Original Article

Age-Related Changes in RBC Indices: A Comparative Cross-Sectional Study

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Abstract

Objective: The primary objective of this comparative cross-sectional study is to elucidate age-related variations in peripheral blood parameters among a cohort of healthy males. Specifically, the study aims to investigate shifts in key hematological indices, with a particular emphasis on hemoglobin levels, across different age strata in males.

Methodology: Data from 88 participants aged 18 to 92 years, all devoid of any known hematological disorders, were included in this study. Key peripheral blood parameters, including hemoglobin, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, were assess edvia a complete blood count test by Boule Medonic AB, Merck, Sweden counter. Participants were categorized into four age groups: I (aged 18 to 37 years),II (38 to 57 years), III (58 to 77 years), and IV (78 years and older).

Results: A highly significant difference (p=<0.05) was noticed between hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration among the four groups compared to a one-way ANOVA test. A negative correlation was observed between age and hemoglobin levels ($r = -.575$, $p < .001$), along with negative correlations between age and red blood cell count ($r = -.439$, $p < .001$) and hematocrit ($r = -.445$, $p < .001$).

Conclusion: Red blood cells parameters varied with increasing age and differed between young males as compared to old age, indicating a decline in erythropoiesis in elderly males.Normocytic and normochromic anemia was observed in groups III and IV.

Keywords: hemoglobin, hematocrit, erythrocyte indices, aging, erythrocytes, male, cross-sectional studies, Hematopoiesis, Age-related Changes, Anemia.

Introduction

Cellular senescence, stem cell depletion, genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, uncontrolled nutrition sensing, and mitochondrial dysfunction are the nine indicators of aging. Organs and tissues affected by these cellular changes include hematopoietic cells, the stromal cells that support them in the common bone marrow microenvironment, and a variety of immunocompetent cells.[1] Homeostasis and function of hematopoietic stem and progenitor cells are controlled by BM endothelial cells (ECs) and the angiocrine chemicals they secrete. Stress caused by aging, inflammation, bone diseases, or bone malignancies can change vascular morphology and endocrine signaling, which has a significant impact on osteogenesis, bone angiogenesis, and hematopoiesis.[2] A balanced production of all blood cells is ensured by Hematopoietic stem cells (HSCs) throughout life. As they age gradually, HSCs lose their self-renewal and regenerative potential, and there is an intense increase in the occurrence of cellular derailment.[3] The term "anemia of the elderly" (AE) has historically been widely accepted since it has long been thought that declining Hb levels are a nearly inevitable result of aging.[4] In a recent consensus agreement, the European Hematology Association recommended AE as a key research issue. In general, one-third of elderly anemia patients have nutritional deficiencies, primarily iron, folate, or vitamin B12 insufficiency; one-third have anemia with unknown causes; one-third have chronic subclinical pro-inflammatory states and chronic kidney disease.[5] According to the study, anemia prevalence is also higher among those who smoke, have a worse socioeconomic position, and have less education.[6] In elderly persons, fragility is closely related to hemoglobin concentration. The likelihood of being frail is reduced by 14% by an increase of one point in hemoglobin concentration.[7] The deformability of red blood cells (RBCs) is determined by three key factors: the shape and structure of the RBCs themselves, the thickness and flow characteristics of the fluid inside the cells (cytosol viscosity), and the viscoelastic properties of the RBC membrane.[8] Health policies on anemia screening should be used to prevent, delay, or even reverse these adverse effects of anemia. Identifying, assessing, and treating anemia in this susceptible population is necessary.[9]

The rationale for this comparative cross-sectional study resides in elucidating the age-related dynamics of peripheral blood parameters in healthy males. The primary aim of this investigation is to address this knowledge gap, considering the scarcity of comprehensive data regarding age-related shifts in hematological indices within this specific demographic entity.

Methodology

This comparative cross-sectional study was conducted from September 2018 to December 2019.An institutional review board of the University of Lahore (letter# NO: UOL/IMBB/Sample/19/106 on 11th February 2019) gave permission to conduct the study according to the Helsinki Declaration. The Gujranwala Medical College Principal and the DHQ Hospital Gujranwala, a teaching affiliate of the college, authorized the sample collection to be carried out. Eighty-eight male volunteers, ranging in age from 18 to 92, were chosen by convenient sampling technique for this study. Cochran's equation[10] had been used to calculate the sample size, considering the population of Punjab, which was 110 million[11] in 2020. This method is suitable for small populations with known sizes and includes a population correction factor.A precision level of ±5% and a confidence level of 95% was chosen to ensure a margin of error within five percentage points of the true population value with 95% confidence. The estimated proportion was set to 0.5 to account for maximum variability. Based on these parameters, the calculated sample size was 86.The decision to include only males in this study was driven by the research objectives and practical considerations, ultimately aiming to provide a focused investigation into age-related variations in RBC parameters among males within a homogenous sample, minimizing confounding factors related to sex-specific differences in hematological parameters.The study did not include participants who had any infectious or chronic conditions, including chronic liver and thyroid illnesses, or who were on long-term medications like steroids. Subjects with serious cognitive or communicative impairments were also disqualified.

Subjects were grouped according to age12, as follows:

Group I: 18 to 37 years

Group II: 38 to 57years

Group III: 58 to 77 years

Group IV: 78 or >78 years

Physical measurements and blood collection:

Each time, formal informed consent, which was duly signed, was obtained prior to blood sample collection. Additionally, a custom form was utilized to document each subject's medical history, including information about past medications and any existing medical conditions. Blood samples were obtained from the cubital vein using single-use syringes for each participant. Approximately 3 milliliters of blood were withdrawn from each individual and transferred into centrifuge tubes containing heparin as an anticoagulant and complete blood count analyses were done on the same day. A CBC counter was used to perform a complete blood count (Boule Medonic AB, Merck, Sweden).The method for determining hemoglobin levels involved adding N/10 HC1 to a graduated dilution tube up to the 20% mark, then transferring heparinized blood into a hemoglobin pipette and mixing it with the HC1 in the tube. Distilled water was added until the color matched

the standard tube. Red blood cell (RBC) count was conducted by drawing blood with an anticoagulant into an RBC diluting pipette, then adding diluting fluid and rotating the tube horizontally to disperse blood cells evenly. Packed cell volume (PCV) was measured using heparinized blood in capillary tubes, sealed, and centrifuged. Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration(MCHC), were calculated using the following formulas:

MCV = Hematocrit × 10 / RBC count

MCH = Hemoglobin × 10 / RBC count

MCHC = Hemoglobin × 100 / Hematocrit13

Statistical Analysis:

ANOVA (F-test) analysis, conducted using Statistical Package for the Social Sciences 25, was employed to assess the significance of inter-group differences, followed by documentation of Pearson correlation among key variables. Statistical significance was deemed present when the p-value fell below 0.05.

Results

Age-related changes in the hematopoietic profile of four groups are shown in Table 1.This table presents data categorized into four groups: Groups I, II, III, and IV, consisting of 24, 26, 28, and 10 participants, respectively. The average age of participants in each group increases progressively from Group I to IV, with mean ages of 27, 48, 67, and 84 years respectively. Hemoglobin (Hb) levels, hematocrit (HCT) percentages, and red blood cell (RBC) counts show a significant decrease as age advances, indicated by the decreasing mean values across the groups. Statistical analysis using an F test indicates significant differences in Hb, HCT, and RBC counts among the groups ($p < 0.001$), as displayed in Figure 1. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) show less variation across the age groups, with no significant differences observed in MCV and MCH ($p > 0.05$). However, there are significant differences in MCHC among the groups ($p = 0.003$).

Age-related changes in hemoglobin and hematocrit: Both hemoglobin (Hb) and hematocrit (HCT) exhibited a decrease with advancing age. Notably, a significant decrease in Hb level was observed in the oldest age group (Group IV) compared to the youngest group (Group I) (11.3±0.4 vs. 13.2±1.43g/dl, respectively). Similarly, mean serum levels of hematocrit tended to significantly decrease with increasing age, even in subjects with normal Hb levels.

Age-related changes in RBC and MCHC: A significant decrement in red blood cell (RBC) count and mean corpuscular hemoglobin concentration (MCHC) was observed from age Groups I to IV. Specifically, the RBC count decreased from 4.61±0.04 in Group I to 4.17±0.13 in Group IV. Moreover, a pronounced decline in the levels of MCHC was noted in subjects aged 80 years or more, likely attributable not only to decreased RBC levels but also to lower Hb levels.

Age-related changes in MCV and MCH: The values of mean cell volume (MCV) and mean cell hemoglobin (MCH) did not exhibit significant decreases with increasing age. However, MCV increased in Group II compared to Group I (76.71±0.8 vs. 75.77±1, respectively), suggesting microcytic anemia in the oldest group. Meanwhile, MCH decreased in Groups II and III, then increased again in Group IV.

To measure the strength of the linear relationship between age and RBC indices markers, correlation was applied as shown in Table2.

The analysis revealed several negative correlations between key hematological parameters. Firstly, there

Table 1. Comparison of Red Blood Cell (RBC) Indices among Different Groups

Group	I	II	III	IV	P (f test)
	n=24	n=26	n=28	n=10	
Age(years)	27±5	48±6	67±5	84±6	
Hb (g/dl)	13±0.8	12.7±1.2	11.5±1.3	11.3±1.4	0.001*
HCT (%)	35±1.9	33±3.4	31±3.7	31±4	0.001*
RBC (million/mm3)	4.6±0.2	4.3±0.4	4.1±0.4	4.1±0.4	0.01*
MCV(FL)	75±5	76±4.5	74±8.1	74±5.2	0.46
MCH(µm3/FL)	28±2	29±2	27±3.3	27±2.3	0.06
MCHC (g/dl)	37±1.3	37±1.1	36±1.3	36±1.4	0.003*

Data is represented as mean ±SD. "n" represents the number of cases in each group. Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular Volume, MCH: Mean corpuscular hemoglobin,MCHC: Mean corpuscular hemoglobin concentration. p=<0.05 is taken as significant.*

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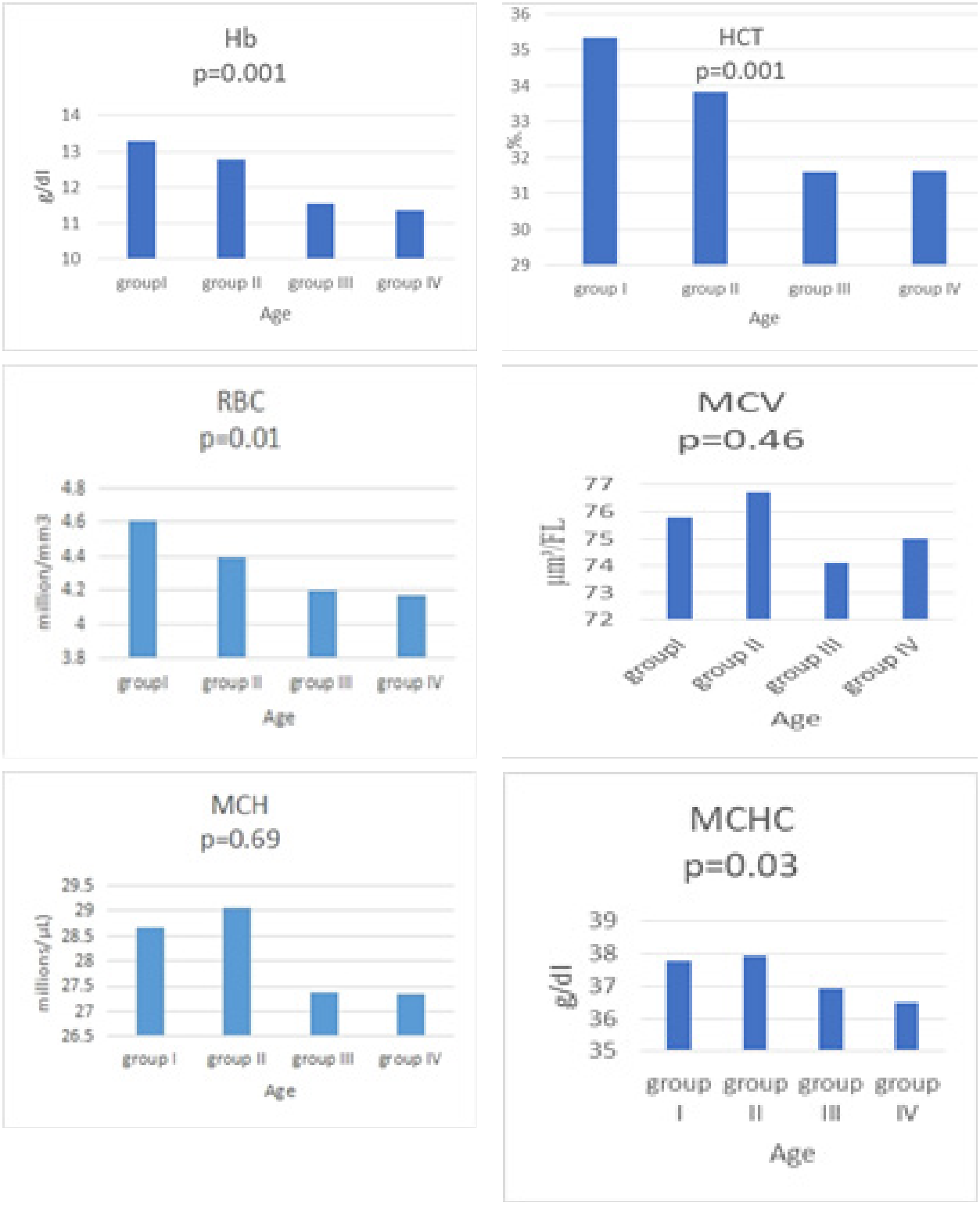

Figure 1: Comparison of Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular Volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration among different age groups

Table 1. Comparison of Red Blood Cell (RBC) Indices among Different Groups

Correlations								
Parameters		**Age**	**Hb**	**RBC**	**MCV**	**MCH**	**MCHC**	**HCT**
Groups(I-IV)	r	.957**	-.530**	-.407**	-.104	-.221*	-.344**	-.416**
	p	.001	.001	.001	.337	.039	.001	.0001
Age	r		-.575**	-.439**	-.097	-.230*	-.380**	-.445**
	p		.001	.001	.367	.031	.001	.001
Hb	r			.743**	.447**	.499**	.334**	.938**
	p			.001	.001	.001	.001	.001
RBC	r				-.122	-.139	-.062	.822**
	p				.257	.197	.565	.001
MCV	r					.920**	.204	.391**
	p					.001	.056	.001
MCH	r						.567**	.328**
	p						.001	.002
MCHC	r							.026
	p							.812

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*
Hb: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin,MCHC: Mean corpuscular hemoglobin concentration. p=<0.05 is taken as significant.*

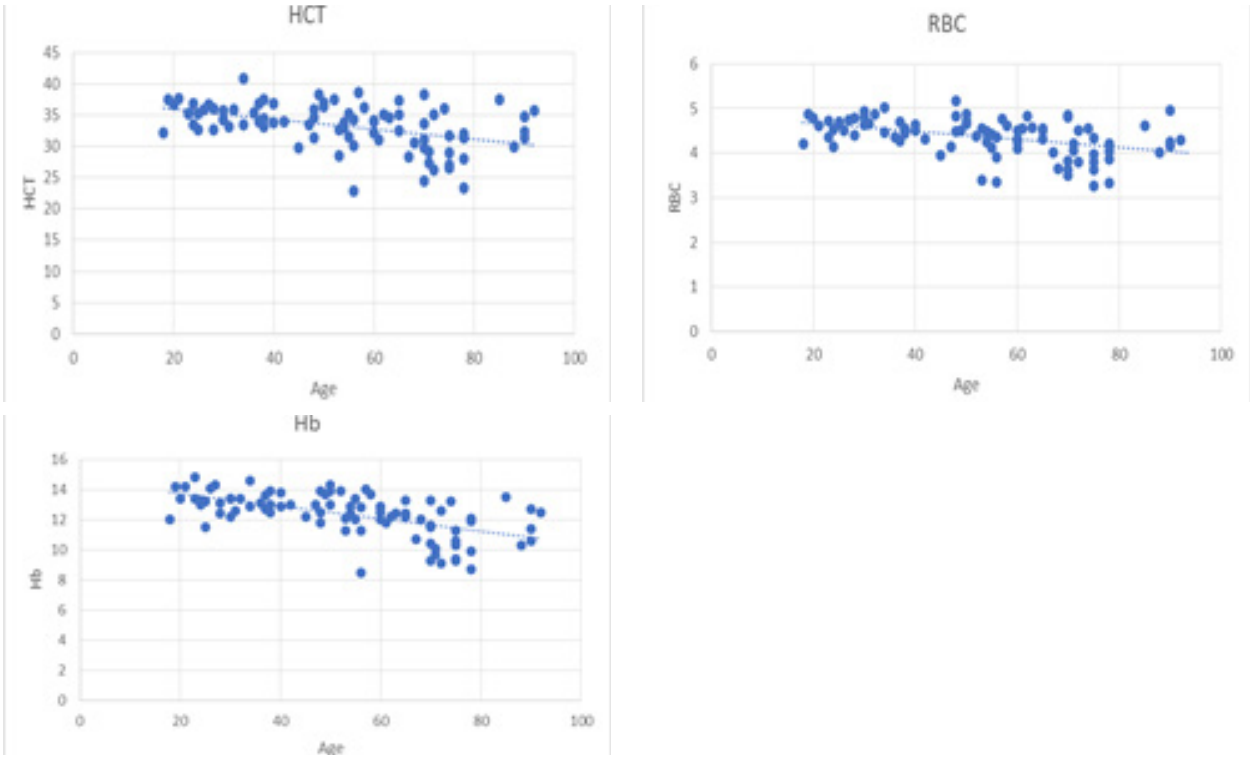

Figure 2: Scatter plots showing a negative correlation between age, hematocrit, hemoglobin, and red blood cell count

was a significant negative correlation between Age and Hemoglobin (Hb) levels (r = -.575, p < .001), indicating that as age increased, hemoglobin levels tended to decrease. Similarly, age exhibited a negative correlation with red blood cell count (RBC) (r = -.439, p < .001) and hematocrit (HCT) (r = -.445, p < .001), suggesting a decline in these parameters with advancing age. A significant correlation has been displayed graphically in Figure 2.

Discussion

In the current study, the age-related changes in hematological parameters were investigated across four groups spanning different age ranges. Hemoglobin (Hb) levels, hematocrit (HCT) percentages, and red blood cell (RBC) count displayed a significant decrease with advancing age, as indicated by decreasing mean values across the groups. Statistical analysis revealed significant differences in Hb, HCT, and RBC counts among the groups (p < 0.001). Conversely, MCV and MCH did not exhibit significant variations across age groups (p > 0.05), while MCHC showed significant differences (p = 0.003). Correlation analysis demonstrated negative correlations between age and Hb levels, RBC counts, and HCT percentages, highlighting a decline in these parameters with increasing age.Normocytic and normochromic anemia was identified in groups III and IV.

Anemia poses a significant health challenge among the elderly population in Indonesia due to age-related physiological changes that disrupt nutrient absorption. A descriptive study aimed to elucidate the characteristics of anemia among elderly patients at Siti Rahmah Hospital, Padang. Hematology profiles of 52 elderly patients, with a mean age of 68.31 years, revealed mild anemia with normochromic normocytic features, while leukocyte, hematocrit, and platelet counts remained within normal ranges. Fourteen cross-sectional descriptive studies conducted at Combined Military Hospital, Multan, aimed to assess the prevalence and morphological types of anemia among geriatric patients. After analyzing data from 175 elderly patients, it was found that normocytic anemia was the most common type, with an overall anemia prevalence of 23.43%. These findings underscore the significant prevalence of anemia in older patients, particularly normocytic anemia, highlighting the need for effective management strategies in geriatric healthcare. This was followed by macrocytic anemia, which accounted for 19.51% of cases, and microcytic anemia, which accounted for 4.88% of cases.[15]

Similar results were obtained in another study in individuals older than 60 years, revealing normocytic anemia (75%); the most common type, microcytic anemia (21.7% each), came next, followed by macrocytic anemia (3.3%).[16] However, the current study was done in central Punjab, and it showed a decline of Hb with advancing age with almost constant MCV, MCH, and MCHC values only in males. In another study, annual average decrease in hemoglobin and hematocrit levels, coupled with an increase in mean corpuscular volume (MCV), was evident, with greater prominence observed among men.[17]

In Bahrain, a study of hospitalized patients revealed that in terms of severity, moderate anemia was the most prevalent type, affecting 56.1% of the patients. Again, anemia associated with chronic disease emerged as the most common etiological type, accounting for 48.1% of cases. Anemia was found to be as prevalent as other comorbidities, such as hypertension (71.4%) and diabetes mellitus (53.7%). Patients with anemia experienced significantly longer hospital stays (p < 0.001), with the duration being inversely correlated to hemoglobin levels. Additionally, one-year mortality rates were markedly higher among anemic patients (p < 0.001). Patients with moderate to severe anemia were at a significantly greater risk of mortality compared to those with mild anemia (odds ratio [OR] = 2.2, 95% confidence interval [CI]: 1.27–4.92).[18]

Comparable findings were observed in West Bengal, revealing an incidence rate of anemia at 85.2%, with the highest prevalence noted among individuals aged over 80 years. In contrast, 71.2% of females were affected by anemia, indicating a higher prevalence in females compared to males.[19] In a study involving diabetic patients, individuals with type 2 diabetes exhibited notably lower average RBC counts, HB, HCT, and MCHC compared to the control group (p < 0.001 for RBC counts, Hb, HCT, p = 0.002 for MCHC). However, a significant disparity between the type 2 diabetic patient group and the control group was observed regarding the mean value of red cell distribution width (RDW) (P < 0.001). Furthermore, diabetic subjects displayed a significant inverse correlation between glycemic control and RBC count, Hb, and HCT levels, indicating that improved glycemic control correlated with higher RBC parameters.[20]

The possible mechanism of reduced hemoglobin (Hb) levels in the elderly is intricate and involves multiple factors. An investigation indicates that subclinical chronic low-grade inflammation, resistance of bone marrow to erythropoietin (EPO), and alterations in hepcidin levels are pivotal mechanisms contributing to this phenomenon, consequently impacting iron metabolism and leading to diminished serum iron levels. Among the biomarkers studied IL-1β, TNFα, and hepcidin exhibited the most significant diagnostic efficacy, while CD34 and CD38 demonstrated utility in assessing the compensatory response to the diminished oxygen-carrying capacity observed in inflammation-related anemia.[5]

Conclusion:

The study revealed compelling evidence of age-related variations in peripheral blood parameters among a cohort of healthy males. Significantly, the analysis highlighted a progressive decrease in key hematological in-

dices, particularly hemoglobin levels, across different age strata. Notably, the findings strongly underscored the hypothesis that there is a progressive decrease in RBC parameters with advancing age, even in the absence of any known illness. Normocytic and normochromic anemia was detected in males above age 58.

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