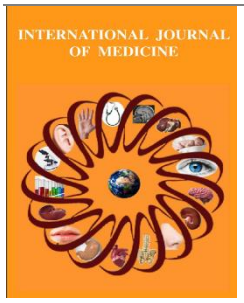
International Journal of Medicine 2026; 8(1): 25-29



International Journal of Medicine

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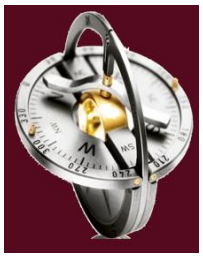

Research Article

Clinical & haematological profile of Vitamin B12 deficiency in rural population of Northwestern Rajasthan: a cross-sectional study

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Abstract

Introduction: Vitamin B12 deficiency is a common health concern around the globe. There are limited data on clinical profile of vitamin B12 in Indian rural population. Aim: to study the clinical profile of vitamin B12 deficiency, its relation to various hematological parameters and co morbid conditions. ***Methods***: This was a cross sectional study conducted in 427 participants selected by stratified random sampling from rural population of Bikaner from December 2023 to May 2024. Various data were collected by interviewing the participants with a predesigned questionnaire and 3 ml venous blood samples were collected and analyzed for serum B12 level and for hematological parameters. Statistical analysis was performed by using SPSS Statistical Software version 21.0. Level of statistical significance were taken as p value <0.05. ***Results***: In this study total participants were 427 (M: F ratio 3:4) and among them 32.08% participants had vitamin B12 deficiency. Statistically significant correlation of symptoms like abnormal sensation, irritability, numbness, myalgia, decreased vision, fatigability, and imbalance was found vitamin B12 deficiency. Out of 137 participants with vitamin B12 deficiency 65.69% participants had anemia, 1.03% had severe anemia, and 31.39% participants had thrombocytopenia. 4.37% each had leucopenia and pancytopenia. We found a significant correlation of anemia, mean hemoglobin level ($p<0.05$), mean MCHC ($p<0.05$) with vitamin B12 deficiency. ***Conclusion***: This study provides critical data on vitamin B12 deficiency status and its clinical profile and relation to various hematological parameters, which will help in developing effective prevention and early management strategies for the Indian population.

Keywords: Vitamin B12 deficiency, Rural, Anemia, India.

Introduction:

Vitamin B12 is a water soluble micronutrient, essential for the normal functioning of nervous and haematological system of our body.1 It is produced naturally by microorganisms, and the main dietary sources are of animal origin.2 Vitamin B12 has the most complex and largest chemical structure among all vitamins; adenosylcobalamin and methylcobalamin are biologically active forms. Hydroxycobalamin and cyanocobalamin are two other forms that may be metabolized into either methylcobalamin or adenosylcobalamin and play a functional roles in the human cells. Vitamin B12 is used in the transfer of methyl group in a methionine synthase requiring reaction, which converts homocysteine to methionine. This reaction activates folate, which is needed for DNA synthesis of red blood cells (RBC's), which helps in the maturation of nucleus.1 Vitamin B12 is essential for fatty acid and amino acid metabolisms and it also plays a significant role in the synthesis of neurotransmitters, phospholipids and methylation of myelin.3 Diagnostic criteria for vitamin B12 deficiency include a serum cobalamin level < 200 picogram/mililiter (148 picomol/liter) in the presence of signs and symptoms and/or haematological indices of vitamin B12 deficiency.4 Common causes of vitamin B12 deficiency are low intake, food bound cobalamin malabsorption (FBCM), autoimmune and in various conditions causing malabsorption. Pregnancy and drugs like metformin, proton pump inhibitors, oral contraceptives, hormone replacement therapy also leads to vitamin B12 deficiency.1 Vitamin B12 is stored in the liver in a sufficient amounts, development of symptoms of deficiency usually takes five or more years. Deficiency of vitamin B12 is characterized by haematological, neurological and neuropsychiatric features, ranging from milder manifestations like fatigue, paraesthesia, to severe manifestations like pancytopenia and subacute combined degeneration of spinal cord. Usually symptoms develop early before a decrease in serum vitamin B12 below the reference value. The patient may have shortness of breath, pallor, dizziness, tinnitus, fatigability, lethargy and palpitation. Since the underlying pathologic process consists of demyelination of peripheral nerves, the spinal cord and the cerebrum, the signs and symptoms include numbness, paraesthesia, weakness, ataxia, poor finger coordination, diminished reflexes and loss of vision. Participants may come up with severe mental problems like depression, memory loss, irritability, behavioral changes, panic attacks and insomnia. Untreated vitamin B12 deficiency can cause pulmonary embolism, deep vein thrombosis, suppression of immune system and bone marrow failure.5 The prevalence of vitamin B12 Deficiency in north Indian population is 47 %.6 Data documenting vitamin B12 status in India in general population is limited. Documentation of more data from different parts of country may spark well informed debate on replacement strategies and also on food fortification. With this in mindset, we had conducted a cross-sectional study on population of rural part of north western Rajasthan, India, to know the clinical profile and hematological parameters correlation with vitamin B12 deficiency among them.

Aim: to study the clinical profile of vitamin B12 deficiency, its relation to various hematological parameters and co morbid conditions..

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DOI: 10.61336/im/26-1-07
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METHODOLOGY

This study was conducted on rural population of Bikaner district in Rajasthan. Research population was divided into different strata geographically and then sample population taken by grid sampling from 6 villages (i.e. Bholasar, Bikkampur, Diatra, Kakda, Panchu and Uttamdesar) selected randomly from 3 tehsils selected randomly. It was a cross sectional study conducted within 6 months between December 2023 and May 2024 and samples collected by stratified random sampling. Calculated sample size was 421 but we had tested some extra samples in view of inadequacy or spillage of blood samples during the processing and we got results of 427 samples, which we had included in our study. We had excluded person with age <14 years, person not willing to participate in the study and subject residing in selected rural area of north western Rajasthan since less than 6 months.

Sample Collection:

We went to a total of 603 people and asked them to participate in the study among them 548 given consent for participation in the study and only 481 participants had given consent for blood sample collection, among them 457 samples were sent to laboratory and rest were found clotted and broken vials during transportation, out of them 427 samples were tested in laboratory and rest were having inadequate sample volume for testing, so we had included data of these 427 samples in our study. All participants were interviewed with a pre-designed questionnaire including age, sex, clinical symptoms, any concomitant illness history etc. The questionnaires were completed with the assistance of a trained person, in the local language. 3 ml venous blood samples were drawn from peripheral vein with aseptic precaution from every subject after obtaining an informed verbal and written consent and transferred from collection site to testing site in cold storage box for biochemical assessment of serum vitamin B12 level (reference range 120-914 pg/ml) by using UniCelDxl 800, Access Immunoassay System S/N 607846, Version 5.3.1 in Immunoassay laboratory, and complete blood counts by automated hematology analyzer in, S.P. Medical College, Bikaner.

RESULT

In our study total 427 participants had participated with a male female ratio of 3:4. Mean age of the study participants was 36.86+ 17.42 years with an age range of 14 to 86 years.

Table-1 - Distribution of severity of vitamin B12 deficiency

Severity of vitamin B12 deficiency (pg/ml)	Male (n=187)		Female (n=240)		Total (n= 427)	
	No.	%	No.	%	No.	%
Border line deficiency (200–299)	31	16.58	49	20.42	80	18.73
Mild deficiency (150 –199)	4	2.14	6	2.5	10	2.34
Moderate deficiency (100–149)	11	5.88	14	5.83	25	5.85
Severe deficiency (<100)	6	3.21	16	6.67	22	5.15
Total Deficient	52	27.81	85	35.42	137	32.08

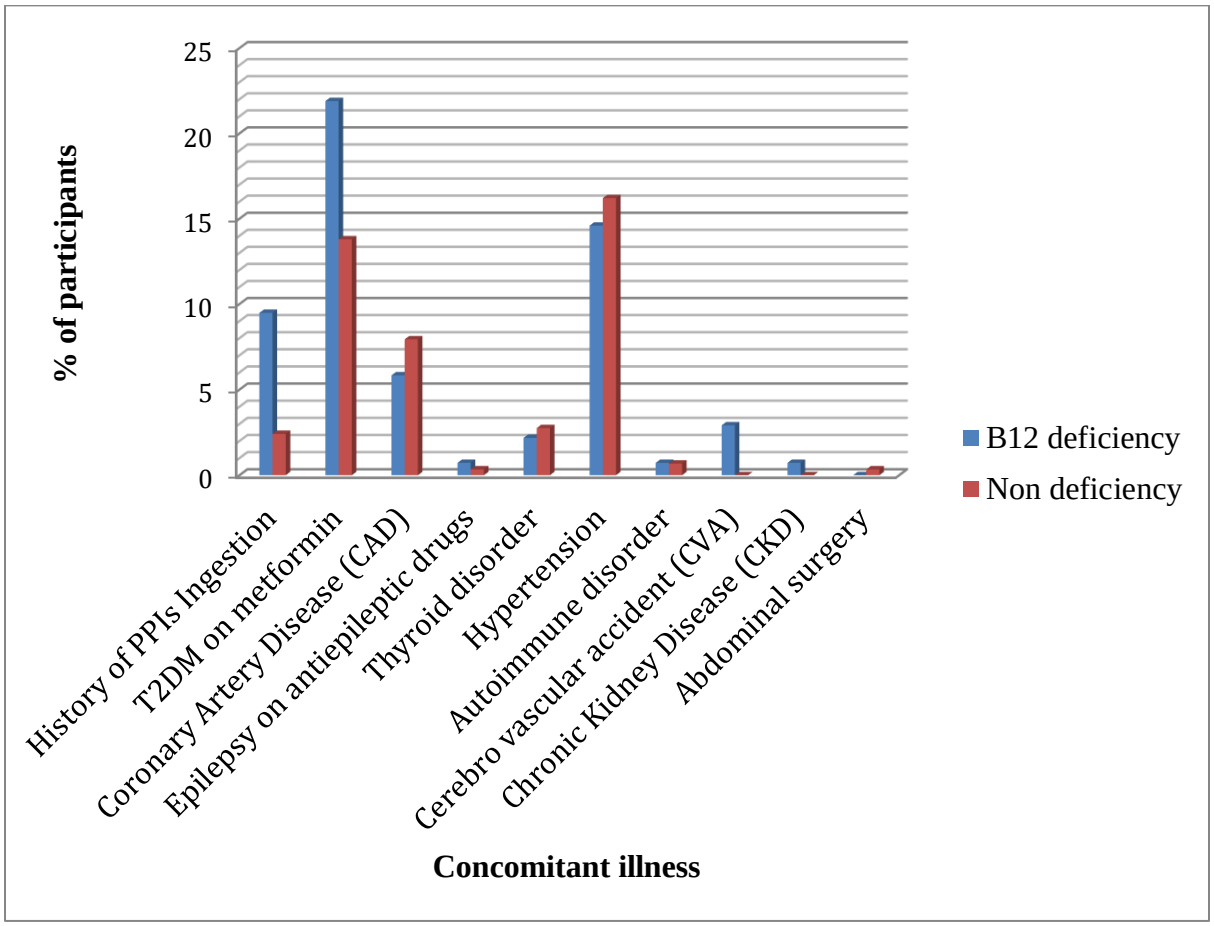
In this study we observed that out of 427 participants, 137 participants (32.08%) had vitamin B12 deficiency with different levels of severity as shown below.

Table 2 – Correlation of Clinical symptoms with vitamin B12 deficiency and non deficiency participants

Clinical symptoms	B12 deficiency (n=137)	Non deficiency (n=290)	p value
Fatigability	62 (45.25%)	96 (33.10%)	0.01
Myalgia	60 (43.79%)	77 (27.24%)	0.003
Numbness	56 (40.87%)	79 (27.24%)	0.004
Back pain	45 (32.84%)	79 (27.24%)	0.23
Muscle Cramps	44 (32.12%)	97 (33.44%)	0.78
Abnormal Sensation	27 (17.70%)	30 (10.34%)	0.007
Decreased Vision	21 (15.32%)	24 (8.27%)	0.02
Imbalance	20 (14.59%)	27 (9.31%)	0.05
Irritability	19 (13.86%)	17 (5.86%)	0.005
Decreased Sleep	16 (11.67%)	28 (9.65%)	0.52
Breathlessness	13 (9.48%)	32 (11.03%)	0.62
Decreased Memory	11 (8.02%)	11 (3.79%)	0.06
Weight Loss	7 (5.11%)	0 (0%)	–
Palpitation	6 (4.37%)	10 (3.44%)	0.63
Ringing Sensation in Ear	3 (2.18%)	4 (1.37%)	0.53

Mean vitamin B12 level was 199.08 + 88.09 pg/ml in B12 deficient. In this study we observed that in vitamin B12 deficient population most common symptom was fatigability followed by myalgia, numbness, back pain and muscle cramps. We observed a statistically significant correlation of many symptoms like abnormal sensation, irritability, numbness, myalgia, decreased vision, fatigability, and imbalance with vitamin B12 deficiency.

Fig. 1 – Correlation between participants with vitamin B12 deficiency and non–deficiency on the basis of other concomitant illness



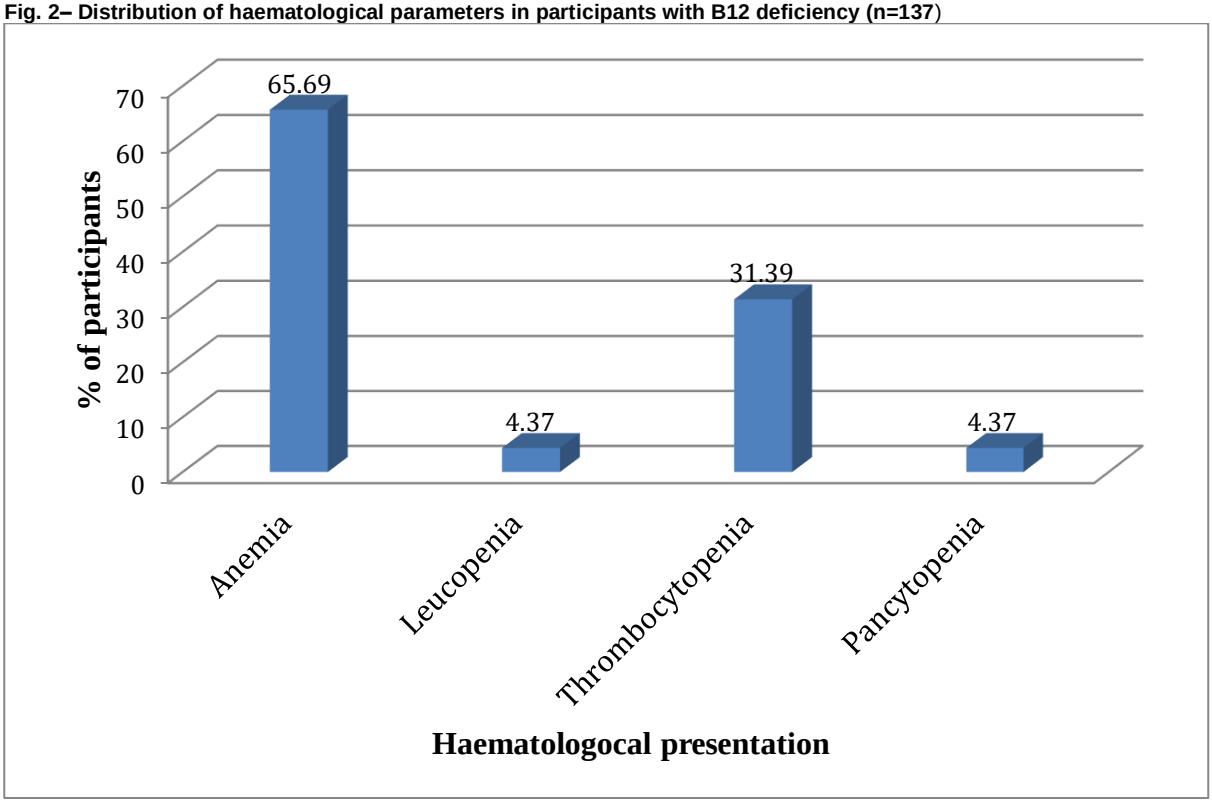
We observed in this study that among participants with vitamin B12 deficiency, 21.89% participants had type 2 diabetes mellitus and all are taking metformin.

Table 3 – Distribution of anaemia in vitamin B12 deficiency and non–deficiency participants

	B12 deficiency (n=137)		Non deficiency (n=290)		p value
Hb (gm%)	No.	%	No.	%	
Hb >12	47	34.31	172	59.41	0.0001
Hb <12	90	65.69	118	40.69	
Hb <7	3	1.03	–	–	

Statistically significant correlation was observed in participants having acid peptic disease on PPIs and type 2 diabetes mellitus on metformin with vitamin B12 deficiency. We reported in our study that out of 137 participants with vitamin B12 deficiency 90 participants had anaemia, 3 had severe anaemia.

Fig. 2– Distribution of haematological parameters in participants with B12 deficiency (n=137)



We found a statistically significant correlation of anaemia with vitamin B12 deficiency. Comparative analysis of mean hemoglobin value and different RBC indices were done. We found a significant correlation of mean haemoglobin level, mean MCHC with vitamin B12 level. There was no significant correlation of mean MCV and MCH value with B12 deficiency.

Table 4: Correlation of haemoglobin, RBC Indices and vitamin B12 assay in B12 deficient and non-deficient group

Haematological Parameters (Mean + SD)	B12 deficiency (n=137)	Non Deficiency (n=290)	P-value
Haemoglobin (gm%)	11.61 +4.06	12.17 + 1.85	0.05
MCV (femtoliter)	72.59 + 11.67	73.05 + 8.44	0.64
MCH (picogram/cell)	24.39 + 8.08	23.84 + 5.67	0.41
MCHC (gm/dl)	31.34 + 1.87	31.64 +1.26	0.05
Vitamin B12 level (pg/ml)	199.08 + 88.09	494.01 + 141.74	0.0001

In this study we showed a significant correlation of mean haemoglobin level and mean vitamin B12 level with B12 deficiency with anaemia.

Table 5– Correlation of different parameters with anaemia in vitamin B12 deficient and non-deficient participants

Haematological Parameters (Mean + SD)	B12 deficiency with anaemia(n=90) Mean +S.D	Non deficiency with anaemia (n= 118) Mean +S.D	P. value
Haemoglobin (gm %)	10.02 + 1.51	10.43 + 1.27	0.003
MCV(femtoliter)	70.68 + 13.47	70.12 + 10.64	0.64
MCH (picogram/cell)	24.53 +9.86	23.82 + 8.57	0.57
MCHC (gm/dl)	31.21 + 2.24	31.44 + 1.79	0.41
Vitamin B12 level (pg/ml)	207.24 + 83.64	480.80 + 136.97	0.0001

There was no significant correlation of mean MCV, MCH, and MCHC value between B12 deficient with anaemia

DISCUSSION

After getting laboratory results we analyzed all our data and compared the results of our study with previously conducted studies, with a special emphasis on comparison with other studies conducted in India. In our study female participants were more as compared to male, possible reason behind this may be because, most of the male population in rural area go out from home for work on daily basis, in our study we had visited house to house to take samples, so less male population were participated. In this study we had observed that 32.08% participants had vitamin B12 deficiency. In this study we had taken a cut-off of 299pg/ml to include most of the possible deficiency population. Similar observation was made by Yao, Y et al on 100 consecutive, unselected geriatric out patients of a primary care setting in Ulster County, New York, they found that 16% of the participants had serum cobalamin levels of 200 pg/ml or below, and 21% had levels between 201 and 299 pg/ml, which was 37% total but they had taken only geriatric outpatients but our study had data on whole population with age range of 14 to 86 years.7 Meena S et al also reported that prevalence of vitamin B12 deficiency was 19.52%, which was also consistent with our observation difference was due to different cut off for deficiency.8 In 2019 Singh G et al also found overall prevalence of vitamin B12 deficiency in urban northern Indian population was 45%, which was higher as compared to our study. In present study 27.81% males and 35.42% females was vitamin B12 deficient.9 According to gender distribution deficiency was more among females in our study as compared to males because female participants were more and also we can correlate with dietary habits of females in rural area, most of the females were vegetarian by diet contributing to their deficiency status. Our results were in contrast to study done by Meena S et al in which they reported that prevalence of vitamin B12 deficiency was more in males (18.33%) as compared to females (15.83%) but data was statistically not significant.8 Sharma P et al in 2018 also found that vitamin B12 deficiency was higher in males i.e. 29.6% as compared to females i.e. 22.2%, and deficiency was much higher in urban males than urban females as compared to rural males and females with a significant difference and p value of 0.05.10 Vitamin B12 deficiency manifests with a wide variety of symptoms ranging from mild fatigue to severe neuropsychiatric symptoms. Concordant with other similar studies, in our study vitamin B12 deficient participants had most common constitutional symptom as fatigability followed by myalgia and neurological symptoms like numbness, back pain, muscle cramps, abnormal sensation, imbalance, decreased sleep, decreased vision, irritability, decreased memory which was consistent with findings of other similar studies. In 2018 Sharma P et al also reported that majority of participants (78.9%) had constitutional symptoms like weakness, fatigue, anorexia, weight loss, while gastrointestinal symptoms were present in 53.33% participants. Maximum deficiency was seen in females presenting with late neurological deficit like dementia or seizures (42.8%) as compared to males but the difference was not statistically significant (p=0.405) followed by males (42.3%) presenting with early neurological deficit like tingling and loss of sensation with a significant statistical difference than female (p=0.007).10 Aaron, S et al reported in their hospital-based study on patients admitted with vitamin B12 deficiency-related neurological disorders. Most common neurological manifestation was myeloneuropathy (54%), followed by myeloneuropathy with cognitive dysfunction (34%), and peripheral neuropathy (9%). They also reported neuropsychiatric manifestations in 38% and dementia in 19% of patients.11 Issac TG et al done a study studied neuropsychiatric symptoms on patients with vitamin B12 deficiency and found that out of 259 patients with vitamin B12 deficiency (<220 pmol/L), 60 had neuropsychiatric symptoms. They identified different types of dementias, psychiatric disorders, parkinson's disease and alcohol dependent syndromes among participants. Behavioural disturbances was the first symptom of presentation found in 30 (50%) patients particularly in elderly followed by memory loss in 20 (33.9%), and sensorimotor and movement disorders in 9 (15.3%). Memory loss was present in 16 (84.2%) patients of severe vitamin B12 deficiency.12 Overall; these studies highlight the varied neurological and psychiatric presentations of vitamin B12 deficiency, emphasizing the importance of timely diagnosis and treatment, particularly in vulnerable populations like the elderly. In this study most common concomitant illness associated with B12 deficiency was type 2 diabetes mellitus and all of them were on metformin other positive association was found with acid peptic disease taking proton pump inhibitors. 21.89% participants had type 2 diabetes mellitus and all were taking metformin, other diseases were not significantly linked to vitamin B12 deficiency. Similar observations was made by Ramesh V et al In the year 2020 that among the 200 vitamin B12 deficiency participants, the presence of hypertension (15%), peptic ulcer (7.5%), autoimmune diseases (2.5%), abdominal surgery (17.5%), ileal disorder (2.5%) and drug intake (10%) would had reduced the vitamin B12 level in both vegetarian and non vegetarian population.13 Similar interpretation was made by Hasan, Najam Ul et al in a study on 72 patients of diabetes mellitus, that mean vitamin B12 level was 360±185.2 pg/ml in patients taking metformin while among non-metformin group mean vitamin B12 level was 619±176 pg/ml (p value=0.0001). They concluded that long term (>2 years) use of metformin is significantly associated with vitamin B12 deficiency.14 We reported in our study that most of the patients with B12 deficiency had anaemia and other hematologic abnormality like thromobocytopenia, leucopenia, and pancytopenia. Severity of anaemia was also associated with severity of vitamin B12 deficiency. According to our study RBC indices are not much significant indicator of B12 deficiency because concomitant presence of other causes like iron deficiency and other micronutrient deficiency may alter these parameters so their use may not be much helpful. Meena S et al showed that prevalence of vitamin B12 deficiency in participants with pancytopenia and without pancytopenia was 24.16% and

10.00% respectively, showing chance of high vitamin B12 deficiency in patients with pancytopenia which is similar to our results.8 Yajnik C S et al also found in their study found that low vitamin B12 concentration was related to lower blood haemoglobin concentration and higher MCV, but macrocytic anaemia was rare.15 Jain, R. et al in 2012 retrospectively analyzed the records of serum vitamin B12 and MCV of both inpatients and outpatients over a period of one year, to identify the correlation between vitamin B12 level and MCV, they concluded that there was no correlation between vitamin B12 levels and MCV in majority of the cases.16 MCV should not be the only criteria for ordering vitamin B12 for patients with anaemia under evaluation. Our study results were also consistent with these studies.

Limitations:
In this study we had not excluded people who might have already taken supplements containing vitamin B12. Same limitation was faced by other researchers worldwide. Clinical profile and hematological profile may vary because of concurrent presence of other factors like dietary deficiency of iron or any other micronutrients.

Conclusion
This is one of the largest studies on vitamin B12 status in rural population of India. Study data were taken from general population of rural part of north western Rajasthan; which was likely to be representative of north Indian population at large. We had found 32.08% prevalence of vitamin B12 deficiency in our study. This study also provides critical data on clinical features, hematological profile and factors associated with vitamin B12 deficiency will aid in the development of effective prevention, and management techniques tailored to the Indian population. Preventive methods can be adopted to reduce the burden of vitamin B12 deficiency by food fortification for general population. Furthermore, health administrators might use this information for planning of health programs and resource distribution.

Acknowledgments & Conflict of interest: No conflict of interest.
Ethics:
This study was taken approval by the Institutional Ethics and Research Board of S.P. Medical College & PBM Hospital, Bikaner, vide letter no.- F.29.(Acad)SPMC/2022/4763.

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