ORIGINAL ARTICLE

FREQUENCY OF PANCYTOPENIA AMONG PATIENTS WITH VITAMIN B12 DEFICIENCY

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ABSTRACT

Objective: To determine the frequency of pancytopenia among patients with vitamin B12 deficiency in a tertiary-care hospital, in Peshawar.

Materials and Methods: A descriptive cross-sectional study was carried out at the Department of Hematology, Hayatabad Medical Complex, Peshawar from 18/4/2021 to 18/10/2021. In this study, a total of 252 patients were observed. From all patients, a sample of venous blood was obtained and sent to the hospital laboratory for the peripheral smear examination. Pancytopenia was diagnosed based on a peripheral smear showing Hemoglobin below 10g/dL, Total leukocyte counts less than 4,000 cells per mL, platelets count less than150,000 cells per mL, and reticulocyte count <2%. All the peripheral smears were done by an expert hematologist. Strictly exclusion criteria were followed to avoid cofounders and make the study results clear of any bias.

Results: In this study mean age was 42 years with a standard deviation of ± 15.84. Thirty-eight percent of patients were males and 62% of patients were females. Moreover, 5% of patients had pancytopenia and 95% of patients didn't have pancytopenia.

Conclusion: The frequency of pancytopenia was 5% among the local patients with vitamin B12 deficiency.

Keywords: pancytopenia, B12 deficiency, Peshawar, Pakistan.

This article may be cited as: Niaz HT, Jan ZS, Jan NS, Jan KA, Ali M. Frequency of Pancytopenia Among Patients with Vitamin B12 Deficiency. J Med Sci 2023 January;31(1):62-66

INTRODUCTION

Pancytopenia is defined as a decrease in leukocytes, platelets, and erythrocytes in all three peripheral blood lineages. It is a group of findings rather than a disease that can be brought on by several different underlying disease processes. Pancytopenia can be caused by these conditions, which can either directly or indirectly affect bone marrow [1]. Pancytopenia may be acquired or inherited (genetic but not necessarily present at birth). Pancytopenia is caused by either a failure to produce hematopoietic progenitors or peripheral destruction of cellular elements caused by infection, immune-mediated damage, or hypersplenism [2]. To evaluate the overall cellularity and morphology of a bone marrow biopsy specimen and a marrow aspirate in pancytopenia, a microscopic examination is required. Bone marrow examination is one of the important diagnostic procedures for many hematological disorders. In most cases, it gives a specific diagnosis; however, in a few cases, additional tests are required. The presenting symptoms are often attributable to anemia, thrombocytopenia, or leucopenia. The etiological spectrum of pancytopenia varies according to geographical distribution and genetic disturbances [3].

Anemia caused by vitamin B12 (cobalamin) insufficiency is most typically caused by pernicious anemia and occurs far less frequently because of dietary inadequacy. However, as the adoption of veganism and vegetarian

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Date Received: 31-10-2022
Date Revised: 23-02-2023
Date Accepted: 25-02-2023

diets rises, so does the prevalence of B12 deficiency [4]. The tissues that experience high cell turnover are most impacted by a vitamin B12 deficit since this vitamin, which is predominantly found in animal products, is a crucial component in the synthesis of DNA and RNA. It normally takes one to four years for a deficit to have clinical effects, with hematologic and neurologic signs being the most noticeable [5].

Implications of a B12 inadequacy may include hematologic or neurologic effects. A defining symptom of B12 deficiency is megaloblastic anemia, which is frequently accompanied by hyper-segmented neutrophils on a peripheral blood smear. The most common symptoms are low levels of white blood cells, red blood cells, platelets, or a combination of these, such as pancytopenia and hemolysis. Glossitis, exhaustion, palpitations, a pale complexion, weight loss, and infertility were some additional systemic symptoms. A B12 deficiency, on the other hand, is also associated with neurological changes such as sensory impairments, paresthesia, ataxia, weakness, and gait instability. Severe cases may also lead to spasticity and paraplegia [6]. The most concerning neurologic outcome of subacute combined degeneration is demyelination of the spinal cord's dorsal, lateral, and spinocerebellar tracts. Other symptoms of vitamin B12 deficiency include paresthesia, loss of fine touch, vibrational and pressure sensations, vision loss, mental changes, bilateral spastic paresis, or paralysis [7].Since vitamin B12 deficiency is an easily curable cause of demyelinating neurological disease and bone marrow failure, it is crucial to identify and treat it[8]. Vitamin B12 (cobalamin), which cannot be produced by humans, is made by the normal flora in the gut. Foods of animal origin also contain trace amounts of it[(7)]. Megaloblastic anemia is brought on by folate and vitamin B12 deficiency[9]. In peripheral blood granulocytes, macrocytosis, immature nuclei, and hyper-segmentation are caused by an imbalance between the cytoplasmic and nuclear maturation processes[10]. As a reversible cause of bone marrow failure, vitamin B12 deficiency must be promptly identified and treated. Finding the underlying cause of vitamin B12 deficiency after a diagnosis is crucial for individualized treatment[11]. In one study, out of 94 patients presenting with low vitamin B12 levels, macrocytosis was seen in 29.8%, and a dimorphic blood picture of microcytic hypochromic cells and macrocytes was seen in 17%. 27.7% of patients had a microcytic and hypochromic picture while 25.5% had a normochromic, normocytic picture. 5.4% had pancytopenia[12].

The purpose of the current study was to determine the prevalence of pancytopenia in patients with vitamin B12 deficiency. Vitamin B12 deficiency is common in our population, and because of its importance in bone marrow cell lines and the nervous system, it is critical to assess the complications associated with it. Reduction in cell lines due to bone marrow failure secondary to vitamin B12 deficiency is critical and therefore, we designed this study to determine the burden of pancytopenia among the local population with vitamin B12 deficiency.

MATERIAL AND METHODS

A descriptive cross-sectional study was designed and carried out at the Department of Hematology, Hayatabad Medical Complex, Peshawar, to achieve the goal of the study. The duration of the study was 06 months i.e., from 18/4/2021 to 18/10/2021. The sample size was calculated using an online World Health Organization (W.H.O) sample size calculator i.e., a total of 252 subjects were included in the study using a 4.27% proportion of pancytopenia among patients with vitamin B12 deficiency[10], 95% confidence level, and 2.5% margin of error. A simple non-probability consecutive sampling technique was employed. All patients with severe vitamin B12 deficiency were eligible for the study i.e., with serum level less than 118 pmol/L, with duration more than 2 years, patients in the age range 18-60 years of either gender while patients already on treatment for diseases like cancer chemotherapy, already diagnosed cases of iron deficiency anemia, aplastic anemia, leukemias, and infectious diseases were excluded from the study. Permission was taken from the head of the department before starting the study. Written informed consent was taken from all patients. The objectives of the study and the risks involved were informed and explained to patients. All the information including name, age, sex, duration of vitamin B12 deficiency, and serum B12 Levels was recorded on pre-designed Performa. The privacy implications of the collected information were clearly explained to the patients at the time of taking written consent. From all patients, a sample of venous blood was obtained and sent to the hospital laboratory for the peripheral smear examination. Pancytopenia was diagnosed based on a Peripheral smear showing Hemoglobin <10g/dL, total leukocyte count <4,000 per mL, Platelets <150,000 per mL, and reticulocyte count <2%. All the peripheral smears were done by an expert hematologist. Similarly, the presence of megaloblastic anemia was diagnosed based on mean corpuscular volume (MCV) great-

er than 115fL. Strictly exclusion criteria were followed to avoid cofounders and make the study results clear of any bias. All the collected information on proforma was entered and analyzed through SPSS 22. Mean and Standard Deviation were calculated for numerical variables i.e. age, duration of vitamin B12 deficiency, and serum B12 Levels. Frequency and percentages were calculated for gender, malabsorption, nutritional deficiency, and pancytopenia. Pancytopenia was stratified among the age, gender, duration of vitamin B12 deficiency, malabsorption, and nutritional deficiency to examine the effect modification using the chi-square test, with a p-value of 0.05 considered significant. Tables and graphs were used to present all the results.

RESULTS

Among 252 patients, 96(38%) patients were males while 156(62%) patients were females. The distribution of patients concerning age is shown in Table 1. The mean age was 42 ± 15.84 years. The status of pancytopenia among 252 patients was analyzed and 13(5%) patients had pancytopenia while 239(95%) patients didn't have pancytopenia. Table 2 shows the distribution of pancytopenia with clinical parameters. Stratification of pancytopenia with age and gender is shown in Table 3 while stratification of pancytopenia with vitamin B12 deficiency and megaloblastic anemia is shown in Table 4.

Table 1: Distribution of patient

Age (Yrs.)	Frequency	Percentage	Mean Age
18-30	30	12%	42± 15.84 Years
31-40	50	20%	
41-50	81	32%	
51-60	91	36%	

Table 2. Distribution of Patients W.R.T Clinical Parameter

Observation	Vitamin B12 Deficiency (n)	Megaloblastic Anemia (n) (%)
Present	252	165 (65.48)
Absent	0	87 (34.52)

Table 3: Stratification of pancytopenia w.r.t age and gender distribution

Stratification of Pancytopenia W.R.T Age Distribution						
Pancytopenia	18-30 Yrs.	31-40 Yrs.	41-50 Yrs.	51-60 Yrs.	Total	**p-value**
Present	2	3	4	4	13	0.9546
Absent	28	47	77	87	239	
Stratification of Pancytopenia W.R.T Gender Distribution						
Pancytopenia (n)	Male (n)	Female (n)	Total	p-value		
Present	5	8	13	0.9777		
Absent	91	148	239			

Table 4: Stratification of pancytopenia with Vit B12 Deficiency and Megaloblastic Anemia

Pancytopenia (n)	Vitamin B12 Deficiency (n)	Percentage (%)	**p-value**
Present	13	5.16	0.7590
Absent	239	94.84	
Stratification of Pancytopenia W.R.T Megaloblastic Anemia			
Pancytopenia (n)	Megaloblastic Anemia (n)	Percentage (%)	p-value
Present	165	65.48	0.7590
Absent	87	34.52	

DISCUSSION

Even though vitamin B12 (cobalamin) deficiency has been known for more than a century, it can still be challenging to diagnose and treat properly. Lack of vitamin B12 can cause a variety of symptoms, from neurologic to psychiatric. Many people who don't get enough vitamin B12 could suffer from megaloblastic anemia[13]. Numerous cases of vitamin B12 deficiency are ignored or even misdiagnosed in clinical settings[14]. A plasma concentration below 118 pmol/L (160 pg/mL) is deficient in vitamin B12. However, in general practice, plasma vitamin B12 levels greater than 140 pmol/L are commonly considered normal by physicians, but many symptomatic patients may have such levels, which may be because of taking oral vitamin supplementation. The range of signs and symptoms for a vitamin B12 deficiency is fairly established[15]. However, many of the symptoms are vague and could be brought on by other illnesses. As of right now, no studies have shown how well certain symptoms or symptom scores can predict whether a person has a vitamin B12 deficiency[16]. Previously, according to a study conducted by Marin JDM et al., vitamin B12 deficiency manifests as anemia, neuropathy, and myelopathy. More than 10% of people over the age of 65 suffer from anemia (defined by WHO as serum hemoglobin (Hb) levels below 12 g/dl in women and 13 g/dl in men). 17% of these cases are caused by vitamin B12 deficiency. Anemia (21%), leukopenia (11%), thrombocytopenia (9%), and pancytopenia (6.5%) are the most common hematologic manifestations in patients with vitamin B12 deficiency (200 pg/ml levels)[17].

Pancytopenia must be diagnosed using a thorough diagnostic process that is tailored to the clinical setting. Pancytopenia with anemias due to vitamin B12 deficiency is evaluated using a combination of known hypersensitivity, clinical, cytological, and biomarker factors. Pancytopenia has a variety of causes that vary in presentation and severity; its prevalence varies greatly across countries[18]. On the hand, megaloblastic anemia has been identified as the leading cause of pancytopenia worldwide. Megaloblastic anemia is diagnosed simply using a complete blood count, a peripheral blood smear, and bone marrow cytology, which is a low-cost procedure. Nutritional variables, recurrent infection, vitamin B12, and folate deficits appear to be closely linked to megaloblastic anemia[19]. In South Asia, megaloblastic anemia and aplastic anemia are major causes of pancytopenia. However, In Pakistan, aplastic anemia was found more common hematological disorder (20.2%) than megaloblastic anemia (14.6%) whereas iron deficiency anemia and idiopathic thrombocytopenic purpura were the other common causes with the frequency of 7.6% and 15.7% respectively[20]. Similarly, pancytopenia was found in 70% of individuals with megaloblastic anemia in a cross-sectional observational research in Pakistan[21]. In the current study, megaloblastic anemia was found in 65.48% of patients with serum vitamin B12 deficiency.

Although patients with low serum vitamin B12 levels may not exhibit any symptoms, they are highly likely to do so. When determining the etiology of pancytopenia in the beginning, the serum vitamin B12 level should be considered. The prevalence of vitamin B12 deficiency in pancytopenia patients ranges from 3% to 5% in the general population, and it ranges from 5% to 20% in those over 65 [22]. According to our findings, 5% of patients had pancytopenia and 95% did not experience a decrease in blood cells. Another study found comparable results conducted by Bhatia P et al., in which out of 94 patients presenting with low vitamin B12 levels, macrocytosis was seen in 29.8%, dimorphic blood picture of microcytic hypochromic cells and macrocytes was seen in 17%. 27.7% of patients had a microcytic and hypochromic picture while 25.5% had a normochromic, normocytic picture. 5.4% had pancytopenia [23]. In a study conducted by Mezalek ZT et al., 268 consecutive patients who were hospitalized for cobalamin deficiency from January 2000 to December 2015, their medical data identified and retrospectively examined which also showed that pancytopenia affected 104 (8.8%) of the total patient's [24].

CONCLUSION

Our study concluded that there was a low prevalence (5%) of severe pancytopenia in the local population with vitamin B12 deficiency based on a single institution with a significant number of consecutive patients with well-documented cobalamin deficiency. We haven't yet found a reason for those findings. These findings show that severe vitamin B12 deficiency can mimic a malignant hematologic disorder and that prompt diagnosis and supplementation result in the resolution of symptoms and blood abnormalities.

ACKNOWLEDGEMENT

The authors acknowledge the technical support provided by the staff at the Department of Hematology, Hayatabad Medical Complex, Peshawar, Pakistan for technical support during the study.

REFERENCES

1. Gnanaraj J, Parnes A, Francis CW, Go RS, Takemoto CM, Hashmi SK. Approach to pancytopenia: Diagnostic algorithm for clinical hematologists. Blood reviews.

2018;32(5):361-7.

2. Sharma R, Nalepa G. Evaluation and management of chronic pancytopenia. Pediatr Rev. 2016;37(3):101.
3. Vargas-Carretero CJ, Fernandez-Vargas OE, Ron-Magaña AL, Padilla-Ortega JA, Ron-Guerrero CS, Barrera-Chairez E. Etiology and clinico-hematological profile of pancytopenia: experience of a Mexican Tertiary Care Center and review of the literature. Hematol. 2019;24(1):399-404.
4. Green R, Mitra AD. Megaloblastic anemias: nutritional and other causes. Medical Clinics. 2017;101(2):297-317.
5. Socha DS, DeSouza SI, Flagg A, Sekeres M, Rogers HJ. Severe megaloblastic anemia: Vitamin deficiency and other causes. Clevel ClinJ Med. 2020;87(3):153-64.
6. Hariz A, Bhattacharya PT. Megaloblastic anemia. StatPearls [Internet]: StatPearls Publishing; 2021.
7. Pavlov CS, Damulin I, Shulpekova YO, Andreev E. Neurological disorders in vitamin B12 deficiency. Terapevticheskii arkhiv. 2019;91(4):122-9.
8. Sako S, Tsunogai T, Oishi K. Thiamine-responsive megaloblastic anemia syndrome. GeneReviews®[Internet]. 2022.
9. Green R, Allen LH, Bjørke-Monsen A-L, Brito A, Guéant J-L, Miller JW, et al. Vitamin B12 deficiency. Nat Rev Dis primers. 2017;3(1):1-20.
10. Wolffenbuttel BH, Wouters HJ, Heiner-Fokkema MR, van der Klauw MM. The many faces of cobalamin (vitamin B12) deficiency. Mayo clinic proceedings: innovations, quality & outcomes. 2019;3(2):200-14.
11. Green R. Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood. 2017 May 11;129(19):2603-2611. doi: 10.1182/blood-2016-10-569186. Epub 2017 Mar 30. PMID: 28360040.
12. Kamath SU, Bhaskaranand N, Rao A. B12 Deficiency in a Breastfed Infant Due to Maternal B12 Deficiency: A Case Report. JCDR. 2018;12(1).
13. Langan RC, Goodbred AJ. Vitamin B12 deficiency: recognition and management. Am Fam Physician . 2017;96(6):384-9.
14. Margalit I, Cohen E, Goldberg E, Krause I. Vitamin B12 deficiency and the role of gender: a cross-sectional study of a large cohort. Annals of Nutrition and Metabolism. 2018;72(4):265-71.
15. Harrington DJ. Laboratory assessment of vitamin B12 status. J Clin Pathol. 2017;70(2):168-73.
16. Sukumar N, Saravanan P. Investigating vitamin B12 deficiency. BMJ. 2019;365.
17. Martínez Marín JD, Henao Riveros SC, Rey Tovar MH. Levels of vitamin B12 in Colombian patients with chronic atrophic gastritis. Rev Colomb Gastroenterol. 2010;25(3):261-4.
18. Depuis Z, Gatineau-Sailliant S, Ketelslegers O, Minon J-M, Seghaye M-C, Vasbien M, et al. Pancytopenia Due to Vitamin B12 and Folic Acid Deficiency—A Case Report. Pediatr Rep. 2022;14(1):106-14.
19. Geissler K. Pancytopenia—a diagnostic challenge? memo-Mag Euro Med Onco. 2021;14(4):355-9.
20. Ahmad A, Idrees M, Afridi IG, Rehman G. To determine etiology and frequency of pancytopenia in pediatric population and compare it with other studies. J Med Sci. 2016;9(2):186-9.
21. Khattak MB, Ismail M, Marwat ZI, Khan F. Frequency and characterisation of pancytopenia in megaloblastic anaemia. J Ayub Med Coll Abbottabad. 2012;24(3-4):53-5.
22. Rao VR. Vitamin B12 deficiency presenting with hyperpigmentation and pancytopenia. J Family Med Prim Care. 2018;7(3):642.
23. 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(6.2012).
24. Mezalek ZT, Ammouri W, Maamar M, Bourkia M, Harmouche H, Adnaoui M, et al. Vitamine B12 Deficiency Presenting As Severe Pancytopenia in Adult Patients. a Monocentric Study of 104 Cases. Blood. 2016;128(22):4812.

CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE: NIL

AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Niaz HT:	Conception of idea, Manuscript writing
Jan JS:	Data Collection, Laboratory Work
Jan NS:	Data Collection, Laboratory work
Bahadur L:	Statistical analysis, Bibliography
Jan KA:	Laboratory work, Data analysis
Ali M:	Results compilation, statistical analysis

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.