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Age and Gender-Wise Distribution of Normochromic Normocytic Anemia Subtypes in Adults: A Cross-Sectional Study

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Abstract

Objective: To determine the frequency of the two main subtypes of normochromic normocytic anemia (NNA), anemia of chronic disease (AOCD) and mixed deficiency anemia (MDA), across different adult age groups and genders, and to compare the severity of anemia between these groups.

Methodology: This descriptive cross-sectional study was conducted at the Department of Hematology, Pakistan Railway Hospital, Rawalpindi, from September 2024 to June 2025. Using non-probability convenience sampling, 174 patients aged 15–65 years with NNA on complete blood count and peripheral blood smear were enrolled. Patients were classified as AOCD or MDA on smear morphology, confirmed by serum ferritin, vitamin B12, and folate, with C-reactive protein and erythrocyte sedimentation rate as inflammatory markers.

Results: The mean age was 42.8 ± 13.2 years, and 110 (63.2%) patients were female. AOCD was found in 136 (78.2%) and MDA in 38 (21.8%) patients. Patients with MDA were younger than those with AOCD (36.4 ± 17.1 vs 44.6 ± 11.3 years; p=0.008). MDA accounted for 65.0% of NNA in the 15–25-year group, compared with 7.4–23.3% in older groups, while AOCD peaked at 46–55 years (36.8% of AOCD cases) (p<0.001). The gender distribution did not differ between subtypes (females 62.5% vs 65.8%; p=0.71). MDA was associated with lower mean hemoglobin (8.8 ± 1.3 vs 9.5 ± 1.6 g/dL; p=0.021) and more moderate-to-severe anemia (89.5% vs 55.1%; p<0.001). Severity did not differ significantly by age group (p=0.53) or gender (p=0.23).

Conclusion: AOCD was the predominant cause of NNA and was concentrated in middle-aged adults, whereas MDA predominated among adolescents and young adults and produced more severe anemia. A normocytic blood picture in a young patient should prompt evaluation of iron, vitamin B12, and folate status rather than being attributed to chronic disease.

Keywords: Anemia; Anemia of chronic disease; Mixed deficiency anemia; Normochromic normocytic anemia; Age factors; Sex factors; Pakistan.

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Introduction

Anemia is a reduction in hemoglobin concentration or red cell mass below the range expected for an individual's age and sex; the World Health Organization (WHO) defines it in non-pregnant adults as a hemoglobin concentration below 12 g/dL in women and below 13 g/dL in men.[1] In 2021, anemia affected an estimated 1.92 billion people worldwide, with women and populations in South Asia among the most affected.[2] Anemia is classified morphologically by mean corpuscular volume (MCV) into microcytic, normocytic and macrocytic types; normochromic normocytic anemia (NNA) is characterized by an MCV of 80–100 fL with normal mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration.[3,4] Because automated red cell indices are averages, examination of the peripheral blood smear (PBS) remains essential to detect mixed or dimorphic red cell populations that the indices conceal.[5,6]

NNA has two principal etiologies in adults. In anemia of chronic disease (AOCD), also termed anemia of inflammation, cytokines such as interleukin-6 induce hepcidin, which blocks intestinal iron absorption and traps iron in macrophages and hepatocytes; erythropoietin response is blunted and red cell survival shortened, producing iron-restricted erythropoiesis despite adequate iron stores.[7–9] Mixed deficiency anemia (MDA), in contrast, results from the coexistence of iron deficiency with vitamin B12 and/or folate deficiency; the opposing effects of microcytosis and macrocytosis can yield a normal MCV, while the smear shows a dimorphic picture.[5,10] MDA usually reflects inadequate dietary

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intake, malabsorption or gastrointestinal disease, including *Helicobacter pylori* infection, which is associated with iron deficiency.[11,12]

Distinguishing these subtypes is clinically important because treatment differs. A serum ferritin above 100 ng/mL with low transferrin saturation favours AOCD, a ferritin below 30 ng/mL indicates absolute iron deficiency, and intermediate values are difficult to interpret.[13,14] As ferritin is an acute-phase reactant, it must be interpreted together with inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), alongside vitamin B12 and folate assays.[15,16]

The relative contribution of these subtypes is likely to vary with age and gender. In older adults, about one-third of anemia is attributable to chronic inflammation or chronic kidney disease[17], whereas nutritional anemia predominates among adolescents and women of reproductive age in low- and middle-income countries, including Pakistan.[11,18–20] Most hospital-based studies from Pakistan have, however, examined anemia in the elderly or within single diseases such as diabetes, hypothyroidism or systemic lupus erythematosus[21–23], and data on how the subtypes of NNA are distributed across the adult age span and between genders are lacking. This study was therefore conducted to determine the frequency of AOCD and MDA among adults with NNA across different age groups and genders, and to compare the severity of anemia between these groups.

Methodology

Study design and setting: This descriptive cross-sectional study was conducted at the Department of Hematology, Pakistan Railway Hospital (PRH), from September 2024 to June 2025.

Ethical approval: The study was approved by the Institutional Review Committee (IRC) of Islamic International Medical College, Riphah International University, Rawalpindi (Ref. No. Riphah/IIMC/IRC/24/1057, dated 12 August 2024). Written informed consent was obtained from all participants (and from a guardian for participants under 18 years).

Sample size and sampling: The sample size of 174 was calculated using Cochran's formula, $n = Z^2p(1-p)/d^2$, taking an expected prevalence of the normochromic normocytic blood picture of 13% [24], a 95% confidence level (Z = 1.96) and a 5% margin of error. Patients were recruited by non-probability convenience sampling.

Inclusion criteria: Patients aged 15–65 years of either gender attending the medicine outpatient or inpatient departments whose complete blood count (CBC) showed anemia by WHO criteria[1] with normal red cell indices (MCV 80–100 fL, MCH 27–32 pg), and whose anemia was attributable to an inflammatory or systemic chronic disease (rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus, chronic kidney disease, hypothyroidism) or to nutritional deficiency or malabsorption (poor dietary intake, celiac disease, Crohn's disease, ulcerative colitis, *H. pylori* infection).

Exclusion criteria: Pregnancy; current hematinic therapy; history of blood transfusion; chronic blood loss; malignancy; tuberculosis; vasculitis; hypopituitarism; and chronic liver disease.

Data collection and laboratory methods: A detailed history and examination were recorded on a structured proforma. Venous blood was collected in EDTA, plain and citrate tubes following standard operating procedures; samples were checked for hemolysis, clotting, adequate volume and labelling. CBC was performed on a Mindray BC-5000 hematology analyzer with daily internal quality control. A peripheral smear was prepared from each sample, stained with Leishman stain and examined under oil immersion by the principal investigator for red cell morphology. Serum ferritin, vitamin B12 and folate were measured by enzyme-linked immunosorbent assay on an RT-6000 microplate reader (Rayto), CRP by a semi-quantitative latex agglutination slide test (a result >6 mg/L taken as raised), and ESR by the Westergren method.

Operational definitions: Reference ranges were: serum ferritin 10–120 ng/mL in females and 10–240 ng/mL in males[25]; serum folate 3.56–22.40 ng/mL[25]]; serum vitamin B12 206–678 pg/mL [26]; CRP <6 mg/L; and ESR up to 15 mm/h in men and 20 mm/h in women.[27] *AOCD* was defined as NNA with a normochromic normocytic smear in a patient with a documented chronic inflammatory or systemic disease, with normal or raised serum ferritin, normal vitamin B12 and folate, and raised ESR and/or CRP. *MDA* was defined as NNA with a smear showing a mixed red cell population (hypochromic microcytes with macrocytes, with or without macro-ovalocytes and hyper

segmented neutrophils), with low serum ferritin together with low vitamin B12 and/or folate. Severity of anemia was graded using the WHO hemoglobin cut-offs of 10.0 and 7.0 g/dL as mild (Hb ≥10.0 g/dL), moderate (7.0–9.9 g/dL) or severe (<7.0 g/dL), as previously applied in adult patients.[28,29] Participants were grouped by age into 15–25, 26–35, 36–45, 46–55 and 56–65 years.

Statistical analysis: Data were entered and analyzed in SPSS version 27. Continuous variables were expressed as mean ± standard deviation and compared with the independent-samples t-test (Welch's correction where variances were unequal). Categorical variables were expressed as frequencies and percentages and compared with the chi-square test. A p-value <0.05 was considered statistically significant.

Results

A total of 174 patients with NNA were included. The mean age was 42.8 ± 13.2 years (range 16–64), and 110 (63.2%) were female. AOCD was identified in 136 (78.2%) and MDA in 38 (21.8%) patients. The demographic and hematological characteristics of the two subtypes are shown in Table 1. Patients with MDA were significantly younger and had a lower mean hemoglobin than those with AOCD, whereas MCV and MCH did not differ. The proportion of females was similar in both subtypes (p=0.71).

AOCD: anemia of chronic disease; MDA: mixed deficiency anemia; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin. Chi-square test.

The distribution of the subtypes differed significantly across age groups (p<0.001; Table 2). MDA was concentrated in the youngest group, accounting for 13 of 20 (65.0%) patients aged 15–25 years, whereas its share was 22.5% at 26–35 years and only 7.4% at 36–45 years. AOCD became more frequent with age, with the largest share of AOCD cases (50/136, 36.8%) in the 46–55-year group. Notably, all six male patients aged 15–25 years had MDA, as did 7 of 14 (50.0%) females in this age group.

Column percentages for AOCD and MDA; chi-square test (χ^2=28.2, df=4).

Overall, 65 (37.4%) patients had mild, 91 (52.3%) moderate and 18 (10.3%) severe anemia. Severity differed significantly between subtypes (p<0.001): mild anemia was more frequent in AOCD (44.9% vs 10.5%), whereas moderate (73.7% vs 46.3%) and severe (15.8% vs 8.8%) anemia were more frequent in MDA. Severity did not differ significantly between genders (p=0.23) or across age groups (p=0.53), although one-quarter of patients aged 15–25 years had severe anemia (Table 3).

Row percentages; chi-square test. Mild: Hb ≥10.0 g/dL; moderate: 7.0–9.9 g/dL; severe: <7.0 g/dL.

Discussion

This study shows that AOCD accounted for more than

Table I: Demographic and hematological characteristics of patients by subtype of normochromic normocytic anemia. (n=174)

Characteristic	AOCD (n=136)	MDA (n=38)	Total (n=174)	p-value
Age (years), mean ± SD	44.6 ± 11.3	36.4 ± 17.1	42.8 ± 13.2	0.008
Gender, n (%)				
Female	85 (62.5)	25 (65.8)	110 (63.2)	0.71
Male	51 (37.5)	13 (34.2)	64 (36.8)	
Hemoglobin (g/dL), mean ± SD	9.5 ± 1.6	8.8 ± 1.3	9.3 ± 1.5	0.021
MCV (fL), mean ± SD	86.5 ± 5.0	87.2 ± 5.3	86.6 ± 5.1	0.49
MCH (pg), mean ± SD	29.6 ± 1.4	29.6 ± 1.5	29.6 ± 1.4	0.95

Table II: Distribution of normochromic normocytic anemia subtypes across age groups (n=174)

Age group (years)	AOCD n (%)	MDA n (%)	Total n	MDA within age group (%)	p-value
15–25	7 (5.1)	13 (34.2)	20	65.0	<0.001
26–35	31 (22.8)	9 (23.7)	40	22.5	
36–45	25 (18.4)	2 (5.3)	27	7.4	
46–55	50 (36.8)	7 (18.4)	57	12.3	
56–65	23 (16.9)	7 (18.4)	30	23.3	
Total	136 (100)	38 (100)	174	21.8	

Table III: Severity of anemia according to subtype, gender and age group. (n=174)					
Variable	Mild n (%)	Moderate n (%)	Severe n (%)	Total	p-value
Subtype					
AOCD	61 (44.9)	63 (46.3)	12 (8.8)	136	<0.001
MDA	4 (10.5)	28 (73.7)	6 (15.8)	38	
Gender					
Female	37 (33.6)	63 (57.3)	10 (9.1)	110	0.23
Male	28 (43.8)	28 (43.8)	8 (12.5)	64	
Age group (years)					
15–25	5 (25.0)	10 (50.0)	5 (25.0)	20	0.53
26–35	16 (40.0)	21 (52.5)	3 (7.5)	40	
36–45	10 (37.0)	15 (55.6)	2 (7.4)	27	
46–55	24 (42.1)	29 (50.9)	4 (7.0)	57	
56–65	10 (33.3)	16 (53.3)	4 (13.3)	30	
Total	65 (37.4)	91 (52.3)	18 (10.3)	174	

three-quarters of NNA in adults, and that the two subtypes followed distinct age patterns: MDA was concentrated among adolescents and young adults, whereas AOCD rose steadily to a peak in the sixth decade. MDA was also associated with lower hemoglobin and more moderate-to-severe anemia, while the gender distribution was similar in both subtypes.

The predominance of AOCD is consistent with the view of anemia of inflammation as the commonest anemia among chronically ill and hospitalized patients.[8] Hospital-based studies from Pakistan report a similar pattern within individual chronic diseases: among diabetic inpatients in Karachi, anemia was normocytic in 59.8% and attributable to chronic kidney disease in 44%, while mixed deficiency accounted for only 6%[21]; in primary hypothyroidism, normocytic anemia was the commonest type (38.6%)[22]; and in systemic lupus erythematosus, AOCD was the leading cause of anemia (40%).[23] Our findings extend these observations to a mixed adult population and show that, even in a setting where nutritional deficiency is common, a normocytic blood picture in an adult with chronic disease most often reflects inflammation-driven anemia.

The age pattern is the principal finding of this study. AOCD is traditionally regarded as a disease of older people; in a large survey of adults aged 65 years and above, about one-third of anemia was due to chronic inflammation or renal disease.[17] By contrast, in a nationally representative sample of Indian adults aged 60 years and above, 63.5% of anemia was nutritional and anemia of chronic disease was the commonest non-nutritional type[30], suggesting that the predominance of AOCD in our cohort partly reflects its hospital-based case mix. In our cohort, which excluded patients older than 65 years, more than half of AOCD cases were nonetheless aged 46–65 years and the mean age of AOCD patients was 44.6 years, indicating that inflammation-related anemia is already common in middle age. This is likely to reflect the early onset of diabetes, chronic kidney disease and autoimmune disease in South Asia, conditions that drive hepcidin-mediated iron restriction and, in kidney disease, erythropoietin deficiency.[7,31] Conversely, MDA dominated the 15–25-year group, where it accounted for two-thirds of NNA. Adolescence and early adulthood are periods of high micronutrient demand, and in low- and middle-income countries this age group is particularly vulnerable to nutritional anemia because of poor dietary diversity, menstrual losses in women and gastrointestinal infection.[11,18] In Pakistan, pooled data show anemia in more than half of non-pregnant adolescent and adult women[32], and the National Nutrition Survey found vitamin B12 and folate deficiency each in about half of women of reproductive age[33], so combined deficiencies are to be expected in young adults. A high prevalence of anemia has also been reported among university students in Bangladesh, most commonly at 20–22 years of age.[34]

The observation that all six men aged 15–25 years had MDA suggests that dietary inadequacy and malabsorption are not confined to women. This is consistent with national data from India showing that three in ten rural men aged 15–54 years are anemic, with underweight men at higher risk.[35] Community data from rural India similarly show that the burden and pattern of anemia vary sharply with age and that gender differences emerge after puberty.[36] Taken together, these findings argue against assuming that NNA in a young adult is due to chronic disease; in this age group, iron, vitamin B12

and folate should be assessed even when indices are normal.

Women formed nearly two-thirds of both subtypes. Higher anemia rates in women are well documented in Pakistan and are linked to menstrual and reproductive losses, poverty, undernutrition and intra-household food inequity.[19,20,37] Among non-pregnant Pakistani women of reproductive age, iron deficiency anemia alone affects nearly one in five and is associated with household food insecurity and high parity.[38] However, the female excess in our AOCD group is better explained by its etiological mix: autoimmune diseases, which are strongly female-predominant[39], formed a substantial proportion of AOCD, whereas diabetes and chronic kidney disease were more common among men (data reported separately). Rheumatoid arthritis is itself frequently complicated by anemia, with mild anemia reported in 33–60% of patients.[40] In MDA, the female predominance is consistent with the nutritional vulnerability of women of reproductive age[19,20], whereas the similar severity of anemia in both genders indicates that, once NNA is established, its severity is driven more by etiology than by sex.

MDA produced more severe anemia than AOCD. AOCD is typically mild to moderate because erythropoiesis is restricted rather than abolished[8,9], whereas combined iron and vitamin B12 and/or folate deficiency impairs both hemoglobin synthesis and DNA synthesis in erythroid precursors.[11] This difference was significant using the cut-offs of 10.0 and 7.0 g/dL. These WHO thresholds were developed for pregnant women and young children[29] but have previously been applied to adults.[28] When the WHO grades specific to non-pregnant adults were applied (mild 11.0–11.9 g/dL in women and 11.0–12.9 g/dL in men; moderate 8.0–10.9 g/dL; severe <8.0 g/dL) [29], the difference was in the same direction but not statistically significant (p=0.22), largely because few patients met the adult criterion for mild anemia; with these grades, severity again did not differ by gender (p=0.25) or age group (p=0.07). However, mean hemoglobin, which does not depend on any grading, was significantly lower in MDA (8.8 vs 9.5 g/dL; p=0.021), supporting a true difference in severity. Clinically, the greater severity of MDA, together with its normal red cell indices, means that it can easily be missed if PBS examination and micronutrient assays are omitted.[5,6]

Strengths and limitations: The strengths of this study include the combination of PBS morphology with biochemical confirmation (ferritin, vitamin B12, folate, CRP and ESR) for every patient, and its focus on the working-age adult population, which is under-represented in anemia research. Its limitations include the cross-sectional design, which precludes causal inference; non-probability convenience sampling from a single hospital laboratory, which limits generalizability to the community; and the restriction of the sample to patients whose anemia was attributable to the pre-specified chronic or nutritional conditions, so the figures represent the distribution of subtypes within NNA rather than the population prevalence of NNA. The MDA group was small, particularly in some age strata, the upper age limit of 65 years excluded the elderly, and advanced markers such as transferrin saturation, soluble transferrin receptor and hepcidin were not available to identify AOCD with coexisting iron deficiency. In addition, long-term metformin therapy is associated with vitamin B12 deficiency in more than one-third of Pakistani patients with type 2 diabetes[41], so a nutritional component within the diabetic AOCD group cannot be fully excluded. Finally, severity was graded using WHO cut-offs designed for pregnancy and early childhood; results with the adult-specific WHO grades are therefore also reported.

Conclusion

AOCD was the predominant subtype of normochromic normocytic anemia in adults and was concentrated in the middle-aged, whereas MDA predominated among adolescents and young adults of both genders and was associated with more severe anemia. Age is therefore a useful guide in the evaluation of NNA: a normocytic blood picture in a young patient should prompt peripheral smear examination and assessment of iron, vitamin B12 and folate status, while NNA in middle-aged adults should prompt a search for underlying chronic disease. Larger community-based studies including older adults and advanced iron markers are recommended.

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