

HAEMATOLOGICAL MANIFESTATIONS IN VITAMIN B₁₂ DEFICIENCY CASES- SINGLE CENTRE STUDY IN NORTH INDIA

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ABSTRACT

In this study vitamin B₁₂ deficiency is correlated with the haematological manifestations which are common in the human population of the northern parts of India. The importance of haematological manifestations in relation to vitamin B₁₂ deficiency is not recorded in the literature so far. Estimation of the clinical profile of vitamin B₁₂ deficiency in the human population of north region of India and to stabilized association/relationship between hematologic manifestations with vitamin B₁₂ deficiency. All symptomatic patients of pernicious anemia requiring blood transfusions and vitamin B₁₂ who had either raised their mean corpuscular volume (MCV) or bicytopenia/pancytopenia on complete blood count were symptomatic screened for vitamin B₁₂ deficiency. A total of 110 cases and 110 controls were diagnosed those have anemia records. Total 110 (50.0%) were males and 110 (50%) were females of all the age groups. The average mean age was found 35.5±9.7. Vitamin B₁₂ deficiency was recorded in 120 (70%) patients. This deficiency was severe in 40 (3.12%) cases and moderate Vitamin B₁₂ deficiency was recorded in 10 (12.5%) cases. In the present study vitamin B₁₂ deficiency has found a strong co-relation with the as pernicious anemia. This study has clear indications to evaluate to B₁₂ status in cases manifesting with such features especially in cases.

KEYWORDS: Pernicious Anemia, Vitamin B₁₂ Deficiency, North Indian Population

Deficiency of vitamin B₁₂ has been an acknowledged clinical aspect since long time (Kumar *et al.*, 1989). In megaloblastic anemia may be due to unusual maturation of hematopoietic cells caused by damaged DNA synthesis. Two vitamins, cobalamin (vitamin B₁₂) and folic acid are essential for DNA biosynthesis. Another serious disease is also known as pernicious anaemia due to vitamin B₁₂ deficiency (John *et al.*, 2009). The traditional manifestations of B₁₂ deficiency was first acknowledged in pernicious anaemia, the exact cause was not known that time. Pernicious anaemia initially acquired its suitable environment and ultimately may be fatal with haematological manifestations of the disease mainly due to vitamin B₁₂ deficiency (Iqbal *et al.*, 2009). Deficiency of vitamin B₁₂ is also responsible for the malfunctioning in the maturation of the nucleus and cytoplasm of hastily regenerating cells. Inside hematopoietic system, this abnormality causes irregular nuclear maturation with normal cytoplasmic maturation (Linker and Damon, 2012). Such type of irregularities apoptosis, ineffective erythropoiesis, intramedullary haemolysis, pancytopenia and typical morphological abnormalities in the blood and marrow cells may be common (Khanduri *et al.*, 2005).

In the reported studies the effects of B₁₂ deficiency are mainly recorded in the blood and nervous system. The biochemical evidence of vitamin B₁₂ deficiency in the structure of increased blood and tissue levels of MMA and homocysteine and waning levels of the portion of vitamin B₁₂ leap to trans-cobalamin

preceded the manifestation of any deficiency. This condition has also been known to as subclinical vitamin B₁₂ deficiency (Tanner *et al.*, 2003).

In the northern region of India the common causes of anemia is due to vitamin B₁₂ deficiency and congenital hemolytic anemia. Severe anemic patients are requiring regular blood transfusions under the supervision of expert health workers. Many studies have been mentioned that vitamin B₁₂ deficiency could be more widespread in this region than anticipated (Tanner and Liz Bisson, 2004). This provoked us to carry out a scientific study in this area. We perform a case study between April 2018 to May 2019 and documented the prevalence of vitamin B₁₂ deficiency with anemia in this hospital.

The aim of this study was to resolve the actual relationship between the vitamin B₁₂ deficiency and the manifestation of haematological symptoms along with other related factors.

MATERIALS AND METHODS

In this case single centre study over a period of 11 months (April 2018 to May 2019) at a tertiary care hospital of government institute of medical sciences, Greater Noida, U.P. (India). Before the collection of samples from patient's a written consent form was taken. The patients of anemia were managed over a period of 11 months. In these study pregnant and lactating women, children, adults and olds were considered randomly. The

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patients who are already on Vit B₁₂ and folic acid substitution were excluded from study. Hemogram was estimated using 2 ml of ethylene diamine tetraacetic acid (EDTA) anti-coagulated blood in programmed Beckman Coulter 500 FC series analyzer. Anemia was considered as hemoglobin (Hb) < 13 g/dL in males and Hb < 12 g/dL in females, as per WHO recommendations of anemia. Harsh anemia was estimated as Hb < 8 g/dL. The patients of anemia with mean corpuscular volume (MCV) > 100 fL were considered with peripheral blood smear (PBS) (Pappo *et al.*, 1992), bone marrow examinations, and estimation of Vit B₁₂ and folic acid levels. Peripheral blood smear reminiscent of MA was estimated by attendance of macro-ovalocytosis, anisocytosis, poikilocytosis, hyper-segmented neutrophils and pancytopenia. The diagnosis of MA was established with bone marrow aspiration studies where occurrence of hyper-cellular marrow with enlarged erythroid/myeloid ratio, megaloblasts, giant bands and metamyelocytes and reduced megakaryocytes support the diagnosis of MA. Vitamin B₁₂ and folic acid levels were calculated by the using chemi-luminescence approaches (Nizamani *et al.*, 2014). Normal levels of Vitamin B₁₂ and folic acid were estimated at 211-911 pg/ml and >5.38 ng/ml correspondingly as the protocol of WHO. Comprehensive dietary histories of all the patients were recorded. Diet record such as non vegetarian diet was considered as diet consisting mainly of animal food. Mixed diet was considered as food containing of both plant and animal source. Similarly, vegetarian diet was considered of food of plant origin and milk, while pure vegetarian diet was recorded as vegetarian diet (Premkumar *et al.*, 2012).

A prescribed questionnaire was developed to record the family history of eating of drugs including proton pump inhibitor was considered with along with surgery record. Alcohol intake and amount of alcohol intake was recorded. Any other diseases such as neurological symptoms, Liver function tests, Lactate dehydrogenase, Upper gastrointestinal endoscopy and biopsy from stomach and duodenum were recognized.

By using the statistical formula we have calculated 220 sample size. Total sample size was 110 cases and 110 were controls for this study.

Statistical Analysis

For the authentication and validation of our data we used SPSS version 16. Different proportions and qualitative variables were estimated using chi-square test. A P value of less than or equal to 0.05 was measured statistically significant (Aminoff *et al.*, 1999).

RESULTS

A total of 110 cases patients and 110 controls were diagnosed those have anemia records. Total 110 (50.0%) were males and 110 (50%) were females of all the age groups. The average mean age was found 35.5±9.7. Vitamin B₁₂ deficiency was recorded in 120 (70%) patients. This deficiency was severe in 50 (3.12%) cases and moderate Vitamin B₁₂ deficiency was recorded in 30 (12.5%) cases. The deficiency of Vitamin B₁₂ was recorded in 10 (29.18) cases. Normal Vitamin B₁₂ levels were found in around 10 (25.0%) individuals. The deficiency of vitamin B₁₂ was a serious concerned in the females of reproductive age group (18-40 years) constitute 80 % (Total pregnant women were studied, 109) of their category.

In this study red blood cell indices are grouped as per diverse groups of vitamin B₁₂ levels. Highest value of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were found 140 fl and 45.9pg respectively in the harshly in vitamin B₁₂ deficient group. In this study a strong and positive correlation was observed between vitamin B₁₂ deficiency and platelet count in the patients. Calculated P value of this correlation was recorded statistically significant (P<0.001). Vitamin B₁₂ deficiency and leukocyte counts (WBC) were also recorded significant positive correlations with P value ≤0.001. There was also a significant negative correlation was estimated between vitamin B₁₂ deficiency and Mean Corpuscular Volume (P value=0.020). Similarly a strong negative significant correlation was also observed between Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration. In some cases 208 (13%) pancytopenia was recorded with vitamin B₁₂ deficiency persons which was found statistically significant (P<0.001). In the peripheral blood film assessment macrocytes were pragmatic in 460 smears (28.75%) with hyper-segmented neutrophils in 516 smears (32.25%).

Table 1: Estimation of Vitamin B₁₂ levels (n = 220)

S.N.	Amount of Vitamin B ₁₂	Cases (N)	Mean	Standard Deviation
1.	Normal (> 240pg/ml)	60 (35 %)	710.25	585.94
2.	Borderline Vitamin B ₁₂ deficiency (170-240 pg/ml)	92 (11.2%)	195.29	25.83
3.	Vitamin B ₁₂ deficiency (< 170pg/ml)	40 (25.0 %)	205.37	29.90
4.	Severe Vitamin B ₁₂ (<100pg/ml)	28 (28.0 %)	238.08	26.07
	N=	220 (100%)		

Table 2: Estimation of Red Blood cell indices

S.N.	Amount of Vitamin B ₁₂	MCV	MCH	MCHC
1.	Normal (> 240pg/ml)	106.55± 9.04	33.10± 6.8	31.00± 4.3
2.	Borderline Vitamin B ₁₂ deficiency (170-240 pg/ml)	107.81± 10.01	34.1 ±6.0	29.98± 6.8
3.	Vitamin B ₁₂ deficiency (< 170pg/ml)	106.45± 6.8	33.0± 7.5	31.08 ±1.8
4.	Severe Vitamin B ₁₂ (<100pg/ml)	110.01 ±6.58	35.01 ±7.66	32.25 ±5.6
	N=	220 (100%)		

Table 3: Estimation of Pearson’s correlation of Vitamin B₁₂ deficiency (N=220)

S.N.	Parameter	MCV	MCH	MCHC	WBC	Platelets
1.	Correlation coefficient	-0.231	-0.185	-0.108	0.594	0.802
2.	P-value	0.017	0.056	0.263	<0.001	<0.001

DISCUSSION

In this study hematological aspects were correlated with vitamin B₁₂ deficiency clinically. It commonly presents haematologically as pernicious anaemia as disorders. In Northern part of India vitamin B₁₂ deficiency is an important cause of pernicious anaemia. In the present study the role of vitamin B₁₂ deficiency recorded in a significant level. In the literature it has stated that 49.5% of non-anaemic adult cases have either deficient level of vitamin B₁₂ alone or combined with folic acid deficiency (Nazeem and Ultra, 2007).

The data obtained in our study were mentioned in Table 1 showing that prevalence of vitamin B₁₂ deficiency to be 64.2%. Similar data were mentioned by Khanduri and Sharma (2007) (65%). Similarly, Ahmed *et al.*, (2012) has estimated vitamin B₁₂ deficiency in 72.6% in their study. With the support of this study Sarode *et al.*, (1989), Sen *et al.*, (2015) and Hashim and Tahir (2006) had also recorded the occurrences of B₁₂ deficiency to be 76% in their studies. A study conducted by Garewal *et al.*, (1972) has mentioned increased prevalence of B₁₂ deficiency 88% in the studies conducted at Delhi. On the

other hand some authors have mentioned that low prevalence (39.9%) and (36.5%) of vitamin B₁₂ deficiency such by Gilgit Agency of Pakistan, and Bhatia *et al.*, (2012) of Karnataka correspondingly. The variations of vitamin B₁₂ may be due to the dietary deficiency associated with various cultural and may be due to difference geographical regions. But in northern India preponderance of the population are vegetarians and mixed dietary habits. A population based study at West Bengal and Karachi (Summer *et al.*, 1996) have mentioned vitamin B₁₂ deficiency in 79 % and 85% of non-vegetarians correspondingly. Thus vitamin B₁₂ deficiency may also noticed in non-vegetarians too. There are some extra features may also responsible to create deficiency of B₁₂ are mal-absorptive states such as Tropical sprue, Giardiasis, gastrointestinal infections due to *H. pylori*, autoimmune gastritis, gastric surgeries, surgery beneath nitrous oxide anaesthesia, anti-convulsants, proton pump inhibitors etc (Jain *et al.*, 2012).

To avoid sex discrimination we have selected no of males and females in equal ratio in our study. There

are 800 males representing 50% of our study population. On some other studies females reported predominately and found that females are more prone to vitamin B₁₂ deficiency. Similarly, women in the child bearing group and lactating mothers groups (18-40 years) constitutes around 80% of vitamin B₁₂ deficiency. Similar data has been obtained in the present study whereas women 75 %. In the study conducted by Bhatia *et al.*, (2012). they reported low level of vitamin B₁₂ in the reproductive age group is a subject of apprehension as low maternal vitamin B₁₂ status is associated with increased risk for hematological defects. This may be due to intra uterine augmentation retardation and a cause of low birth weight. According to the WHO recommendation a serum B₁₂ value of 300pg/ml should be required before a woman get pregnant.

In the present study vitamin B₁₂ deficiency affects the cases mainly haematologic, gastrointestinal and nervous system. Major haematological manifestations were anaemia, leukopenia, thrombocytopenia, pancytopenia, macrocytosis and hyper-segmented neutrophils in peripheral smear and megaloblastosis in bone marrow which were recorded. Yuksel *et al.*, (2006) have found in their study that haematological disorders at the rate of 96% in an assessment of B₁₂ deficient groups. We have recorded vitamin B₁₂ and folate deficiency and high Mean corpuscular volume (MCV) values in erythrocyte indices in the cases. Wheeler *et al.*, (1977) have recommended that vitamin B₁₂ should be resolute in anaemic patients with MCV higher than 100 fl. Thus we have selected inclusion criteria $MCV \geq 96$ fl for cases in our study. Erythrocyte indices were grouped based to levels of vitamin B₁₂ and highest mean values of MCV and MCH were experimental in the cases (vitamin B₁₂ \leq 100pg/ml). A statistical significant negative correlation of MCV with vitamin B₁₂ levels has been experimental in our study. Similar data has strong correlation with few other studies.

CONCLUSION

In the present study vitamin B₁₂ deficiency has found a strong co-relation with the as pernicious anemia. This study has clear indications to evaluate to B₁₂ status in cases manifesting with such features. In this region some amount of antenatal programmes including iron and folate are being provide to the pregnant and lactating mothers. But from our study it should be clear that fortifying foods with vitamin B₁₂ may be suggested to cure pernicious anemia. Finally, for the exact suggestion

and recommendations further studies would be needed in large population size.

Conflicts of Interest

There is no any conflict of interest associated with this study.

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