National Journal of Health Sciences, 2025, *10*, 183-187

Research Article

Frequency of Pancytopenia among Patients with Vitamin B_{12} Deficiency

Sabir Rehman*, Momin Khan, Fawad Ali, Karim Ullah, Attaur Rehman

Department of Medicine, Saidu Teaching Hospital, Saidu Medical College, Swat, Pakistan.

Abstract: Background: B_{12} deficiency is a common yet nutritional ailment prevalent among the aged ages of sixty years and above and amongst women. Major hematological result with vitamin B_{12} deficit is pancytopenia (PCP) which is reversible in most cases with an early treatment. The paucity of local data with regard to the incidence of PCP among the vitamin B_{12} deficient patients highlights the fact that, additional research is required.

Objective: To evaluate the frequency of pancytopenia in patients with vitamin B_{12} deficiency.

Materials and Methods: A descriptive cross-sectional study of 270 patients (18-60 years old) was conducted in Saidu Group of Teaching Hospitals, Swat, Pakistan, between July and December 2024 to study vitamin B_{12} deficiency in patients. A patient with a <150 pmol/L serum vitamin B_{12} level was considered vitamin B_{12} deficient. The presence or absence of PCP was marked on the peripheral smear of blood, i.e., hemoglobin<10 g/dL, total leucocyte count <4,000/µL, platelets<150,000/µL, and reticulocyte count<2%. Chi-square test was applied to see the association of PCP with demographic and clinical variables among patients with vitamin B_{12} deficiency, taking $p<0.05$ as significant.

Result: In a total of 270 patients, 160 (59.3%) were male, and 110 (40.7%) female. The mean age, and BMI were 35.4±10.1 years, and 25.5±3.6 kg/m^2, respectively. Residential status of 156 (57.8%) was urban. Diabetes mellitus was diagnosed in 46 (17.0%) patients. The mean vitamin B_{12} levels was 110.4±18.9 pmol/L. The frequency of PCP was noted in 57 (21.1%) patients. Stratification of PCP with respect to gender, age groups, BMI categories, residential status, diabetes melitus, and smoking status did not reveal any significant associations ($p>0.05$).

Conclusion: The frequency of PCP was high in adult patients with vitamin B_{12} deficiency.

Keywords: Anemia, Pancytopenia, Hemoglobin, Red cell count, Mean corpuscular volume, Vitamin B_{12} deficiency.

INTRODUCTION

Methylcobalamin and adenosylcobalamin are the two biologically active forms of vitamin B_{12} [1, 2]. The body's need for vitamin B_{12} is met through dietary intake, as it is not synthesized by the human body itself [3]. Foods obtained from animal sources, i.e., dairy products, meat, fish, and eggs, are rich in vitamin B_{12}, which is acquired indirectly from synthesizing microorganisms [4]. Insufficient intake or defects in the absorption, transport, and processing of the vitamin can cause vitamin B_{12} deficiency [5]. Literature from different parts of the world has revealed that vitamin B_{12} deficiency can be manifested in a variety of physiological conditions and stages of life [6]. Vitamin B_{12} deficiency can clinically be assessed on the basis of hematological and neurological symptoms. Pernicious anemia, an autoimmune disease that damages the stomach mucosa and subsequently affects intrinsic factor secretion, was the first to be shown to exhibit the characteristic symptoms. Megaloblastic anemia and macrocytosis are hematology-related signs and symptoms of vitamin B_{12} deficiency [7].

A number of diseases can manifest as pancytopenia (PCP), and laboratory evaluations can help in diagnosing the condition [8]. Although, PCP can be treated by suggesting vitamin B_{12}, if caused by vitamin deficiency, its diagnosis on occasions requires advanced techniques like bone marrow biopsy [9, 10]. Using laboratory tests, radiological imaging, and invasive procedures, where needed, a diagnosis can be made. Expertise and knowledge of the physician, as well as the cause of PCP, determine the time needed for the diagnosis.

Literature shows that the overall global burden of vitamin B_{12} deficiency encompasses 6%, with a 1.6 -10% occurrence rate among European populations. Data from various countries suggest that adults over 60 years of age, with women dominating the male gender, are more likely to have vitamin B_{12} deficiency (10-19%) [11]. Another study revealed that PCP was noted in 5.4% of patients presenting with low vitamin B_{12} levels [12].

Vitamin B_{12} deficiency is a frequently encountered and often under diagnosed cause of PCP [6-8]. This hematological manifestation may closely resemble more serious bone marrow disorders, leading to diagnostic uncertainty and potential delays in appropriate therapy. In Pakistan, the prevalence of nutritional deficiencies, including vitamin B_{12}, remains high due to dietary habits and socioeconomic factors. When taking into consideration that pancytopenia due to the deficiency of vitamin B_{12} is reversible, then the lack of local statistics can cause delays or completely overlooked diagnoses. This research attempt will close this knowledge gap because it examines the prevalence of PCP among patients with vitamin B_{12} deficiency, which eventu-

* Address correspondence to this author at the Department of Medicine, Saidu Teaching Hospital, Saidu Medical College, Swat, Pakistan.
Email: rehmansabir006@gmail.com

ally will help increase the awareness of clinicians and promote timely diagnosis and management. This can ultimately help improve patient outcomes by preventing unnecessary interventions and promoting targeted treatment strategies in our healthcare setting. This study aimed to determine the frequency of PCP in patients with vitamin B_{12} deficiency. Results of this study would not only furnish the local data with fresh statistics about the magnitude of the problem but also serve as firsthand local evidence for further studies, from which prevention and control suggestions can be formulated.

MATERIALS AND METHODS

This is a descriptive cross-sectional study that was done in the outpatient department of medicine, Saidu Group of Teaching Hospitals, Swat, Pakistan, between July 2024 and December 2024 after obtaining ethical approval of Institutional Ethical Review Committee (approval no. 169-ERB/023, dated December 15, 2023).

The participants were 18-60 years old patients with a known vitamin B_{12} deficiency (< 150 pmol/L) who had the following symptoms of vitamin B_{12} deficiency: Weakness, fatigue, pale skin. Malignancy, aplastic anemia, leukemia, iron deficiency anemia, megaloblastic anemia, and infectious diseases were listed as the exclusion criteria.

Sample size was estimated using Open-EPi sample size calculator which came out to be 270 patients at a level of confidence 95%, and absolute precision 2.7 percent, and PCP frequency of 5.4% [12]. Patients were recruited by using non-probability consecutive sampling after their written informed consent.

The eligible subjects went through documentation of demographics and medical history. For laboratory assessments, 10cc blood sample was drawn using the strict aseptic technique and was immediately sent to the institutional laboratory to assess relevant laboratory investigations including random blood sugar, and complete blood count. An experienced hematologist with a minimum of five years of experience performed all investigations. PCP was labeled if peripheral smear of blood showed hemoglobin<10 g/dL, total leukocyte count<4,000 per microliter, platelets<150,000 per microliter, and reticulocyte count<2% [12]. A specifically predesigned proforma was used to collect all of the necessary information related to this study.

STATISTICAL ANALYSIS

The statistical analysis was done using "IBM-SPSS Statistics" version 26.0. For the qualitative variables frequency and percentages were computed. The quantitative variables were represented as mean and standard deviation after assessing normality assumption wit Shapiro-Wilk test. Effect modifiers like age, sex, BMI, smoking history, DM, years of schooling, and residential status were controlled through stratification. Post-stratification, chi-square test was applied to see the impact of effect modifiers on outcome variable (PCP) taking p-value<0.05 as statistically significant.

RESULT

In a total of 270 patients, 160 (59.3%) were male. The mean age, and BMI were 35.4±10.1 years, and 25.5±3.6 kg/m^2, respectively. Residential status of 156 (57.8%) was urban. Diabetes mellitus was diagnosed in 46 (17.0%) patients. Smoking history was present in 42 (15.6%) participants. Characteristics of patients are mentioned in Table **1**.

Table 1. Characteristics of Patients with Vitamin B_{12} Deficiency (n=270).

Characteristics		Frequency (% age)
Gender	Male	160 (59.3%)
	Female	110 (40.7%)
Age Groups (years)	18-30	91 (33.7%)
	>30-40	101 (37.4%)
	>40-60	78 (28.9%)
BMI Categories (kg/m^2)	20-24.9	106 (39.3%)
	>24.9-29.9	134 (49.6%)
	>29.9-33	30 (11.1%)
Residential Status	Urban	156 (57.8%)
	Rural	114 (42.2%)
Diabetes Mellitus	Yes	46 (17.0%)
	No	224 (83.0%)
Smoking Status	Yes	42 (15.6%)
	No	228 (84.4%)

Mean vitamin B_{12} level was 110.4 ± 18.9 pmol/L. The presence of pancytopenia (PCP) was identified in 57 patients (21.1%, 95% CI: 16.4%-26.5%). Additional stratification, shown in Table **2**, indicates PCP frequency was not significantly different among subgroups, such as, gender, age groups, BMI categories, residential status, diabetes status, smoking status, and the levels of vitamin B_{12}.

Table 2. Pancytopenia Stratification with Respect to Study Variables in Patients with Vitamin B_{12} Deficiency (n=57).

Variables		Pancytopenia		p-value
		Yes (n=57)	No (n=213)	
Gender	Male	35 (61.4)	125 (78.1)	0.711
	Female	22 (38.6)	88 (80.0)	
Age (years)	18-30	22 (38.6)	69 (75.8)	0.551
	30.1-40	18 (31.6)	83 (82.2)	
	40.1-60	17 (29.8)	61 (78.2)	
BMI Categories (kg/m^2)	20-24.9	18 (31.6)	88 (83.0)	0.408
	25-29.9	32 (56.1)	102 (76.1)	
	30-33	7 (12.3)	23 (76.7)	

Continued

Continued

Residential Status	Urban	34 (59.6)	122 (78.2)	0.757
	Rural	23 (40.4)	91 (79.8)	
Diabetes Mellitus	Yes	9 (15.8)	37 (80.4)	0.778
	No	48 (84.2)	176 (78.6)	
Smoking History	Yes	7 (12.3)	35 (83.3)	0.442
	No	50 (87.7)	178 (78.1)	

Data is expressed as n(%).

DISCUSSION

The current study revealed that overall 21.2% of the vitamin B_{12} deficient patients had PCP. Gulnaz *et al.* showed vitamin B_{12} deficiency to be the commonest finding in patients with PCP [13]. A study among 137 cases showed that 17% of the PCP patients were deficient in vitamin B_{12} [10]. The frequency of PCP (21.1%) found in this study aligns closely with the 20.5% reported by Cordan and Tukek [14], who identified vitamin B_{12} deficiency and hypersplenism as the leading causes of PCP in Turkey. A local study carried out by Niazi *et al.* mentioned that 5% of their study patients had PCP [15]. Studies focusing on children such as Tahir *et al.* [16] and Sarbay [17] who noted rates of B_{12}-associated PCP as 6.2%, and 27%, respectively, which may reflect different etiological spectrums in pediatric populations. In such cases, infections, inherited marrow failure syndromes, and nutritional deficits other than B_{12} contribute more significantly to the pathogenesis of cytopenias [16]. Another plausible explanation for the higher PCP rate is the more profound deficiency of vitamin B_{12} observed in this study, with a mean serum level of 110.4±18.9 pmol/L. Studies like those by Jain *et al.* [18] and Kaur *et al.* [19] documented that lower vitamin B_{12} levels were significantly associated with more severe hematologic abnormalities, including PCP and macrocytosis. Although causality cannot be definitively established in a cross-sectional design, the association between profound B_{12} deficiency and marrow suppression appears biologically plausible and clinically consistent [20]. The discrepancy might be partially explained by differences in diagnostic thresholds, regional variations in micronutrient deficiency, and the underlying prevalence of chronic illnesses influencing marrow suppression. The current study adopted a strict definition of PCP, consistent with most hematologic literature and this uniform diagnostic threshold enhances comparability across studies, yet subtle variations in cut-off values and laboratory methods cannot be overlooked as a confounding variable in literature comparisons.

This study found male predominance among patients with PCP (59.3%). Jain *et al.* [18] observed a female predominance (60%) in PCP in Indian cohort, and Cordan and Tukek [14] also reported a female-to-male ratio of 1.8:1. The discrepancy may be attributable to regional healthcare-seeking behaviors and differences in dietary habits, particularly in Pakistan, where sociocultural factors might delay female presentation to tertiary healthcare facilities. The mean age in the present study was 35.4±10.1 years, significantly younger than the cohort analyzed by Cordan and Tukek [14], where the mean age was 56.5 years, with the most affected group being 70-79 years old. This demographic difference may reflect the different referral patterns and age-based susceptibility to vitamin B_{12} deficiency. The younger age group in this study may also suggest earlier onset or higher exposure to dietary deficiencies, which can have public health implications regarding nutritional surveillance and fortification strategies. The stratification by BMI, residential status, diabetes mellitus, and smoking history did not yield any significant associations with PCP. These findings suggest that while PCP is prevalent in B_{12}-deficient individuals, its occurrence may not be strongly dependent on these demographic or lifestyle variables, but more likely on the severity and chronicity of the deficiency.

From a clinical standpoint, the relatively high burden of PCP in vitamin B_{12}-deficient patients reported in this study has several implications. It necessitates early detection and correction of B_{12} deficiency to prevent progression to marrow failure [21]. These finding challenge clinicians to avoid premature conclusions about hematologic malignancies in young adults with PCP, particularly when megaloblastic changes or macrocytosis are present. It calls for robust dietary assessments and perhaps public health strategies such as vitamin B_{12} fortification in at-risk populations, particularly in rural or underserved regions where dietary diversity may be lacking [22]. Although bone marrow examination remains the gold standard for confirming the cause of PCP, especially in ambiguous or non-responding cases, peripheral smear analysis supported by serum B_{12} testing can preclude the need for invasive diagnostics in a significant proportion of patients, particularly when the clinical context and laboratory findings strongly suggest megaloblastic anemia [23, 24].

STRENGHT

A key strength of the current study is its relatively large sample size (n=270) and the use of standardized diagnostic criteria, which enhance the reproducibility of findings. The use of stratification to control for potential effect modifiers also adds methodological strength, despite the absence of significant associations in subgroup analyses.

LIMITATIONS

This study suffers with few limitations. The cross-sectional design precludes any causal inferences, and the study's reliance on non-probability consecutive sampling may introduce selection bias. The exclusion of patients with overlapping hematologic conditions such as iron deficiency or chronic infections might limit generalizability to real-world clinical scenarios where nutritional and infectious etiologies often coexist. The reliance on a single measurement of serum B_{12} and clinical symptoms may underestimate functional B_{12} deficiency, particularly in cases with borderline values or where methylmalonic acid and homocysteine levels are not measured [25]. Another potential limitation is the absence of data on dietary patterns to correlate with B_{12} deficiency and PCP risk. The follow-up data were not collected to assess hematologic recovery post-B_{12}

supplementation, which could have strengthened the clinical relevance of the findings by linking diagnosis with treatment response. Moreover, simple univariate analysis were conducted without adjusting multiple testing which should be considered when interpreting results.

CONCLUSION

The frequency of PCP was high in adult patients with vitamin B_{12} deficiency. This study reinforces the clinical imperative to include B_{12} deficiency in the differential diagnosis of PCP without overt signs of malignancy or chronic disease. Given the treatable nature of megaloblastic anemia, timely diagnosis and intervention can avert unnecessary invasive diagnostics and improve patient outcomes. Future longitudinal studies incorporating dietary assessments, functional B_{12} markers, and therapeutic follow-up are warranted to further elucidate the clinical spectrum and recovery patterns of PCP in vitamin B_{12}-deficient patients in resource-limited settings.

AUTHORS' CONTRIBUTION

Sabir Rehman: Conceptualization, Study Design, Writing Draft, Final approval, final proof to be published.

Momin Khan and Fawad Ali: Writing Draft.

Karim Ullah and Attaur Rehman: Methodology, Data analysis and interpretation.

ACKNOWLEDGEMENTS

Declared none.

DECLARATIONS

Data Availability

Data will be available from the corresponding author upon a reasonable request.

Ethical Approval

The study was commenced with the approval of Institutional Review Board of Saidu Teaching Hospital/ Saidu Medical College, Swat (IRB#169-ERB/023).

Consent to Participate

All the study participants were enlisted with their written informed consent.

Consent for Publication

All authors give consent for the publication of this work.

Conflict of Interest

Declared none.

Competing Interest/Funding

Declared none.

Use of AI-Assisted Technologies

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were utilized in the writing of this manuscript, in the creation of images/graphics/tables/captions, or in any other aspect of its preparation.

REFERENCES

[1] Hannibal L, Lysne V, Bjørke-Monsen AL, Behringer S, Grünert SC, Spiekerkoetter U, *et al.* Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency. Front Mol Biosci 2016; 3: 27. doi: 10.3389/fmolb.2016.00027

[2] Gherasim C, Lofgren M, Banerjee R. Navigating the B(12) road: Assimilation, delivery, and disorders of cobalamin. J Biol Chem 2013; 288(19): 13186-93. doi: 10.1074/jbc.R113.458810

[3] Green R. Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood 2017; 129(19): 2603-11. doi: 10.1182/blood-2016-10-569186

[4] Fedosov SN, Brito A, Miller JW, Green R, Allen LH. Combined indicator of vitamin B12 status: Modification for missing biomarkers and folate status and recommendations for revised cut-points. Clin Chem Lab Med 2015; 53(8): 1215-25. doi: 10.1515/cclm-2014-0818

[5] Briani C, Dalla Torre C, Citton V, Manara R, Pompanin S, Binotto G, *et al.* Cobalamin deficiency: Clinical picture and radiological findings. Nutrients 2013; 5(11): 4521-39. doi: 10.3390/nu5114521

[6] Ahmed MA. Metformin and vitamin B12 deficiency: Where do we stand?. J Pharm Pharm Sci 2016; 19(3): 382-98. doi: 10.18433/J3PK7P

[7] Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. Br Med J 2014; 349: g5226. doi: 10.1136/bmj.g5226

[8] Jian LQ, Weng EHQ, Wee CS. Case series of pancytopenia secondary to pernicious anemia. Borneo J Med Sci 2019; 13(2): 55-9. doi: 10.51200/bjms.v13i2.1274

[9] Mohsin KM, Kalsuramath MV. A clinical and etiological profile of pancytopenia -one year prospective study in a tertiary care hospital. Glob J Res Anal 2019; 8(3): 53-7. doi: 10.36106/gjra

[10] Yokuş O, Gedik H. Etiological causes of pancytopenia: A report of 137 cases. Avicenna J Med 2016; 6(4): 109-12. doi: 10.4103/2231-0770.191447

[11] Kumar V, Prasad J. Clinical assessment of pancytopenia in peripheral blood smears identified in patients from Bihar region. Int J Med Biomed Stud 2019; 3(8): 281-6. doi: 10.32553/ijmbs.v3i8.573

[12] Bhatia P, Kulkarni JD, Pai SA. Vitamin B12 deficiency in India: Mean corpuscular volume is an unreliable screening parameter. Natl Med J India 2012; 25(6): 336-8.

[13] Khalid G, Moosani MA, Ahmad L, Farooqi AN. Megaloblastic anemia seen in 48 cases of pancytopenia. Ann ASH-KMDC 2005; 10(2): 742-44.

[14] Çordan İ, Tukek T. Evaluation of etiological causes and incidence of pancytopenia: A case series from a tertiary care center. Cureus 2025; 17(2): e79485. doi: 10.7759/cureus.79485

[15] Niazi HT, Jan ZS, Jan, NS, Bahadur L, Khan KA, Ali M. Frequency of pancytopenia among patients with vitamin B12 deficiency. J Med Sci 2023; 31(1): 62-6. doi: 10.52764/jms.23.31.1.12

[16] Tahir MN, Athar H, Shahid M, Umer R, Haq AU. Frequency of vitamin B12 deficiency in patients of pancytopenia. Pak J Med Health Sci 2023; 16(12): 40-2. doi: 10.53350/pjmhs2022161240

[17] Sarbay H. Comparison of the severity of cytopenias with etiologic factors in patients with pancytopenia and bicytopenia. Pan Afr Med J 2019; 34: 149. doi: 10.11604/pamj.2019.34.149.18749

[18] Jain A, Garg R, Kaur R, Nibhoria S, Chawla SPS, Kaur S. Clinico-hematological profile of pancytopenic adult patients in a tertiary care teaching hospital. Tzu Chi Med J 2021; 34(1): 95-101. doi: 10.4103/tcmj.tcmj_17_21

[19] Kaur N, Nair V, Sharma S, Dudeja P, Puri P. A descriptive study of clinico-hematological profile of megaloblastic anemia in a tertiary care hospital. Med J Armed Forces India 2018; 74(4): 365-70. doi: 10.1016/j.mjafi.2017.11.005

[20] Yavuz S, Kaya H, Sert A, Yigit O. Retrospective evaluation of patients with vitamin B12 deficiency in the pediatrics outpatient clinic. J Surg Med 2024; 8(3): 51-4. doi: 10.28982/josam.7587

[21] Farooque R, Iftikhar S, Herekar F, Patel MJ. Frequency and etiology of pancytopenia in patients admitted to a tertiary care hospital in Karachi. Cureus 2020; 12(10): e11057. doi: 10.7759/cureus.11057

[22] Niklewicz A, Smith AD, Smith A, Holzer A, Klein A, McCaddon A, *et al.* The importance of vitamin B12 for individuals choosing plant-based diets. Eur J Nutr 2023; 62(3): 1551-9. doi: 10.1007/s00394-022-03025-4

[23] Dastidar R, Sikder K. Diagnostic reliability of serum active B12 (holo-transcobalamin) in true evaluation of vitamin B12 deficiency: Relevance in current perspective. BMC Res Notes 2022; 15(1): 329. doi: 10.1186/s13104-022-06224-8

[24] Lacombe V, Chabrun F, Lacout C, Ghali A, Capitain O, Patsouris A, *et al.* Persistent elevation of plasma vitamin B12 is strongly associated with solid cancer. Sci Rep 2021; 11(1): 13361. doi: 10.1038/s41598-021-92945-y

[25] Wolffenbuttel BHR, Wouters HJCM, Heiner-Fokkema MR, van der Klauw MM. The many faces of cobalamin (Vitamin B12) deficiency. Mayo Clin Proc Innov Qual Outcomes 2019; 3(2): 200-14. doi: 10.1016/j.mayocpiqo.2019.03.002

Received: July 22, 2025 Revised: September 09, 2025 Accepted: September 13, 2025