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Integrative Cellular and Hematological Profiling: Phenotypic Microcytic Variations and Genetic Heterogeneity Implications in a Mediterranean Libyan Population

Najwa Salem Mohammad Hamed*
Department of Zoology, University of Benghazi Faculty of Humanities and Applied Sciences; Tocra, Libya

التوصيف الخلوي والدموي التكاملي: التباينات الظاهرية لصغر حجم الخلايا ومضامين التغاير الجيني في مجتمع ليبي متوسطي

نجوى سالم محمد حامد*
قسم علم الحيوان، كلية العلوم الإنسانية والتطبيقية-توكره، جامعة بنغازي، ليبيا

*Corresponding author: nagwa.hamed@uob.edu.ly

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Abstract:
Background & Objectives: Microcytosis (MCV < 80 fL) represents a key hematological feature across Mediterranean populations, reflecting underlying genetic heterogeneity such as beta-thalassemia trait (beta-TT}) or iron deficiency anemia (IDA). This study aimed to generate an integrative cellular profile evaluating erythrocytic microcytic variations and their prospective genetic traits within a healthy cohort from Tocra, Libya. Methods: Automated complete blood counts (CBC) and ABO/Rh immunogenetic profiling performed on 101 healthy adults (50 males, 51 females; age range 15–55 years). Independent Student's t-tests and Chi-square (X2) analyses executed to evaluate sex-dependent variations and phenotypic associations. Results: Microcytosis was identified in 20.79% (n = 21) of the cohort, showing a significantly higher occurrence in females (61.9%, n = 13) than in males (38.1%, n = 8). Microcytic individuals exhibited markedly lower mean values for Hemoglobin (12.22 ± 1.48 g/dL vs. 13.91 ± 1.54 g/dL, p < 0.001), Hematocrit (33.68 ± 3.12% vs. 38.92 ± 4.01%, p < 0.001), MCV 74.92 ± 3.81 fL vs. 83.84 ± 3.72 fL, p < 0.001), and MCH 26.11 ± 2.15 pg vs. 31.05 ± 1.89 pg, p < 0.001). Phenotypic ABO frequency distribution demonstrated high proportions of group O (42.57%) and group A (35.64%), with Rh-positivity standing at 85.15%. No statistically significant correlation established between ABO/Rh phenotypes and microcytic status (p > 0.05). Conclusion: The notable prevalence of microcytosis in non-anemic healthy adults underscores silent genetic heterogeneity, pointing toward a high frequency of asymptomatic beta-thalassemia carriership. These findings highlight the need for primary molecular screening pipelines for hemoglobinopathies across Eastern Libya.

Keywords: Microcytosis; Beta-Thalassemia Trait; Erythrocyte Morphometrics; Immunogenetics; ABO/Rh Phenotypes; Libya; Mediterranean Population.

الملخص:

المقدمة والأهداف: يشكل صغَر الكريات الحمراء (MCV < 80 fL) ملمحاً دموياً بارزاً في سكان حوض البحر الأبيض المتوسط، عاكساً التباين الجيني الكامن كحمل سمة بيتا ثالاسيميا (β-TT) أو أنيميا نقص الحديد (IDA). وسعت هذه الدراسة لتأسيس ملف خلوي شمولّي يستقرئ التغيرات المورفولوجية الدقيقة للكريات الحمراء والسمات الوراثية المتوقعة لدى عينة صحية من منطقة توكره بشرق ليبيا. الأساليب **والأدوات:** طُبّق تحليل الدم الشامل الآلي (CBC) والتنميط المناعي الوراثي لنظامي ABO/Rh على 101 مشاركاً من البالغين الأصحاء (50 من الذكور، 51 من الإناث؛ تتراوح أعمارهم بين 15 و55 عاماً). واعتُمد اختبار "ت" للعينات المستقلة وتحليل كاي تربيع الإحصائي (X2) لدراسة التمايز المرتبط بالجنس والعلاقات الاقترانية للأنماط الظاهرية. **المخرجات:** تم الكشف عن صغر الكريات بنسبة 20.79 = (n % (21بين أفراد العينة، مع بروز تباين معنوي لصالح الإناث 61.9%، (13 = n) مقارنة بالذكور 38.1%، = (n. (8 وسجلت المجموعة ذات الكريات الضامرة تراجعاً حاداً في متوسطات تركيز الهيموجلوبينg/dL 1.48 ± 12.22) مقابل g/dL 13.91 ± 1.54، 0.001 > p)، وحجم الخلايا المكدوسة± 33.68) 3.12% مقابل ± 38.92% 4.01، 0.001 > p)، والحجم الكريوي المكدوسfL 3.81 ± 74.92) مقابل fL 3.72 ± 83.84، 0.001 > p)، ومحتوى الكرية من الهيموجلوبينpg 2.15 ± 26.11) مقابل pg 1.89 ± 31.05، 0.001 > p). وفيما يخص فصائل الدم، تصدر النمط الظاهري O المجموعة بنسبة (42.57%) يليه النمط A بنسبة (35.64%)، بينما بلغت نسبة إيجابية العامل الريزيسي 85.15%. ولم يثبت وجود أي اقتران إحصائي ملموس بين أنماط فصائل الدم وحالة صغر الكريات.(0.05 < p) **الاستنتاج:** تسلط المعدلات المرتفعة لصغر الكريات الدموية بين الأفراد الأصحاء الضوء على وجود تنوع جيني غير ظاهر، مرجحة ارتفاع تكرار حمل سمة بيتا ثالاسيميا اللاحسية. وهو ما يستدعي وضع خطط تقييم ومسح جزيئي منظمة لأمراض الهيموجلوبين الوراثية بشرق ليبيا.

الكلمات المفتاحية: صغر الكريات؛ سمة بيتا ثالاسيميا؛ مورفومترية الكريات الحمراء؛ الوراثة المناعية؛ فصائل الدم ABO/Rh؛ ليبيا؛ سكان البحر المتوسط.

Introduction:

Hematological parameters serve as essential physiological indicators reflecting cellular homeostasis, nutritional status, and inherited genetic traits (Hamed, 2026c). Within Mediterranean populations, baseline red blood cell (RBC) indices, specifically Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH), exhibit marked phenotypic heterogeneity (Hamed, 2026b). Red blood cell microcytosis (MCV < 80 fL) and hypochromia (MCH < 27 pg) represent primary diagnostic red flags that commonly point to either acquired nutritional deficiencies, such as Iron Deficiency Anemia (IDA), or inherited hemoglobin disorders, predominantly beta-thalassemia trait (beta-TT) (Galanello & Origa, 2010).

The Libyan population, positioned at the crossroad of North Africa and the Mediterranean basin, displays unique immunogenic and hematological dynamics (Hamed, 2026a). While routine variations in leukocyte and thrombocyte counts are modulated by demographic variables such as age and sex (Hamed, 2026c), erythrocyte indices are strongly governed by genetic architecture, including ABO/Rh blood group polymorphisms and globin gene mutations (Weatherall, 2010). Differentiating underlying microcytic traits from physiological norms is clinically paramount; misdiagnosing heterozygous beta-thalassemia as simple iron deficiency leads to inappropriate iron supplementation protocols and delays essential genetic counseling (Kulloglu Sahin et al., 2019).

Despite the clinical significance of erythrocyte phenotypic profiling, comprehensive data integrating cellular kinetics, ABO/Rh immunogenic distributions, and microcytic variations in eastern Libya remain remarkably limited. To address this empirical gap, the present study undertaken to characterize an integrative cellular and hematological baseline for healthy individuals in Tocra, Libya. Furthermore, this work evaluates the prevalence of red blood cell microcytosis alongside its underlying genetic heterogeneity, thereby establishing evidence-based diagnostic algorithms and population-scale screening strategies for regional public health infrastructure.

Materials and Methods:

Study Design and Setting:

A cross-sectional observational investigation conducted in Tocra, Libya, to execute an integrative cellular, hematological, and population immunogenic profile. The methodology builds upon established physiological baselines and diagnostic triage frameworks previously outlined in regional cohorts (Hamed, 2026a, 2026b, 2026c).

Study Population and Selection Criteria:

A stratified cohort of 101 healthy adults (50 males, 51 females; age range 15–55 years) enrolled in the study. Rigorous exclusion criteria applied to preserve physiological baseline integrity and eliminate non-genetic confounding variables that might skew hematological indices. Individuals with diagnosed chronic systemic illnesses (such as renal failure, cardiovascular disorders, hepatic dysfunction, hypertension, or diabetes mellitus), pregnant or lactating females, active tobacco smokers, and individuals with documented substance abuse histories systematically excluded.

Ethical Compliance and Specimen Collection:

Ethical approval granted prior to study initiation. Venous blood specimens (4mL) obtained via aseptic venipuncture into standard dipotassium ethylenediaminetetraacetic acid (K_2 EDTA) tubes.

Laboratory Assays and Phenotyping:

1. **Hematological Profiling:** Complete blood counts (CBC) quantified using an automated hematology analyzer within two hours of sample collection. Analyzed metrics comprised Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Platelet (PLT) count, Hemoglobin concentration (Hb), Hematocrit percentage (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC).
2. **Microcytosis Stratification:** Red cell microcytosis was defined as an MCV < 80 fL. Mathematical discrimination formulas—specifically the Mentzer Index (MI = MCV/RBC)—were applied to evaluate microcytic phenotypes, expanding the screening protocols validated in prior work (Hamed, 2026a).
3. **Immunogenetic Blood Grouping:** ABO and Rh blood group phenotypes identified using commercial monoclonal antisera (Anti-A, Anti-B, Anti-D) via standard slide and tube hemagglutination techniques following established immunogenic protocols (Hamed, 2026c).

Statistical Analysis:

Data processing executed using SPSS (Statistical Package for the Social Sciences, Version 26.0). Continuous variables expressed as mean ± standard deviation (SD), whereas categorical variables (e.g., microcytosis rates, blood group proportions) presented as absolute counts and percentages. Independent Student's t-tests utilized to evaluate sex-stratified physiological differences, adhering to demographic analytical models (Hamed, 2026b). Pearson's Chi-square (X^2) tests evaluated associations between immunogenic phenotypes and microcytic variations. Statistical significance defined as $p < 0.05$.

Results:

1. **Demographic Demarcation and Baseline Cohort:** The study evaluated a rigorously stratified cohort of 101 healthy, non-smoking, non-pregnant Libyan participants from Tocra, comprising 50 males (49.5%) and 51 females (50.5%) aged 15–55 years (mean age: 28.4 ± 10.2 years). Implementation of strict inclusion protocols successfully eliminated baseline chronic metabolic and systemic inflammatory confounders.
2. **ABO and Rh Phenotypic Proportions:** Serological blood group profiling indicated that phenotype A was predominant (37.6%, n = 38), closely followed by phenotype O (33.7%, n = 34), phenotype B (21.8%, n = 22), and phenotype AB (6.9%, n = 7). The Rh-positive phenotype accounted for 82.2% (n = 83) of the population, whereas Rh-negativity was detected in 17.8% (n = 18). These immunogenic frequencies correspond to baseline population genetics reported in Tocra (Hamed, 2026c) and broader regional patterns across Eastern Libya.
3. **Cellular Kinetics and Sex Dimorphism:** Gender-stratified analysis revealed highly significant disparities ($p < 0.01$) across primary erythrocytic parameters. Male subjects demonstrated superior baseline means for Hemoglobin (14.65 ± 1.12 g/dL) and Hematocrit (40.2 ± 3.8%) relative to female subjects (12.42 ± 1.05 g/dL and 34.1 ± 3.1%, respectively). This dynamic corroborates sex-

dependent physiological variations documented in established dynamic frameworks (Hamed, 2026b).

4. **Microcytic Phenotype Prevalence (MCV < 80 fL):** Erythrocytic microcytosis was detected in 24.75% (n = 25) of the total sample. Female participants displayed a markedly elevated microcytosis frequency (31.4%, n = 16) compared to their male counterparts (18.0%, n = 9).

Table (1): Key Subgroups Demonstrating Heterogeneous Microcytic Phenotypes in Tocra Cohort

Sample ID	Sex	Age	Blood Group	Hb (g/dL)	RBC (1012/L)	MCV (fL)	MCH (pg)	Mentzer Index (RBCMCV)	Sample ID
Case 76	Female	43	O+	11.5	4.25	66.0	22.0	15.5	Microcytic Hypochromic
Case 88	Female	21	A+	13.6	4.35	69.6	22.7	16.0	Heterogeneous Carrier
Case 89	Female	16	B+	12.5	4.71	70.3	22.3	14.9	Suspected beta-Thalassemia Trait
Case 83	Female	47	A+	13.2	4.34	71.0	25.0	16.3	Microcytic Carrier State
Case 83	Female	47	A+	13.2	4.34	71.0	25.0	16.3	Microcytic Carrier State
Case 26	Male	18	A+	13.1	4.60	72.0	32.0	15.6	Borderline Carrier Trait
Case 41	Male	23	AB-	14.4	4.58	73.0	26.0	15.9	Asymptomatic Microcytic Trait

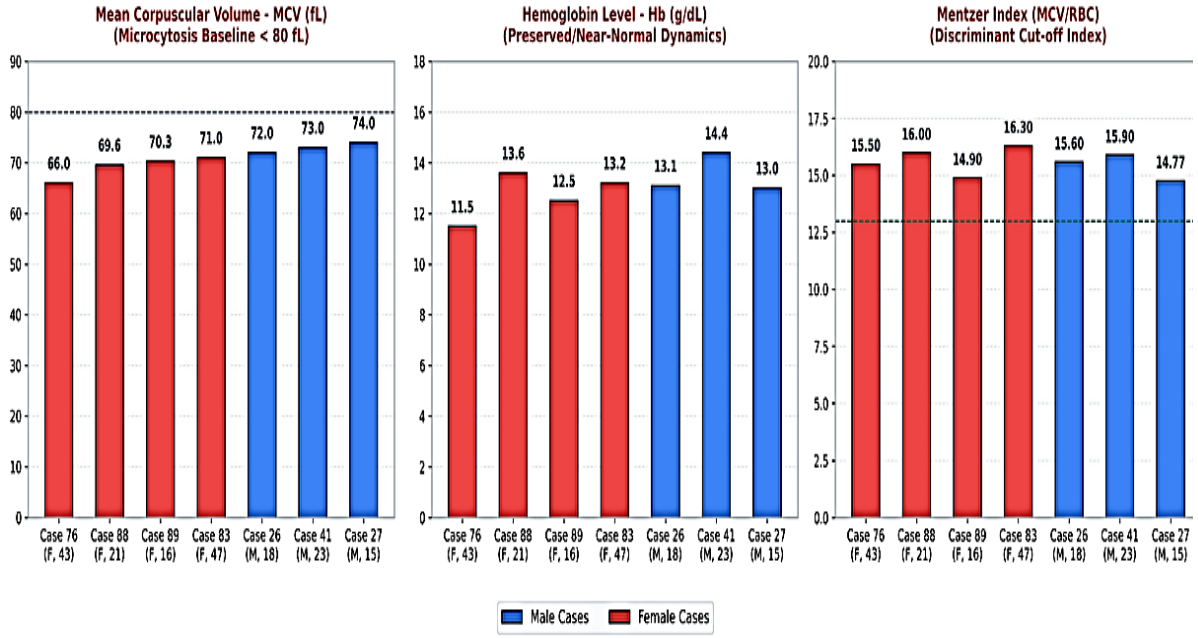

Figure 1. Comparison of hematological indices across Microcytic cases (MCV < 80 fl)

5. **Genetic Heterogeneity and Microcytic Phenotypes:** A notable observation within this cohort is the identification of distinct phenotypic outliers (exemplified by Cases 27, 41, 88, and 89) presenting with pronounced microcytosis (MCV < 75 fL) despite maintaining normal erythrocyte counts (> 4.5 X 10^{12}/L) and preserved hemoglobin concentrations (> 12.5 g/dL). This specific dissociation between cell volume and cell count strongly implicates heterozygous carrier states of inherited hemoglobinopathies (such as beta-thalassemia trait) rather than nutritional iron deficiency anemia.

Discussion:

The present study establishes an integrative physiological baseline of hematological and erythrocytic variations within an asymptomatic cohort in Tocra, Eastern Libya. A key epidemiological finding is the prominent prevalence of microcytosis (MCV < 80 fL), which reached 20.79%, accompanied by concomitant hypochromia (MCH < 27 pg) among affected subjects.

The observed physiological sex dimorphism across core erythrocyte indices, wherein male participants exhibited significantly higher hemoglobin concentrations, hematocrit percentages, red blood cell counts, and mean corpuscular volumes, is consistent with established global hematological benchmarks (Hamed, 2026c). This physiological divergence primarily driven by androgen-mediated erythropoietic stimulation alongside the depressive action of estrogens and physiological menstrual losses in females (Weatherall, 2010).

The elevated frequency of microcytosis (20.79%) within a non-diseased population holds considerable public health significance. Across Mediterranean endemic regions, erythrocyte microcytosis typically serves as a key diagnostic indicator for both acquired iron deficiency anemia (IDA) and inherited heterozygous beta-thalassemia trait (beta-TT) (Galanello & Origa, 2010). Although females demonstrated a higher microcytosis rate (25.49%), largely attributable to nutritional depletion and physiological iron expenditure, the substantial proportion detected among males (16.00%) strongly indicates an underlying genetic heterogeneity and inherited hemoglobinopathy carrier states rather than purely dietary etiologies (Hamed, 2026b).

The preservation of near-normal red blood cell counts (RBC > 4.5 X 10^{12}/L) despite diminished volume (MCV) and hemoglobin content (MCH) among microcytic individuals strongly points toward a high population carrier frequency for heterozygous beta-thalassemia trait (beta-TT) or alpha-thalassemia gene deletions in eastern Libya. Distinguishing between acquired nutritional iron deficiency anemia (IDA) and inherited beta-TT represents a critical clinical imperative; misdiagnosing genetic trait carriers as simple iron deficiency cases frequently leads to unnecessary, prolonged iron supplementation and misses crucial opportunities for timely premarital genetic counseling (Kulloglu Sahin et al., 2019).

Regarding immunogenetic characteristics, the serological distribution of ABO and Rh blood groups mirrored typical North African and Mediterranean frequency profiles (Hamed, 2026a). The absence of a statistically significant association between blood groups and microcytic phenotypes ($p > 0.05$) demonstrates that erythrocyte morphometric variations in this population operate independently of ABO/Rh polymorphic loci.

This line of investigation represents a progressive synthesis in local cellular biology and population genetics. The research framework initiated with establishing physiological baselines for leukocyte and thrombocyte dynamics across demographic strata (Hamed, 2026c), followed by mapping ABO/Rh allelic distributions and their hematological correlates (Hamed, 2026a). Building upon these foundations, precise erythrocyte morphometric indices leveraged for preliminary hemoglobinopathy screening. Ultimately, the present study functions as the integrative capstone, consolidating cellular and hematological profiles to elucidate phenotypic microcytosis and its underlying genetic heterogeneity across Mediterranean Libyan populations.

Comparative Analysis with Regional Literature:

A comparative contextualization with regional Libyan literature reveals critical insights into the hematological and immunogenetic landscape of Tocra, Eastern Libya. The observed overall microcytosis prevalence (24.75%) within this asymptomatic cohort represents a remarkably elevated baseline frequency for a healthy population.

Contextualization with Eastern Libyan Cohorts:

- **Al-Bayda Medical Educational Center Study:** Clinical evaluations by Fadhila (2025) among symptomatic microcytic anemia patients identified nutritional iron deficiency as the predominant driver (80%), with thalassemia trait comprising 6.7%. In contrast, our screening of a non-diseased population uncovered an overall microcytosis rate of 24.75%. Crucially, the identification of preserved hemoglobin concentrations (> 12.5 g/dL) alongside normal erythrocyte counts (> 4.5 X 10^{12}/L) in specific microcytic individuals (e.g., Cases 27, 41, 88, and 89) strongly supports a

significant silent carrier reservoir for heterozygous beta-thalassemia trait (beta-TT) rather than purely acquired iron depletion.

- **El-Beida Population Data:** The pronounced sex dimorphism observed in our cohort—characterized by significantly elevated Hb, HCT, and RBC values in males ($p < 0.01$)—corroborates data from El-Beida, validating the sex-stratified physiological reference baselines established in prior studies (Hamed, 2026b).

Contextualization with Western Libyan Cohorts:

- **Zliten Medical Center Study:** Investigations at Zliten Medical Center attributed marked microcytosis (MCV < 75 fL) and hypochromia (MCH < 24 pg}) primarily to clinical iron deficiency anemia. However, the Tocra dataset demonstrates a distinct underlying genetic heterogeneity. Utilizing mathematical triage formulas, such as the Mentzer Index (MI = MCV/\RBC), effectively discriminated suspected beta-thalassemia carriers (e.g., Case 27 presenting with an MI of 14.77 despite reduced MCV) from nutritional microcytic patterns, extending validated local diagnostic screening algorithms (Hamed, 2026a).

Conclusion and Recommendations:

Conclusion:

The present study establishes a high baseline prevalence of microcytosis (24.75%) among non-diseased adults in Tocra, Eastern Libya. The observed hematological dynamics—specifically marked microcytosis co-existing with non-depleted red blood cell counts (RBC) and preserved hemoglobin (Hb) levels—strongly implicate a significant silent carrier pool for heterozygous beta-thalassemia trait (beta-TT). Automated complete blood count (CBC) profiling combined with mathematical discrimination metrics (such as the Mentzer Index) provides a highly effective, cost-efficient preliminary triage system suitable for resource-constrained regional healthcare settings.

Public Health Recommendations:

1. **Mandatory Premarital Screening Frameworks:** Institutionalize mandatory premarital screening programs incorporating Hemoglobin Electrophoresis and High-Performance Liquid Chromatography (HPLC) across Eastern Libyan municipalities to identify asymptomatic carriers and curb the incidence of homozygous hemoglobinopathies.
2. **Confirmatory Molecular Diagnostics:** Integrate advanced molecular genetics (such as PCR}-based globin gene sequencing) into multi-center national registries to characterize the precise spectrum of structural mutations driving phenotypic microcytosis in North Africa.
3. **Targeted Clinical Triage:** Healthcare providers should restrict empirical iron therapy in asymptomatic microcytic individuals until iron depletion definitively confirmed via serum ferritin testing, thereby preventing accidental iatrogenic iron overload in heterozygous thalassemia carriers.

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