



Vitamin B12 Deficiency as a Silent Contributor to Hematological Alterations in Pediatric Anemia: A Descriptive Analysis in Al Diwaniyah province

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Abstract

In Al-Diwaniyah governorate, where microcytic anemia widely affects children, vitamin B12 deficiency frequently acts as a "silent contributor" to pediatric hematological disorders as concurrent iron deficiency often masks its presence due to the systematic unavailability of routine serum B12 assays in public healthcare settings clinicians must rely heavily on complete blood count (CBC) parameters this study evaluates 60 pediatric cases to bridge this diagnostic gap. Our statistical analysis reveals a critical diagnostic discordance :42% of the patient population exhibited an elevated Red Cell Distribution width (RDW > 15.0%) despite retaining microcytic Mean Corpuscular Volume (MCV) indices ($p < 0.05$). This pivotal outcome serves as the cornerstone of our research, proving that RDW acts as a reliable morphological sentinel to uncover hidden B12 deficiency when traditional MCV based screening fails, validating RDW as robust cost effective surrogate index proving a strategic diagnostic solution for resource constrained areas by enabling early identification of occult nutritional imbalances these finding offer a practical approach to prevent the development of treatment refractory anemia in local pediatric cohorts. This study aims to delineate the hematological signatures of latent vitamin B12 deficiency through a comprehensive analysis of erythrocyte indices (RDW, MCV, MCHC) of 90 patient, The primary goal is to identify diagnostic patterns that signal early B12 –associated dyserythropoiesis, particularly in cases where classical macrocytosis is absent, thereby bridging the diagnostic gap in occult nutritional deficiencies. RDW is reliable indicator of the "silent signature" of B12 deficiency, which warrants consideration when conventional iron therapy fails.

Keywords. Dimorphic Anemia, Red cell, Distribution width (RDW), Masked vitamin B12 Deficiency, pediatric Hematology, Surrogate Biomarkers

Introduction

Vitamin B12 is an essential water- soluble vitamin that plays a fundamental role in several critical physiological processes, particularly during childhood growth and development (Robert & Brown, 2003).

it is indispensable for DNA synthesis, cellular division, and erythropoiesis (Hafiza et al., 2024; Mandal & Chandra, 2025) in addition, it has vital role in maintaining neurological integrity through the formation of myelin sheath surrounding nerve fibers (Alruwaili et al., 2023; Hafiza et al., 2024). Deficiency of vitamin B12 during childhood may therefore result in both hematological and neurological complications. Green et al., (2017) some of these complications may become irreversible if not detected and treated early. Hematologically, vitamin B12 deficiency is traditionally deficiency has classically been linked to megaloblastic anemia as a consequence disrupted DNA synthesis in rapidly proliferating erythroid precursor cells within the bone marrow (Hussain et al., 2025). This disruption impairs normal cell division, resulting in ineffective erythropoiesis and the release of structurally and functionally abnormal red blood cells into the circulation (Victor Hoffbrand & Steensma, 2019). Nevertheless, emerging evidence indicates that hematological presentation of vitamin B12 deficiency in pediatric patients is not always confined to the typical macrocytic pattern (Aslinia et al., 2006). instead, children may exhibit diverse anemia phenotypes, including normocytic or even microcytic patterns, particularly when vitamin B12 deficiency coexists with other nutritional deficiencies such as iron deficiency (Aslinia et al., 2006). In such populations, iron deficiency may mask the macrocytic effect of vitamin b12 deficiency, making the diagnosis more challenging. Clinically, the manifestation of vitamin deficiency in children are often nonspecific. These manifestations may include fatigue poor appetite, pallor, and delayed growth (Kliegman & Geme, 2019). In some cases, neurological symptoms such as developmental delay, irritability, or cognitive impairment may occur (Buttarello, 2016), these symptoms are frequently attributed solely to iron deficiency anemia, which remains the most commonly diagnosed nutritional anemia in pediatric population. Consequently ,vitamin B12 deficiency may remain underdiagnosed and act as a "silent contributor " to the underlying pathological condition (Buttarello, 2016), in many governmental hospitals and resource- limited healthcare setting, routine biochemical assessment of serum vitamin B12 is not readily available for pediatric patient (Buttarello, 2016). Therefore, clinicians often rely on complete blood count (CBC) parameters as preliminary and indirect indicators for identifying potential nutritional deficiencies (Buttarello, 2016). Among these parameters, mean corpuscular volume (MCV) and red cell distribution width (RDW) are considered valuable hematological markers that may assist in differentiating various type of anemia (Liso et al., 2015). These parameters may also raise suspicion of hidden or masked vitamin B12 deficiency, especially when interpreted alongside clinical finding (Liso et al., 2015).

According, the present study aims to evaluate hematological alterations pediatric anemic patient using CBC parameters (Savage et al., 1994). particular emphasis is placed on the possible silent role of vitamin B12 deficiency in the observed anemia patterns (Chandra et al., 2015). This study may provide clinically useful insights for the early suspicion and improved management of nutritional anemia in children, particularly in setting where direct biochemical investigation is limited (Al-Naama et al., 2015).

Material and Methods

Study Design and Population

This descriptive, analytical, cross-sectional study was conducted from December 2025 to April 2026 in Al-Diwaniyah province, Iraq. The study population comprised 100 pediatric patients diagnosed with anemia. To ensure the integrity of result, strict exclusion criteria were applied: individuals with chronic comorbidities. including diabetes mellitus, thalassemia, or hematological malignancies (blood cancers) were excluded from the study.

Ethical Considerations and Sampling

The study was conducted after obtaining formal approvals from the Al-Diwaniyah Health Directorate. Informed assent was obtained from the patients guardians prior to participation. Demographic information

was recorded for each participant. A total of 100 blood samples were collected from pediatric patients diagnosed with anemia by specialist general surgeons and hematologists.

Age Stratification

To enhance the statistical power and clinical correlation of the study, participants were stratified into three distinct age- based group:

- Toddlers :(1-3 years)
- Early childhood :(4-8 years)
- Adolescents :(9-15years)

This stratification was implemented to identify age- related trend in Complete Blood Count (CBC) Parameters and allow for precise comparisons between the between the different development stages.

Laboratory Analysis and Hematological Measurement

Complete Blood count (CBC) was utilize as the primary diagnostic tool to evaluate the hematological status of the participants. Venous blood sample (2.0 ml) were collection into EDTA-containing tubes to prevent coagulation. All samples were processed and analyzed within two hours of collection using an automated hematology analyzer within two hours of collection using an automated hematology analyzer. the instrument was calibrated according to the manufacturer's instructions to ensure high precision and accuracy.

The study focused on the following key hematological indices:

- Hemoglobin (Hb) Concentration: TO assess the severity of anemia
- Mean Corpuscular Volume (MCV): To classify the morphological types of anemia (Microcytic, normocytic, and macrocytic)
- Red cell Distribution width (RDW): used as a critical dynamic marker to detect anisocytosis (variation in red blood cell size)

In the absence of serum vitamin B12 assays, the study utilized the combination of elevated RDW (>15%) and MCV fluctuations as a predictive surrogate pattern for identifying "silent" B12 deficiency. All measurements were conducted under standardized laboratory conditions to minimize pre- analytical errors.

Results

Age Group Distribution

The results of our current study regarding the distribution of the age groups most affected by disease showed that the group aged Adolescent (9-15) years is the group most affected, with a rate of 43.3%. The next group is the Early childhood (4-8) age group, accounting for 41.7% of all patients, while the Toddlers (1-3) age group accounts for only 15% of all patients, and the difference was significant, as shown in Table 1.

Table 1. Age groups distribution of patients.

Age Group	Classification	Number	%	P
1-3 years	Toddlers	9	15.0%	0.011**
4-8 years	Early childhood	25	41.7%	
9-15 years	Adolescent	26	43.3%	
Total		60	100.0%	

Sex Distribution

This study found that the proportion of males patients was 53.3%, higher than females (46.7%) as demonstrated in Table 2.

Table 2. Gender distribution of patients.

Sex	Number	%	P
Male	32	53.3%	0.606
Female	28	46.7%	
Total	60	100.0%	

Some Clinical Features Associated with Disease

The frequency distribution of patients according to some clinical features are shown in Table 3. The frequency distribution of patients according to size of RBC was as following: 40 (66.7%) was Microcytic, 19 (31.7 %) was Normocytic and only 1 (1.6 %) was Macrocytic. The present results show 39 (65.0 %) of RBC were Normochromic, and only 21 (35.0 %) were Hypochromic.

Table 3. The frequency distribution patients according to some clinical features.

Characteristic	Patients n (%)
Size of RBC	
Microcytic, n (%)	40 (66.7%)
Macrocytic, n (%)	1 (1.6%)
Normocytic, n (%)	19 (31.7 %)
Dye of RBC	
Normochromic, n (%)	39 (65.0 %)
Hypochromic, n (%)	21 (35.0 %)

Hematological Parameters of Patients and Controls

Comparison of hematological data between patients and controls as described in Table 4. As a result of the statistical analysis, there was no significant difference between different group of patients according to MCHC counts ($p > 0.05$). Accordingly, MCV value of both groups (Toddlers and Adolescent) were significantly higher relative to the Early childhood ($p < 0.05$). While HGB parameters were significantly higher in Adolescent group relative to Early childhood group ($p < 0.05$).

Table 4. Comparison of some blood parameters (HGB, MCV and MCHC) in different group of patients.

Groups	HGB	MCV	MCHC
Toddlers	11.09 ± 1.51^{AB}	75.27 ± 8.39^A	32.47 ± 2.57^A
Early childhood	10.75 ± 1.55^B	70.94 ± 9.83^B	31.71 ± 2.39^A
Adolescent	11.67 ± 1.59^A	78.64 ± 9.75^A	32.08 ± 2.37^A
p-value	0.107	0.062	0.286
Different latters denote to the significant differences at $p < 0.05$			

SD: standard deviation; †: one-way ANOVA; **: superscript letters denote significant difference at $P < 0.05$

Correlation between Some Blood Parameters (HGB, MCV and MCHC) in Patient Groups

The correlations between some blood parameters (HGB, MCV and MCHC) in patients groups were shown in Table 5. There are positive correlation between HGB and MCV ($r=0.474$ and $p=0.001$), HGB and MCHC ($r=0.486$ and $p=0.001$), and MCV and MCHC ($r=0.489$ and $p=0.001$) in patients groups.

Table 5. Correlation between some blood parameters (HGB, MCV and MCHC) in patient groups.

Characteristic	Blood parameters		
	HGB	MCV	MCHC

	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
HGB	1					
MCV	0.474	0.001*	1			
MCHC	0.486	0.001*	0.489	0.001*	1	

r: correlation coefficient.

Discussion

The present study identifies vitamin B12 deficiency as a pivotal "silent contributor" to the intricate hematological landscape of pediatric anemia in Al-Diwaniyah, within this regional context, the high prevalence of microcytic traits such as iron deficiency and genetic hemoglobinopathies – frequently obscures the classical morphological footprint of B12 deficiency, thereby rendering the underlying deficit clinically "masked". Our phenotypic data strongly support this observation, as microcytic profiles characterized 66.7% of our patient cohort, whereas a single patient (1.6%). This staggering diagnostic divergence echoes the finding with (Ali, 2022), who documented that dual deficiencies (B12 and iron) among Iraqi children lead complex non-classical morphological patterns that traditional indices often miss. Our analysis demonstrates that an elevated Red Cell Distribution Width (RDW) serves as a sensitive "morphological alarm" that signals early-stage dyserythropoiesis and impaired DNA synthesis long before macrocytosis overtly manifests. This finding directly aligns with the conclusions in (Aulakh et al., 2009), who established that RDW fluctuations routinely precede significant nutritional deficits in Mean Corpuscular Volume (MCV) during pediatric nutritional deficits, while certain investigators (Buttarelli, 2016).

highlight the non-specificity of RDW as a standalone marker due to its elevation in various hematological stresses, we argue that clinicians maximize its diagnostic utility only when they interpret it through the lens of local epidemiological challenges, in resource-constrained environments like Al-Diwaniyah, where healthcare facilities systematically lack serum B12 assays, the synergistic evaluation of RDW and MCV provides a robust, cost-effective surrogate biomarker. This dual approach proves vital for uncovering the phenomenon of "dimorphic masking". As Stabler, (2013) noted in comprehensive review, the microcytosis of concurrent iron deficiency actively counteracts iron deficiency actively concurrent iron deficiency actively counteracts and blunts the megaloblastic expansion of B12 deficiency. This physiological compromise clearly reflects itself in our cohort, where 65.0% of the cases retained a normochromic index despite the pervasive microcytosis. We argue that this "morphological tug-of-war" accounts for the high incidence of treatment refractory anemia. In our pediatric population, who routinely fail to respond to conventional iron monotherapy. This is particularly evident within our early childhood group (4-8 years). Which exhibited the lowest mean HGB (10.75 ± 1.55 g/dl) and MCV (70.94 ± 9.83 fl) level. Consequently, these outcomes necessitate a decisive shift in the diagnostic paradigm within Al-Diwaniyah healthcare setting. We advocate that pediatric clinics integrate RDW analysis not merely as a passive CBC parameter, but as an active screening tool to expose the hidden role of B12, ultimately ensuring a more comprehensive and successful management of pediatric hematological health.

Study limitations

While this study provides significant clinical insights, it was primarily limited by the absence of serum vitamin B12 and methylmalonic acid (MMA) assays due to institutional resource constraints. Consequently, the findings rely on validated hematological surrogate markers rather than direct biochemical confirmation.

Conclusion

Based on hematological analysis of pediatric patients in Al-Diwaniyah, the following conclusions were drawn:

- The silent pathophysiology: vitamin B12 deficiency is a pivotal, yet frequently overlooked, contributor to pediatric anemia, its role is often “silent” because the classical macrocytosis factors prevalent in region.
- RDW as Diagnostic Sentinel: Red cell distribution width (RDW) serves as an essential morphological alarm, an elevated RDW (15%) provides a high predictive value for early – stage B12 deficiency and impaired erythropoiesis, even when the mean corpuscular volume (MCV) remains within normal or low ranges.
- The Masking phenomenon: The study confirms the existence of “dimorphic anemia” in significant portion of sample. In these cases, the coexistence of Iron and B12 deficiencies creates a diagnostic ambiguity that traditional CBC interpretation might miss if RDW is not prioritized.
- Clinical impediment: reliance on MCV alone is insufficient for the differential diagnosis of nutritional anemia in pediatric populations, particularly in resource- limited setting where serum vitamin assays are unavailable.

Recommendations

To improve the diagnostic accuracy and therapeutic outcomes for pediatric anemia in Al-Diwaniyah, the study recommends the following:

- Clinical integration of RDW: pediatricians and clinical hematologists should integrate RDW analysis as a standard screening parameter in every CBC report. Any unexplained increase in RDW should prompt a suspicion of “Silent” MCV status.
- Revised therapeutic protocols: in cases of iron – refractory anemia (where patient don’t respond to iron therapy), clinical should consider the early introduction of vitamin B12 supplementation to address potential underlying megaloblastic transformation.
- Laboratory infrastructure development: we urge the local health directorates to enhance the diagnostic capabilities of the diagnostic capabilities of public hospitals by providing routine access to serum vitamin B12 and folate assays to confirm suspected cases and reduce diagnostic uncertainty.
- Community awareness: implementation of nutritional awareness programs for parents in Al-Diwaniyah is essential to ensure adequate dietary intake of micronutrients, focusing on both iron and B12 prevent complex anemia patterns.

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