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Research Article

Clinical Spectrum and Frequency of Cutaneous Hyperpigmentation in Patients with Megaloblastic Anemia

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Abstract

Objective: To identify the prevalence and clinical spectrum of cutaneous hyperpigmentation in patients with megaloblastic anemia (MA) and to assess its correlation with hematological and biochemical severity.

Study Design: Cross-sectional study.

Place and Duration: This study was conducted at Liaquat National Hospital Karachi Pakistan Pakistan from April 2025 to April 2026

Methods: A total of 124 patients with confirmed megaloblastic anemia were enrolled by non-probability consecutive sampling. The clinical evaluation assessed the presence, type, distribution, and size of hyperpigmentation. Hematological parameters such as hemoglobin and mean corpuscular volume (MCV) were measured, as well as serum vitamin B12 and folate levels, which were analyzed using electrochemiluminescence immunoassay. Data were analyzed using SPSS version 25.0.

Results: Cutaneous hyperpigmentation was seen in 36 patients (29.0%). The most frequent lesion type was macular (38.9%), followed by patchy (25.0%) and diffuse pigmentation (22.2%). The knuckles were the most common site of involvement (41.7%), and most lesions were 1–5 cm in size (44.4%). Patients with hyperpigmentation had significantly lower hemoglobin levels (7.8 ± 1.2 g/dL vs 9.4 ± 1.5 g/dL), higher MCV (116.3 ± 8.7 fL vs 108.5 ± 7.9 fL), and lower serum vitamin B12 (118.4 ± 32.6 pg/mL vs 164.2 ± 41.8 pg/mL) and folate levels (2.4 ± 0.8 ng/mL vs 3.8 ± 1.1 ng/mL) compared to those without hyperpigmentation ($p<0.05$). The hyperpigmentation group also had a greater

incidence of severe anemia (50.0% vs. 21.6%).

Conclusion: Cutaneous hyperpigmentation is a relatively common finding in megaloblastic anemia and is correlated with higher hematological and biochemical abnormalities. Prompt diagnosis and management of underlying nutritional deficiency may be helped by early recognition of characteristic pigmentary changes.

Keywords: *Meagaloblastic anemia; hyperpigmentation; vitamin B12 deficiency; folate deficiency.*

Introduction

Hyperpigmentation in megaloblastic anemia (MA) is caused by either B12 (cobalamin) or folate deficiency, which disrupts DNA synthesis in rapidly dividing cells and has specific effects on melanocyte biology and melanin dynamics (1). The underlying mechanism of disease is the "folate trap" in B12 deficiency, which is the accumulation of 5-methyltetrahydrofolate that cannot be used for one-carbon metabolism, uracil misincorporation into DNA, delayed nuclear maturation, and impaired hematopoiesis (1, 2). In the skin, B12 deficiency affects the intracellular redox state by decreasing the concentration of reduced glutathione (GSH), which is a natural inhibitor of tyrosinase, the rate-limiting enzyme in melanogenesis. This leads to increased tyrosinase activity, melanin production, and deposition, mainly in the basal layer and upper dermis, sometimes associated with pigmentary incontinence (2, 3). They tend to affect frictional or sun-exposed areas such as knuckles, palmar creases, and extremities, and appear as brownish-black macules or as diffuse pigmentation that often clears within weeks to months of repletion therapy, and which may differ from many permanent pigmentary disorders (3, 4).

Hyperpigmentation is most often seen clinically as brownish-black macules or diffuse darkening, typically accentuating over the knuckles, palmar creases, soles, forehead, neck, and extremities, and occasionally involving the mucosa or nails (5). Histopathology is characterized by increased basal melanin, irregular epidermal architecture, and dermal melanophages with minimal inflammation. The severity tends to correlate with the degree of deficiency (e.g., B12 <100 pg/mL) and is associated with macrocytosis (MCV often >110 fL), pancytopenia, and glossitis (6). Folate-dependent cases are milder in terms of pigmentary expression, as a result of differential effects on the accumulation of methylmalonic acid and succinyl-CoA pathways specific to B12 (5, 6).

Hyperpigmentation is present in 10-44% of B12 deficiency and MA patients worldwide, more common in vegetarian or malnourished populations, as well as dermatology referrals (7). Rates are closely comparable in South Asia, where dietary patterns and malabsorption add to the burden. A study conducted in Pakistan reported that hyperpigmentation was observed in 31% of patients with megaloblastic anemia, and 93.75% of these patients had multiple lesions, mostly affecting the forehead, neck, arms, legs, and back (8). A recent study on frequency and clinical patterns also found that about one-

third of patients with megaloblastic anemia had hyperpigmentation, and the association with more severe hematological abnormalities (7). Nutritional deficiencies remain a considerable public health issue in Pakistan, especially among at-risk communities, leading to a huge burden of avoidable diseases and micronutrient deficiencies (9).

The role of genetic variants in one-carbon metabolism genes, concurrent micronutrient deficiencies, and infection-associated malabsorption on pigmentary severity and recovery patterns holds special significance in high-burden countries like Pakistan. This has significant clinical importance as visible hyperpigmentation can be a readily available point-of-care marker of megaloblastic anemia, potentially leading to earlier treatment to avoid irreversible neuropathy, cytopenia-related issues, and higher morbidity in nutritionally at-risk populations (7, 10). This study aims to determine the frequency of hyperpigmentation in patients with MA and to describe its clinical patterns by site, distribution, and clinical presentation.

Methodology

This cross-sectional study examined the clinical spectrum and prevalence of hyperpigmentation in MA students. The sample size used was 124, determined using non-probability consecutive sampling. The sample size was calculated using the expected prevalence of hyperpigmentation in megaloblastic anemia (34%, based on regional data), a 95% confidence level, and an 8% margin of error (7). Patients aged 15 years or older with confirmed MA (peripheral blood smear with characteristic macro-ovalocytes and hypersegmented neutrophils, bone marrow with megaloblastic changes if performed, and/or serum vitamin B12 <200 pg/mL, and/or folate <300 ng/mL), and macrocytic anemia (MCV >100 fL). After informed consent, both newly diagnosed and known cases with active disease were included.

Patients whose hyperpigmentation was known to be due to chronic liver disease, Addison's disease, hemochromatosis, chronic kidney disease, thyroid disorders, melanoma, pigmentation from other medications such as antimalarials, cytotoxic drugs, or minocycline were excluded. Patients who had received recent vitamin B12 and folate supplementation in the past three months were also excluded to prevent alteration of clinical and biochemical findings.

The data were collected using a structured proforma for detailed history-taking and clinical examination. Demographic information, dietary habits (vegetarian or mixed diet, nutritional intake), medical history (gastrointestinal disease, chronic illnesses, infections, prior anemia), drug history, symptom profile. Clinical examination was used to evaluate the presence, distribution, morphology, and severity of hyperpigmentation, with emphasis on the knuckles, palms, soles, oral mucosa, face, neck, trunk, and extremities.

Venous blood samples were drawn and sent for laboratory examination in a sterile manner. Measurements of serum vitamin B12 and folate were performed by electrochemiluminescence immunoassay (ECLIA) on an automated analyzer (Roche

Cobas system), with high sensitivity and specificity for determining cobalamin and folate levels. A complete blood count (CBC) with peripheral smear review was performed on an automated hematology analyzer.

Data were entered and analyzed with SPSS version 25.0. Continuous variables such as age, hemoglobin, MCV, and vitamin levels were described as mean ± SD or median (IQR). Frequencies and percentages were used for categorical variables (presence and patterns of hyperpigmentation, gender, dietary habits). P-values < 0.05 were taken as statistically significant.

Results

The overall sample size consisted of 124 patients with MA. The majority of participants were male (n=72, 58.1%), while the rest were female (n=52, 41.9%). The most common age group was 31–45 years (n=41, 33.1%), followed by 46–60 years (n=37, 29.8%), 18–30 years (n=28, 22.6%), and >60 years (n=18, 14.5%). Hyperpigmentation was observed in 36 patients (29.0%), and the majority of patients did not have hyperpigmentation (71.0%) (Table 1).

Table 1. Baseline Characteristics of Study Participants (n = 124)

Variable	Frequency (n=124)	Percentage (%)
Gender		
Male	72	58.1
Female	52	41.9
Age Group (years)		
18–30	28	22.6
31–45	41	33.1
46–60	37	29.8
>60	18	14.5
Hyperpigmentation		
Present	36	29.0
Absent	88	71.0

Among the 36 patients with cutaneous hyperpigmentation, the most common lesion type was macular (n=14, 38.9%), followed by patchy lesions (n=9, 25.0%), diffuse pigmentation (n=8, 22.2%), and mixed pattern lesions (n=5, 13.9%). Regarding anatomical distribution, knuckles were the most frequently involved site (n=15, 41.7%), followed by palms/soles (n=8, 22.2%), face/neck (n=6, 16.7%), generalized or multiple-site involvement (n=4, 11.1%), and oral mucosa (n=3, 8.3%). Most lesions measured 1–5 cm (n=16, 44.4%), while fewer lesions were <1 cm (n=7, 19.4%), 6–10 cm (n=9, 25.0%), or >10 cm (n=4, 11.1%) (Table 2).

Table 2. Clinical Spectrum of Cutaneous Hyperpigmentation (n = 36)

Variable	Frequency (n=36)	Percentage (%)
Lesion Type		
Macular	14	38.9
Patchy	9	25.0
Diffuse	8	22.2
Mixed Pattern	5	13.9
Site Involved		
Knuckles	15	41.7
Palms/Soles	8	22.2
Face/Neck	6	16.7
Oral Mucosa	3	8.3
Generalized / Multiple Sites	4	11.1
Lesion Size (cm)		
<1 cm	7	19.4
1–5 cm	16	44.4
6–10 cm	9	25.0
>10 cm	4	11.1

Patients with hyperpigmentation had lower mean hemoglobin levels (7.8 ± 1.2 g/dL) compared to those without hyperpigmentation (9.4 ± 1.5 g/dL). Similarly, the mean corpuscular volume was higher in the hyperpigmentation group (116.3 ± 8.7 fL) than in the non-hyperpigmentation group (108.5 ± 7.9 fL), indicating more pronounced macrocytosis. Serum vitamin B12 and folate levels were also considerably lower among patients with hyperpigmentation (Table 3).

Table 3. Comparison of Biochemical and Hematological Profile According to Hyperpigmentation Status (n = 124)

Parameter	Hyperpigmentation Present (n=36) Mean ± SD	Hyperpigmentation Absent (n=88) Mean ± SD	P-value
Hemoglobin (g/dL)	7.8 ± 1.2	9.4 ± 1.5	0.001
Mean Corpuscular Volume (fL)	116.3 ± 8.7	108.5 ± 7.9	0.003
Serum Vitamin B12 (pg/mL)	118.4 ± 32.6	164.2 ± 41.8	0.002
Serum Folate (ng/mL)	2.4 ± 0.8	3.8 ± 1.1	0.005

Severe anemia was observed more frequently in the hyperpigmentation group (n=18, 50.0%) than in the non-hyperpigmentation group (n=19, 21.6%), whereas mild anemia was more common among patients without hyperpigmentation

(n=27, 30.7%). Similarly, severe vitamin B12 deficiency was more prevalent in patients with hyperpigmentation (n=20, 55.6%) compared to those without hyperpigmentation (n=24, 27.3%) (Table 4).

Table 4. Severity Distribution of Biochemical and Hematological Parameters by Hyperpigmentation Status

Parameter	Category	Hyperpigmentation Present n (%)	Hyperpigmentation Absent n (%)	P-value
Hemoglobin	Severe	18 (50.0)	19 (21.6)	0.002
	Moderate	14 (38.9)	42 (47.7)	
	Mild	4 (11.1)	27 (30.7)	
Vitamin B12	Severe deficiency	20 (55.6)	24 (27.3)	0.001
	Moderate deficiency	11 (30.6)	35 (39.8)	
	Mild deficiency	5 (13.9)	29 (33.0)	

Discussion

Cutaneous hyperpigmentation is frequently thought of as a cosmetic manifestation but can be a marker of greater biochemical deficiency and longer disease history. Without detection, MA may develop into severe anemia, pancytopenia, glossitis, cognitive dysfunction, and permanent neurological symptoms, including peripheral neuropathy, subacute combined degeneration, and gait ataxia (2, 11). These complications are clinically significant because the neurological changes caused by longstanding vitamin B12 deficiency may be only partially reversible after treatment. Thus, the aim of this study was to determine the prevalence and clinical spectrum of skin hyperpigmentation in patients with MA and to estimate its potential role as a clinically visible disease-severity parameter.

In the present study, the prevalence of hyperpigmentation was 29%. The frequency is similar to that reported in an Indian study by Singh et al., which reported hyperpigmentation in approximately 16.81% of B12-deficient patients, predominantly affecting the knuckles and extremities (5). A pediatric study from India reported knuckle hyperpigmentation in 45% of MA cases, a higher rate than our overall rate, possibly due to high rates of nutritional deficiency in the younger age group (12).

Furthermore, the most prevalent type of lesion was the macular pigmentation, followed by patchy and diffuse patterns. This is consistent with reports of brownish-black macules or patches on acral areas, such as the hands and feet, associated with vitamin B12 deficiency (2, 12). Macular lesions were observed in a high proportion of patients, suggesting that many were diagnosed before extensive generalized pigmentation developed. On the other hand, the palms, soles, face, and oral mucosa have

been described as involved in some case reports, typically following a long diagnostic delay and/or misdiagnosis as an allergy, Addison's disease, or a primary dermatological disease (13, 14).

The most involved site in the current study was the knuckles (41.7%). This is consistent with Srivastava et al., who recognized pigmentation over the knuckles and interphalangeal joints as a characteristic early skin manifestation of vitamin B12 deficiency (15). Oral mucosal involvement was relatively uncommon, and palms and soles were the second most common location in our study. This is in contrast to the findings of Viswanathan et al. and Ankar et al., where the oral cavity was also involved and mucocutaneous pigmentation was the most prominent feature; this difference could be due to different duration of deficiency, level of biochemical depletion, ethnic and skin phototype groups, and timing of clinical evaluation (14, 16).

Our results showed that the vitamin B12 level in the serum of hyperpigmented patients was significantly lower than that of non-hyperpigmented patients, a finding consistent with recent reports identifying skin hyperpigmentation as a visible marker of vitamin B12 deficiency (2, 15). Srivastava et al. reported that knuckle pigmentation was an early cutaneous clue of Vitamin B12 deficiency, noting that the skin pigmentation can be present before significant neurological symptoms (15). Further, severe anemia was found to be more common in hyperpigmented patients than in non-hyperpigmented patients, and severe vitamin B12 deficiency was also significantly higher in hyperpigmented patients in our study. This is consistent with Sharma et al., who found that deeper biochemical deficiency and systemic involvement are associated with more marked pigmentary changes (17).

In our study, the relatively decreased serum folate levels in patients with hyperpigmentation also highlight that combined nutritional deficiencies can amplify clinical manifestations. Clinically, this means that a hyperpigmented patient should not be tested for B12 alone; folate testing should also be included, especially in the South Asian population, where inadequate nutrition and malabsorption can occur simultaneously (18, 19).

The study is single-center, cross-sectional, and thus has limited generalizability and cannot establish causality or measure treatment response over time. Furthermore, recall bias in clinical history and the omission of bone marrow examination in certain cases can also influence interpretation.

Conclusion

This study shows that hyperpigmentation of the skin is a clinically relevant finding in patients with megaloblastic anemia (29%) and is highly correlated with more severe hematological and biochemical abnormalities. Patients with hyperpigmentation had lower hemoglobin levels, higher MCV, and significantly decreased serum vitamin B12 and folate, reflecting more advanced disease. The most frequently involved site was the knuckles, and the most frequent lesions were macular. These results demonstrate the value of the clinical examination, which is essential for diagnosing more severe deficiency states

and underscores the need for early diagnosis to prevent hematological deterioration and neurological complications.

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