Original Article

Evaluation of the Vitamin B12 Deficiency in Megaloblastic Anemia in Tertiary Care Hospital, Lahore.

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ABSTRACT

Background: Megaloblastic anemia is a hematologic disorder characterized by the presence of large, immature red blood cells due to a deficiency in vitamin B12 or folate. It remains a significant public health issue globally, affecting various populations across different age groups and dietary habits.

Objective: The objective of this study was to evaluate the prevalence and implications of vitamin B12 deficiency in the development of megaloblastic anemia in a tertiary care hospital in Lahore.

Methods: A cross-sectional analysis was conducted on 85 patients over a period of four months. Ethical approval was secured in line with the Declaration of Helsinki. Participants included both genders with hemoglobin levels <10 g/dl or mean corpuscular volume >95 fL, excluding pregnant women and individuals with primary diseases such as hepatic, renal, or cancerous conditions. Verbal consent was obtained before blood sample collection. CBC parameters were analyzed using a Mindray hematology analyzer, and vitamin B12 levels were measured via ELISA. Statistical evaluation was performed using SPSS version 25.0.

Results: The mean hemoglobin levels for males and females were 10.06 ± 3.10 g/dl and 9.61 ± 3.25 g/dl, respectively. The mean MCV for males was 99.94 ± 6.63 fL, and for females, it was 100.25 ± 7.90 fL. Vitamin B12 levels averaged at 489.92 ± 468.10 pg/ml for males and 440.19 ± 419.47 pg/ml for females. Of the patients, 47.05% had normal B12 levels, 25.88% were deficient, and 27.05% had high levels of B12.

Conclusion: Vitamin B12 deficiency was prevalent among patients with megaloblastic anemia and was independent of gender or age. The findings suggest a strong need for enhanced nutritional awareness and screening practices to identify and treat this deficiency early in at-risk populations.

Keywords: Megaloblastic Anemia, Vitamin B12 Deficiency, Hematologic Disorders, Nutritional Screening, Hemoglobin Levels, Mean Corpuscular Volume, Cross-Sectional Analysis, Healthcare Implications.

INTRODUCTION

Megaloblastic anemia, recognized as a distinct clinical entity for nearly a century, traces its initial clinical description to Thomas Addison in 1849, who identified pernicious anemia as one of its primary causes (1). Research indicates a significant variation in the prevalence of megaloblastic anemia across Pakistan, ranging from 10% to 52% (2, 3), highlighting its status as a major public health concern, particularly in low- and middle-income countries (4). This form of anemia is primarily caused by a deficiency in folic acid and vitamin B12, leading to the production of abnormally large macrocytic red blood cells in the peripheral blood and oversized erythrocyte progenitor cells, known as megaloblasts, in the bone marrow (6). These deficiencies disrupt the maturation of hematopoietic cells due to impaired DNA synthesis, with cobalamin (vitamin B12) and folic acid playing critical roles in the biosynthesis of DNA (9).

The diagnosis of megaloblastic anemia hinges on recognizing the unique morphological features of blood cells caused by deficiencies in vitamin B12 and folate, vital for the synthesis of pyrimidine and purine, the building blocks of DNA. The hallmark of this condition includes large, oval-shaped red blood cells, oversized platelets, and hypersegmented neutrophils, which emerge due to the

disruption in DNA replication during cell division (11). Notably, while hemoglobin concentration assessments can suggest iron deficiency anemia, it is crucial to acknowledge that anemia can also stem from deficiencies in vitamin B12 and folate, not merely iron (3). With iron deficiency affecting approximately 2 billion individuals globally, it stands as the most common nutrient deficiency. However, vitamin B12's unique synthesis by specific archaea and bacteria and its unavailability for absorption due to its production in the colon highlight the complexity of addressing these nutrient deficiencies (7, 8).

The clinical manifestation of megaloblastic anemia is diverse, ranging from tachycardia, fatigue, and shortness of breath to more severe neurological impairments such as difficulty walking, mood swings, and in advanced cases, memory loss (17). These symptoms, coupled with gastrointestinal issues like bleeding gums, weight loss, and disturbed stomach, underscore the systemic impact of this condition (17). The underlying cause, a deficiency in B12 and folate, not only hampers DNA synthesis, particularly affecting erythropoiesis, but also leads to congenital abnormalities, bone marrow failure, and adult demyelinating nervous system disorders if left untreated (13).

The importance of a clinical examination cannot be overstated, as a high index of suspicion may offer crucial clues for diagnosing macrocytic anemia, particularly when cobalamin or folate deficiency is suspected (16). The distinct cytomorphology observed in the blood and bone marrow, characterized by large and immature nuclei encased by mature cytoplasm, results in unequal erythropoiesis, intramedullary hemolysis, and cell death, further complicating the diagnostic landscape (15).

In light of the significant morbidity and mortality associated with anemia, which diminishes the red blood cells' capacity to transport oxygen, this cross-sectional study aims to meticulously evaluate the prevalence and impact of vitamin B12 deficiency in megaloblastic anemia patients in a tertiary care hospital in Lahore. Through a focused examination of the contributing factors, clinical manifestations, and potential interventions, this study seeks to contribute valuable insights into the management and treatment of this pervasive condition, emphasizing the critical role of dietary vitamins in reversing its course and preventing long-term complications.

MATERIAL AND METHODS

This study was conducted as a cross-sectional analysis, encompassing a sample of 85 patients selected based on specific inclusion criteria. The research took place over a four-month period, from April to July 2023, at the hematology laboratory of Riphah International University, Lahore, following the approval of ethical considerations in line with the Declaration of Helsinki for medical research involving human subjects. The inclusion criteria targeted both male and female patients exhibiting a hemoglobin level (Hb) of less than 10g/dl or a mean corpuscular volume (MCV) greater than 95fL, aiming to identify individuals potentially suffering from megaloblastic anemia. Exclusion criteria were stringently applied to ensure the removal of potential confounding variables, thereby excluding pregnant women and patients with primary conditions such as hepatic disease, cancer, or kidney disease.

Prior to sample collection, verbal consent was obtained from each participant, adhering to ethical guidelines that emphasize respect for the autonomy and dignity of participants. Blood samples were meticulously collected from the antecubital fossa, where a prominent vein was identified and the area sanitized with an alcohol swab. Utilizing a disposable syringe, 5 ml of blood was drawn from each participant and secured in EDTA vials to prevent coagulation, ensuring the integrity of the samples for subsequent analysis.

The study employed a Mindray hematology analyzer for the complete blood count (CBC) parameters, while serum vitamin B12 levels were determined using the Enzyme-Linked Immunosorbent Assay (ELISA) method, recognized for its specificity and sensitivity in detecting micronutrient deficiencies. This comprehensive approach facilitated a detailed examination of the hematological landscape of the participants, aiding in the identification and characterization of megaloblastic anemia.

Data collection was thorough, with all relevant information systematically recorded to facilitate a rigorous statistical analysis. The analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software, version 25.0, enabling a robust examination of the collected data through descriptive and inferential statistics. This analytical process aimed to uncover significant patterns and relationships within the data, contributing to a deeper understanding of vitamin B12 deficiency and its role in megaloblastic anemia among the study population.

The study's methodological rigor, from the ethical approval to the detailed procedures of sample collection and analysis, underscores its commitment to generating reliable and ethically sound scientific knowledge. Through its adherence to established protocols and ethical guidelines, the research provides valuable insights into the prevalence and characteristics of megaloblastic anemia, offering a foundation for future interventions and studies in this critical area of public health.

RESULTS

In the study, an analysis of mean values of complete blood count (CBC) parameters and Vitamin B12 levels in anemic patients revealed gender-based differences. Among the subjects, 41 males (48.23%) and 44 females (51.76%) were evaluated, with the P value indicating no statistically significant difference in gender distribution (Table 1). The mean hemoglobin (Hb) concentration for males was 10.06 g/dl with a standard deviation (SD) of 3.10, while for females, it was slightly lower at 9.61 g/dl (SD 3.25), although this difference was not statistically significant (P=0.51). The mean corpuscular volume (MCV) showed an almost identical mean for both genders, with males at 99.94 fL (SD 6.63) and females at 100.25 fL (SD 7.90), resulting in a P value of 0.84, which does not suggest a significant gender disparity.

Further, the mean corpuscular hemoglobin (MCH) values were closely aligned between males and females, with means of 31.06 pg (SD 3.89) and 31.33 pg (SD 5.78) respectively, and a P value of 0.79. Mean corpuscular hemoglobin concentration (MCHC) also did not differ significantly between males (33.60 g/dl, SD 2.96) and females (33.54 g/dl, SD 2.31), with a P value of 0.91 (Table 1). Vitamin B12 levels also followed this trend, with males having a mean level of 489.92 pg/ml (SD 468.10) and females having a mean level of 440.19 pg/ml (SD 419.47), yielding a P value of 0.64, which again indicates no significant gender difference in Vitamin B12 levels among the anemic patients studied.

Table 1: Association of Mean Values of CBC Parameters and Vitamin B12 Levels in Anemic Patients by Gender

Parameter	Male Mean ± SD	Female Mean ± SD	P value
Gender	41 (48.23%)	44 (51.76%)	0.57
Hb (g/dl)	10.06 ± 3.10	9.61 ± 3.25	0.51
MCV (fl)	99.94 ± 6.63	100.25 ± 7.90	0.84
MCH (pg)	31.06 ± 3.89	31.33 ± 5.78	0.79
MCHC (g/dl)	33.60 ± 2.96	33.54 ± 2.31	0.91
Vit B12 (pg/ml)	489.92 ± 468.10	440.19 ± 419.47	0.64
Note: Hb = Hemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, SD = Standard Deviation.			

Table 2: Distribution and Frequency of Vitamin B12 Levels in Study Subjects (n=85)

Vitamin B12 Levels	Mean (pg/ml) ± SD	Number of Patients	Percentage (%)
Normal B12 Level (187-883pg/ml)	411.07 ± 202.2	40	47.05
B12 Deficiency (<187pg/ml)	128.54 ± 41.55	22	25.88
High Level of B12 (>883pg/ml)	1420.91 ± 354.62	23	27.05
The classification of Vitamin B12 levels is based on the serum B12 concentration, with normal levels defined as 187-883 pg/ml, deficiency as <187 pg/ml, and high levels as >883 pg/ml. SD = Standard Deviation.			

The distribution and frequency of Vitamin B12 levels within the study subjects were further categorized (Table 2). Out of the 85 patients, 40 (47.05%) had normal Vitamin B12 levels, averaging at 411.07 pg/ml (SD 202.2), with normal levels defined as falling between 187-883 pg/ml. A deficiency in Vitamin B12 was observed in 22 patients (25.88%), with a mean level of 128.54 pg/ml (SD 41.55), categorized as less than 187 pg/ml. On the higher end of the spectrum, 23 patients (27.05%) exhibited high levels of Vitamin B12, averaging 1420.91 pg/ml (SD 354.62), defined as levels exceeding 883 pg/ml. These findings provide insight into the Vitamin B12 status of anemic patients, indicating that a substantial proportion of the study population either falls below or exceeds the normal range of Vitamin B12 levels.
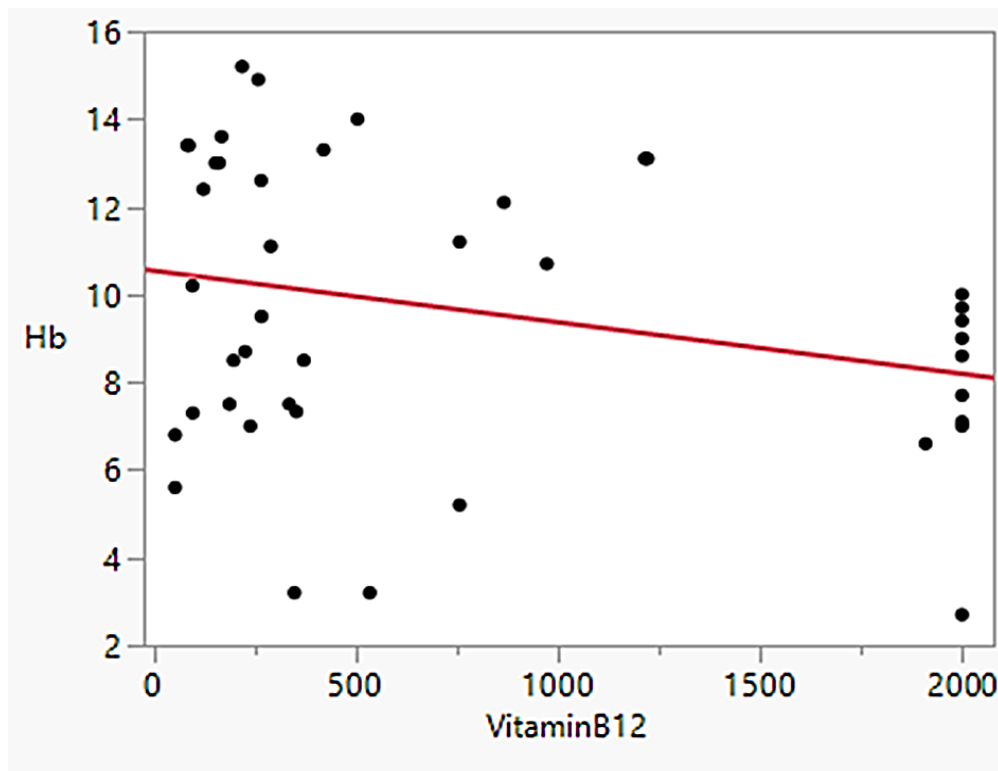
Figure 1 Vitamin B12 levels.

The scatter plot displays a collection of data points representing the relationship between Vitamin B12 levels (ranging from 0 to 2000 pg/ml on the x-axis) and haemoglobin (Hb) concentrations (spanning from 2 to 16 g/dl on the y-axis) in patients. The red line indicates a trend, suggesting a slight negative correlation between Vitamin B12 levels and Hb concentrations. As Vitamin B12 levels increase, there is a subtle decrease in Hb concentrations, although the dispersion of data points implies considerable variability and potentially weak correlation. The majority of data points cluster towards the lower end of the Vitamin B12 scale, with several outliers present at higher Vitamin B12 values.

DISCUSSION

The analysis of data within this research framework has illuminated the critical role of dietary patterns in the prevalence of megaloblastic anaemia, with non-vegetarian subjects presenting significantly lower serum folic acid and haemoglobin levels in comparison to lactovegetarians (20). These findings are pivotal, as they not only reiterate the importance of diet in managing anaemia but also suggest that the inclusion of animal products may be correlated with higher levels of these essential nutrients. The investigation into the prevalence of vitamin B12 and folic acid deficiencies yielded a notable prevalence of neuropsychiatric symptoms among the patients, with depressive symptoms emerging as the most common, signifying the potential neuropsychiatric implications of such deficiencies (21).

Contrasting trends were observed in different age groups; megaloblastic anaemia predominantly afflicted men, with a notable absence of cases reported between the ages of 21-70 years. In stark contrast, a significant proportion of the older population, 71-80 years, were diagnosed with the condition (22). This age-specific distribution of megaloblastic anaemia was further underscored by Rehman S.'s findings from Khyber Teaching Hospital in Peshawar, where vitamin B12 deficiency was prevalent across all age categories above 30 years (23). The interplay between obesity and anaemia was examined in a cohort of morbidly obese Iranian individuals, revealing no direct correlation between iron-deficiency anaemia and age or BMI; however, a substantial 20.9% were found to have vitamin B12 deficiencies (24).

Paediatric data from the District Headquarter Teaching Hospital in Dera Ismail Khan indicated a high incidence of megaloblastic anaemia in children below the age of 12, with a mean age of 9.9 years (25). The gender distribution in our study was equitable among those with normal vitamin B12 levels, as well as among those with deficiencies and high levels of this nutrient. This uniformity across genders suggests that gender may not be a significant variable in the prevalence of vitamin B12 deficiency within the population studied.

The implications of these findings underscore the prevalence of vitamin B12 deficiency as a primary cause of megaloblastic anaemia, transcending the barriers of gender and age. The study's strength lies in its comprehensive analysis of the association between vitamin B12 levels and haematological parameters, while its limitation stems from the absence of longitudinal data to establish causality. Future recommendations include expanding the research scope to include longitudinal studies to better understand the dynamics of vitamin B12 levels over time.

Furthermore, the study suggests that the level of haemoglobin is directly influenced by the level of vitamin B12, positing a potential avenue for intervention in the management of megaloblastic anaemia. To augment the robustness of these findings, it is recommended that subsequent research should explore the impact of vitamin B12 supplementation on haemoglobin levels, considering the influence of dietary patterns and the bioavailability of nutrients. Such research could provide a more nuanced understanding of the etiological factors and offer a strategic basis for dietary recommendations to mitigate the risk of anaemia, particularly in populations with limited access to animal-derived food products.

CONCLUSION

The conclusion of this study highlights that vitamin B12 deficiency is a significant contributor to megaloblastic anaemia, irrespective of gender and age differences within the population. This underscores the importance of vitamin B12 in maintaining healthy haematological function and suggests a potential area of intervention for healthcare providers. It becomes imperative for healthcare systems to focus on the nutritional education of populations at risk, emphasizing the importance of balanced diets rich in vitamin B12, especially for vegetarians and older adults. Additionally, regular screening for vitamin B12 levels could become a standard practice in the early diagnosis and prevention of megaloblastic anaemia, ultimately reducing the healthcare burden associated with this condition and improving patient outcomes through timely and effective treatment strategies.

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