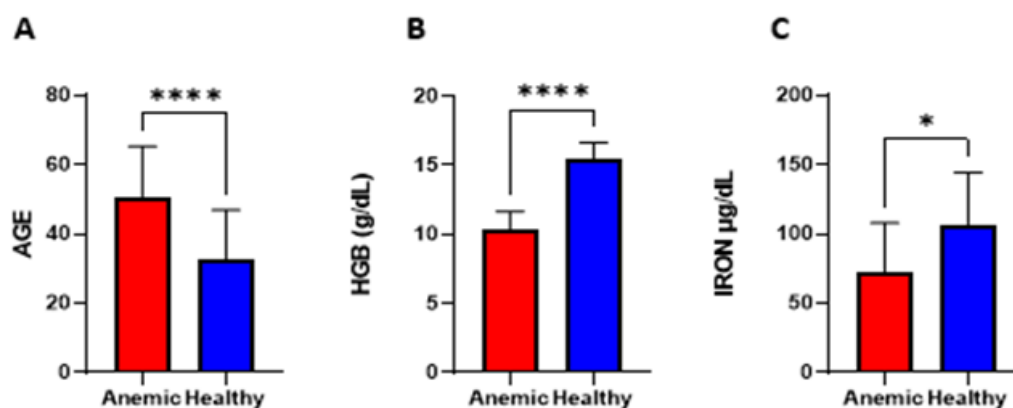
Additionally, out of the 1162 individuals, 86% (n=1005) were Rh (+) individuals carrying the D antigen, while 14% (n=157) were Rh (-) individuals not carrying the D antigen. According to the findings obtained from this study, 2% (n=23) of the 1162 male individuals with hemoglobin levels below 12 g/dL were found to be anemic. Among anemic individuals, the distribution of blood groups was found to be the same as in healthy individuals based on frequency, with A (n:13 / 56.5%), O (n: 5 / 21.7%), B (n:4 / 17.4%), and AB (n:1 / 4.3%) in that order. When each blood group was evaluated within anemic or healthy individuals, individuals with the A Rh (-) blood group had a higher rate of encountering anemic individuals compared to other blood groups (4.4%) (Table 2).

BLOOD GROUP	ANEMIC(n/ %)	HEALTHY (n/ %)	TOTAL(n)
A RH (+)	10 / %2	465 / %97	475
O RH (+)	4 / %1,3	296 / %98	300
B RH (+)	4 / %2,6	145 / %97	149
AB RH (+)	1 / %1,1	86 / %98	87
A RH (-)	3 / %4,4	64 / %95	67
O RH (-)	1 / %1,8	53 / %98	54
B RH (-)	0 / %0	24 / %100	24
AB RH (-)	0 / %0	6 / %100	6
<b>Total</b>	<b>23</b>	<b>1139</b>	<b>1162</b>
<b>Total</b>	<b>2%</b>	<b>98%</b>	<b>100</b>

**Table 2:** Rates of Anemic Individuals According to Blood Groups (n=1162)

### Distribution of Hemoglobin and Iron by Age

When 1162 male individuals were compared in terms of hemoglobin (HGB) and iron, which are primary indicators of anemia, significant differences ( $p < 0.0001$ ) were observed between the group considered anemic and the healthy group. Upon examining the average ages, it was observed that anemia increases with advancing age ( $p < 0.0001$ ) (Figure 2).



**Figure 2:** A. Distribution of healthy and anemic individuals by age ( $p < 0.0001$ ) B. Distribution of HGB levels between healthy and anemic individuals ( $p < 0.0001$ ) C. Distribution of iron levels between healthy and anemic individuals ( $p < 0.0251$ ).

Among the 1162 male individuals included in the study, the average age was 50.5 ( $\pm 14.6$ ), and the average hemoglobin level of the 23 anemic individuals was 10.3 g/dL ( $\pm 1.28$ ). In contrast, among the 1139 healthy individuals with an average age of 32 ( $\pm 14.1$ ), the average hemoglobin level was 15.5 g/dL ( $\pm 1.12$ ) (Figure 3).

When the distribution of blood groups was compared between healthy and anemic individuals, considering the Rhesus factor (D antigen), it was found that the A Rh (+) blood group was the most common in healthy and anemic individuals. In healthy individuals, the least common blood group was O Rh (-) (Figure 4), while among anemic individuals, B Rh (-) and AB Rh (-) blood groups were not encountered (Figure 3, Figure 5).

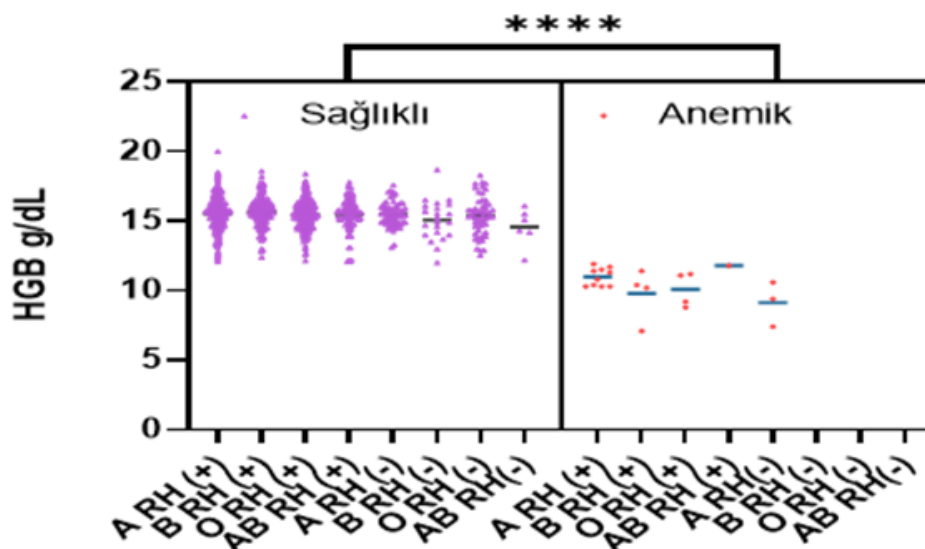


Figure 3: HGB Distributions in Healthy and Anemic Individuals (p<0.0001)

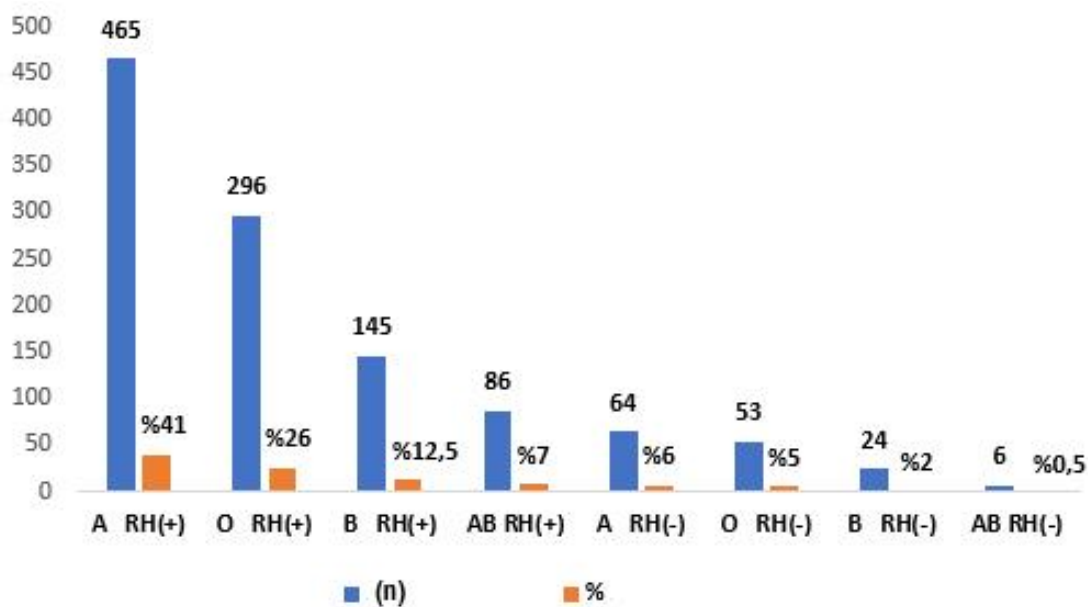


Figure 4: Blood Group Distribution in Healthy Individuals

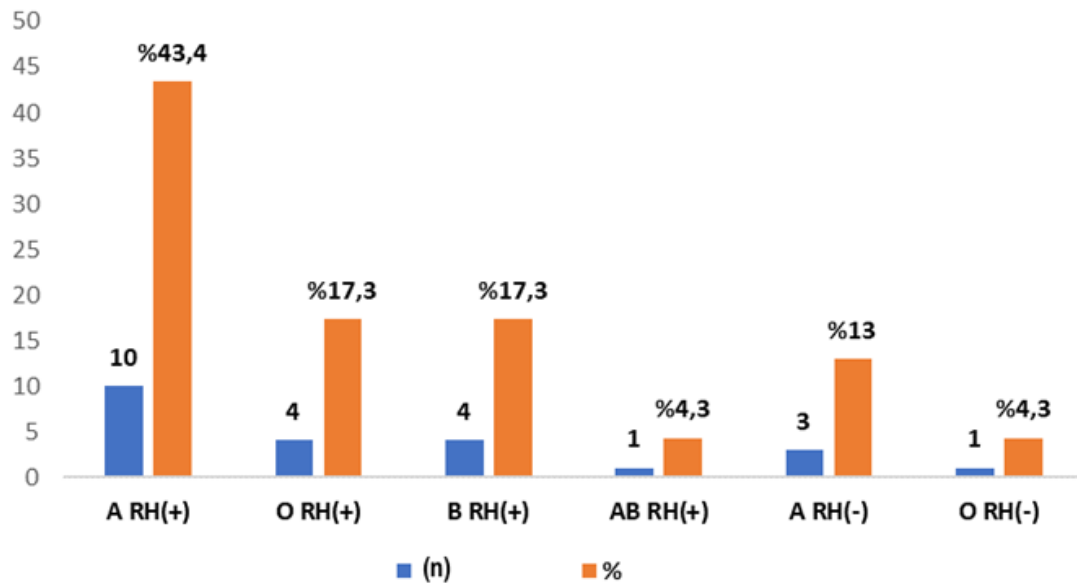


Figure 5: Blood Group Distribution in Anemic Individuals

### Hemoglobin Distributions in Anemic Individuals

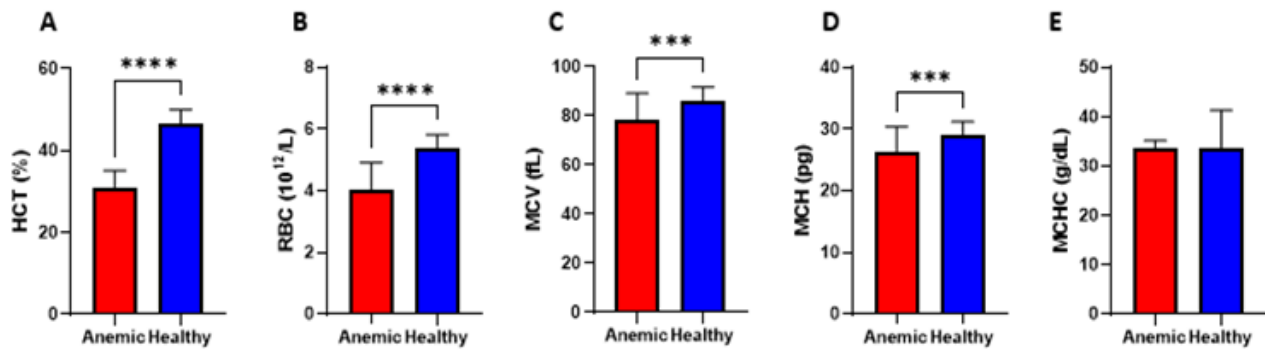
Each blood group was compared based on their HGB levels among the twenty-three anemic individuals. As a result, the lowest mean hemoglobin value was found in the A Rh (-) blood group (Table 3).

BLOOD GROUP	Number of People (n)	HGB Average
A RH(+)	10	10,95± 0,62
O RH(+)	4	10,07± 1,25
B RH(+)	4	9,77± 1,86
AB RH(+)	1	11,8
A RH(-)	3	9,13± 1,61
O RH(-)	1	9,95

Table 3: HGB Averages According to Blood Groups in Anemic Individuals

### Comparison of Erythrocyte Indices

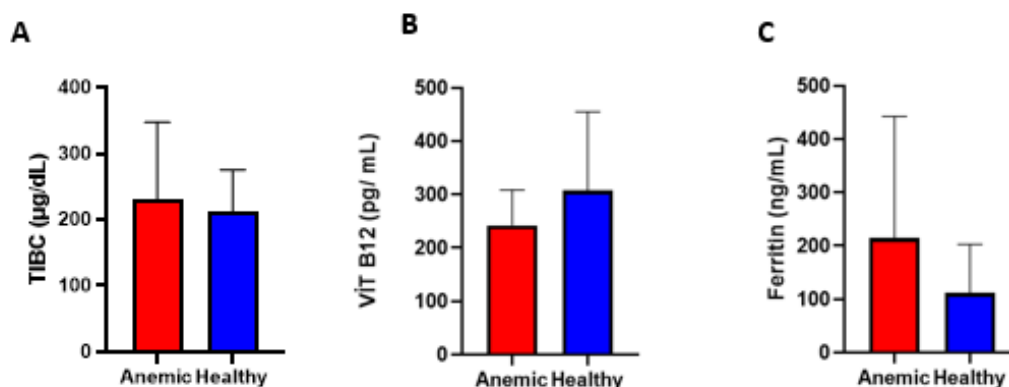
When healthy and anemic individuals were compared in terms of erythrocyte indices, significant differences were found between the two groups in terms of HCT ( $p < 0.0001$ ), RBC ( $p < 0.0001$ ), MCV ( $p < 0.0002$ ), and MCH ( $p < 0.0006$ ), while no significant difference was found between the two groups in the MCHC ( $p < 0.5667$ ) parameter (Figure 6).



**Figure 6:** Erythrocyte Indices in Healthy and Anemic Individuals. A. Distribution of HCT levels between healthy and anemic individuals ( $p < 0.0001$ ) B. Distribution of RBC counts between healthy and anemic individuals ( $p < 0.0001$ ) C. Distribution of MCV values between healthy and anemic individuals ( $p < 0.0002$ ) D. Distribution of MCH levels between healthy

### Comparison of Biochemical Markers

When healthy and anemic individuals were compared in terms of biochemical parameters related to anemia, no significant difference was found between the two groups in terms of Ferritin ( $p = 0.3215$ ), TIBC ( $p = 0.8891$ ), and Vitamin B12 ( $p = 0.1087$ ) (Figure 7).



**Figure 7:** Distribution of biochemical parameters related to anemia in healthy and anemic individuals. A. Distribution of TIBC levels between healthy and anemic individuals ( $p = 0.8891$ ). B. Distribution of Vitamin B12 levels between healthy and anemic individuals ( $p = 0.1087$ ). C. Distribution of Ferritin levels between healthy and anemic individuals ( $p = 0.3215$ ).

### Discussion

The relationship between blood groups and various diseases has been a subject of curiosity for many researchers to date. There are numerous studies on the association between blood groups and diseases such as infectious diseases, hematologic disorders, cancer, and autoimmune diseases [9, 10]. Similar studies to our research can be found in the literature. However, no studies similar to ours, focusing only on male individuals and including erythrocyte indices and biochemical parameters used as anemia markers, have been found. Studies investigating the relationship between blood group and anemia have included both male and female individuals. Since many factors may contribute to anemia in female individuals, we conducted our study with male individuals with more stable factors related to anemia to increase the study's validity.

This study aimed to investigate the relationship between erythrocyte indices, anemia-related biochemical parameters, and blood groups in 1162 male individuals and assess the susceptibility to anemia for each blood group. In the study, individuals were divided into two groups, anemic and healthy, based on their hemoglobin levels, and erythrocyte indices and anemia-related biochemi-



cal parameters were statistically compared between these two groups. As a result of the statistical analyses, the susceptibility to anemia for participants was assessed based on their blood groups.

The first research related to blood groups in Türkiye was conducted in 1918. Subsequent studies in many fields have focused on the phenotypic proportions and gene frequencies of blood groups in Türkiye. In the most comprehensive study conducted on blood group phenotypic distributions in Türkiye, blood groups of 6982 individuals were examined, and it was determined that 39.99% of these individuals had blood type A, 28.26% had blood type O, 17.09% had blood type B, and 14.66% had blood type AB. It was also found that 89.49% of the participants had Rh-positive (Rh+) blood, while 10.51% had Rh-negative (Rh-) blood [11]. Research in the United States indicates that the American population has a distribution of 46.70% blood type O, 37.10% blood type A, 12.10% blood type B, and 4.10% blood type AB. The distribution in the United Arab Emirates is similar to that in the United States, with 46.63% blood type O, 41.78% blood type A, 8.56% blood type B, and 3.04% blood type AB. In contrast, a study conducted in Greece, a neighboring European country, revealed blood group distributions similar to those in the Turkish population. Greece's most common blood groups were 48.9% blood type A, 34.21% blood type O, 12.04% blood type B, and 5.56% blood type AB. An Indian study showed a different distribution of blood groups, with B, O, A, and AB being the most common.

In our study, Turkish males' most common blood groups were A, O, B, and AB. In light of all these studies conducted in various countries and among different races, it is clear that blood group distributions vary among populations, and the Turkish population's blood group distribution bears the closest resemblance to European populations.

In our study, we analyzed the distribution of blood groups and examined the relationship between all blood groups and anemia in 1162 male individuals. Anemia was detected in 2% (n=23) of these individuals. Few studies in the literature specifically link a particular blood group to anemia. In a study conducted in 1956, blood group A was associated with pernicious anemia [14]. The most recent study examining the relationship between blood groups and anemia was conducted in India by Kumar et al. in 2021, involving 100 individuals aged 18-30. According to this study, the B blood group had the highest prevalence of anemia, followed by blood groups O, AB, and A [15]. Our study observed that the most common blood groups among anemic individuals were in the same order as the distribution of blood groups in healthy individuals. However, the frequency of anemia was higher in individuals with A Rh (+), O Rh (+), B Rh (+), and AB Rh (+) blood groups, and the risk was much lower among individuals with the D antigen (-).

This suggests a potential relationship between blood groups and susceptibility to anemia, with certain blood groups having a higher association with anemia. However, more research is needed to understand this relationship's mechanisms and clinical significance.

In Turkish men, individuals with the D antigen (-) blood group, particularly A Rh (-) and O Rh (-) blood groups, have the highest susceptibility to anemia. However, individuals with B Rh (-) and AB Rh (-) blood groups showed no anemic cases. In another study by Kumar et al., the A blood group was identified as the most resistant to anemia [15]. In contrast, our study suggests that B Rh (-) and AB Rh (-) blood groups are the most resistant to anemia in Turkish men.

The A Rh (-) blood group had lower hemoglobin concentrations compared to other blood groups, increasing the risk of anemia for this blood group (Table 2). Following the A Rh (-) blood group, the blood groups with the highest susceptibility to anemia were found to be B Rh (+), A Rh (+), O Rh (-), O Rh (+), and AB Rh (+), in that order. When we look at the study results in parallel with research conducted in different countries, it becomes apparent that susceptibility to anemia varies based on blood groups in different races. A study conducted in Bangladesh showed that the O blood group had the lowest hemoglobin and iron levels [16]. A study conducted in Africa found that the prevalence of anemia was higher in the AB blood group [17]. While the results of our study indicate that D antigen (+) individuals have a higher prevalence of anemia, it is believed that this situation is due to the high prevalence of RH (+) individuals in the population. This study highlights that individuals with the D antigen (-) have a higher risk of susceptibility to anemia.

When anemic and healthy individuals were compared regarding anemia-related test parameters, significant differences were found between the two groups in erythrocyte indices such as HGB, HCT, RBC, MCV, and MCH parameters. The significant differences in the low-value indices found in anemic individuals account for these differences. However, the lack of a significant difference in the MCHC parameter ( $p=0.5667$ ) between anemic and healthy individuals may suggest that the mean corpuscular hemoglobin concentration (MCHC) is not primarily significant in diagnosing anemia. There is no comprehensive study regarding the relationship between anemia and the MCHC parameter. In a study with pregnant women, a particular dietary supplement was effective on MCV and MCH but did not cause any change in MCHC against anemia [18]. In a study that examined the test parameters used to distinguish between iron-deficiency anemia and beta-thalassemia, it was found that there were no significant differences between MCHC and other erythrocyte indices in terms of diagnostic importance [19]. In another study that investigated erythrocyte index values in iron-deficiency anemia, it was found that MCHC was less sensitive compared to MCV (29.2%) and MCH (68.1%) in detecting iron deficiency [20]. Considering all these results related to the MCHC parameter, our study's results also support that the MCHC parameter is not a primary diagnostic test for anemia.

When erythrocyte indices were compared between anemic and healthy individuals, significant differences were found in the MCHC, Ferritin, TIBC, and Vitamin B12 parameters between the two groups. Specifically, the Iron parameter showed significant differences ( $p=0.0251$ ), which suggests that iron has primary importance in diagnosing anemia compared to other parameters. However, no significant differences were found between Ferritin, TIBC, and Vitamin B12 parameters, indicating that these parameters are not primarily crucial in diagnosing anemia.

In this study, unlike other studies, erythrocyte indices and anemia were considered when examining the relationship between blood group and anemia, erythrocyte indices, and anemia-related biochemical parameters other than hemoglobin and iron parameters. Interestingly, it was found that MCHC, Ferritin, TIBC, and Vitamin B12 parameters, which are strongly associated with anemia, did not show significant differences between anemic and healthy individuals. This challenges the conventional belief and suggests that these parameters may not have primary importance in diagnosing anemia.

### Study Limitations

Since there may be many different factors that may cause anemia in female individuals, we conducted our study with male individuals in order to increase the stabilization of the study results. In order to see whether the significant differences in anemic and healthy men will affect the results, female individuals can be included in the study and the differences between anemia-related parameters can be investigated by comparing genders. Similar studies can be expanded by comparing blood groups with the conditions that predispose female individuals to anemia.

### Conclusion

This study investigated the relationship between blood groups, erythrocyte indices, and the biochemical markers used to diagnose anemia. Based on this correlation, the anemia susceptibility risks of blood groups were explored, contributing to the literature. With this study, male individuals with the A Rh (-) blood group, who are at a higher risk of anemia, can be informed and encouraged to take prophylactic measures against anemia, creating awareness of their vulnerability and recommending regular consumption of iron and vitamin-rich foods in their diets.

### Ethics

This study was conducted with the permission of the Cappadocia University Ethics Committee (Decision No: 2021.49) and Nevşehir Provincial Health Directorate.

## Authorship Contribution

Data collection and processing: Z.A., H.D., Literature review: Z.A., H.D., Analysis and Interpretation: Z.A., Writing: Z.A., H.D.

## Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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