



Assessment of Adverse Outcome of Vitamin B₁₂ Deficiency in Selected Adult Population Diagnosed with Varied Clinical Conditions

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Abstract *Back ground:* Vitamin B₁₂ deficiency strike all ages but infants and older population are more vulnerable. Persistent Vitamin B₁₂ deficiency leads to neurological impairment, disability, poor outcome of disease, premature birth or death, psychiatric illness, anemia, vascular occlusion, suppressed immune system, in addition to hindrance in bone marrow development. *Aim:* Present prospective observational study was undertaken to evaluate vitamin B₁₂ levels, determination of underlying clinical condition that causes probable B₁₂ deficiency and symptoms/outcome associated with vitamin B₁₂ deficiencies. *Materials and Methods:* It was a prospective observational study and performed during 1st January 2014 to 1st Dec 2016 with a total of 365 patients (males = 149, females = 216) that were confirmed cases of low vitamin B₁₂ levels (<200pg/ml). Patients were classified according to gender, age groups and vitamin B₁₂ levels as per previously described methodology with three age-groups as > 16 years < 40, > 40 and < 60, > 60 and < 70 years. Patient's history, underlying clinical condition were carefully documented and analyzed as per earlier established methods. Haematological testing for Hemoglobin and MCV was performed on Sysmex analyzer. Serum folic acid and vitamin B₁₂ were assayed on Cobas e411 auto-immunoassay system-ECL technology (Roche Diagnostic, Basel), whereas creatinine and LDH were performed on Cobas 6000 c501 analyzer (Roche Diagnostics). Correlation between ages, gender, clinical conditions was analyzed by multiple linear regressions. Data was analyzed by grouping on the basis of age, gender and vitamin B₁₂ levels. Statistical analyses were performed using the statistical package SPSS-version 15.0 (USA) and results are presented as the percentage. *Results:* Gender and age distribution were noted to be males (total n = 149)- Group >16 <40 yrs, n = 35, 23.48%, Group >40 <60 yrs, n = 47, 31.54% and elderly Group >60 <70 yrs, n = 67, 44.96% and for females (total n = 216); Group >16 <40 yrs, n = 49, 22.6%, Group >40 <60 yrs, n = 86, 39.81%, and elderly Group >60 <70 yrs, n = 81, 37.50%. Around 65.67% (n = 44) males patients and 81.48% (n = 66) female patients in elderly group of >60<70 yrs showed B₁₂ levels of < 150 pg/ml and deficiency was found to be prevalent in females than in male elderly patients. Impaired memory (n = 35, 42.15% in males, n = 61, 44.20% in females) was more frequent clinical finding followed by dyspepsia, ataxia, anemia, drowsiness, weakness, neuropsychiatric symptoms such as urinary or fecal incontinence, polyneuritis and paraesthesia. *Conclusion:* Current presented prospective observational study thus showed deficient vitamin B₁₂ levels in many of the selected population, mostly elders and females, their underlying clinical condition that might have caused probable B₁₂ deficiency and symptoms/outcome associated with vitamin B₁₂ deficiencies, such as dementia both in males and females followed by hypertension, diabetes, and general neuronal dysfunction.

Keywords Vitamin B₁₂, Deficiency, neuropsychiatric, hematologic, clinical conditions



Introduction

Vitamin B₁₂ (B₁₂, Cbl, Cobalamine) is water soluble vitamin, essential for cell growth and several physiological and metabolic functions [1,2]. Some of the reasons or Vitamin B₁₂ deficiencies are mal-absorption, inadequate intake, diet irregularities, drugs, radiation and numerous underlying co-morbid [1-6]. Adequate amount of Vitamin B₁₂ is required and is necessary for DNA synthesis, erythropoiesis, folic acid metabolism, neurological developments [7-9]. It is documented that Vitamin B₁₂ deficiency although strike all ages but infants and older population are more vulnerable [1,5,10,11]. In addition, vegetarian, vegans, bariatric surgery patients, GIT diseases and those with ileal resections were also at risk of developing severe B₁₂ deficiencies [12, 13]. Persistent Vitamin B₁₂ deficiency leads to neurological impairment, disability, poor outcome of disease, premature birth or death, psychiatric illness, anemia, vascular occlusion, suppressed immune system, in addition to hindrance in bone marrow development [5, 14-16]. Furthermore, some commonly reported and well documented clinical conditions associated with vitamin B₁₂ deficiencies are hematological [17,18], paresthesias [17,19], glossitis, anorexia [18, 20], vascular manifestation [this Article 14], and adverse affect on infant growth and development [10, 22-25].

Thus the present prospective observational study was undertaken to evaluate vitamin B₁₂ levels, determination of underlying clinical condition that causes probable B₁₂ deficiency and symptoms/outcome associated with vitamin B₁₂ deficiencies.

Materials and Methods

Patient's selection and research design

Present study is prospective observational and performed during 1st January 2014 to 1st Dec 2016 with a total of 365 patients (males = 149, females = 216) that were confirmed cases of low vitamin B₁₂ levels (<200pg/ml). All patients were shown to posses definite vitamin B₁₂ reporting and related lab investigations, diagnoses and follow ups, including the patients from both indoor and OPDs. Patients were classified according to gender, age groups and vitamin B₁₂ levels as per previously described methodology [26]. Patient's ages ranged from 16 to 70 years in females and 21 to 70 years in males and finally categorized in three age-groups as > 16 years < 40, > 40 and < 60, > 60 and < 70 years. Patient's history, underlying clinical condition that might induce vitamin B₁₂ deficiency and unfavorable outcome due to deficiency were carefully documented and analyzed as per earlier established methods [4, 10, 26].

Vitamin B₁₂ and related Laboratory investigations:

Vitamin B₁₂ analysis and Serum folate, hemoglobin measurements were referred by physicians and practitioners for routine diagnostic check-ups to the laboratory. Blood samples from patients were routinely collected after an overnight fasting. Haematological testing for Hemoglobin and MCV was performed on Sysmex analyzer. Serum folic acid and vitamin B₁₂ were assayed on Cobas e411 auto-immunoassay system-ECL technology (Roche Diagnostic, Basel), whereas creatinine and LDH were performed on Cobas 6000 c501 analyzer (Roche Diagnostics). The total imprecision of the B₁₂ immunoassay is 3.7% Coefficient of Variation and within precision was 3.0%, and for folic acid coefficient variation was 5.0% and within precision 3.0%. Anaemia was defined as a haemoglobin concentration <14.0 g/dL in men and <12.3 g/dL in females, respectively. Low serum levels of folic acid and vitamin B₁₂ were established at < 2.7 (normal 2.7-16.1 ng/ml) and < 220 (normal 220-925 pg/ml), respectively, where B₁₂ < 150 pg/ml is considered as markedly deficient and between 150-220 pg/ml as moderately deficient. Correlation between ages, gender, clinical conditions was analyzed by multiple linear regressions. Data was analyzed by grouping on the basis of age, gender and vitamin B₁₂ levels. Statistical analyses were performed using the statistical package SPSS-version 15.0 (USA) and results are presented as the percentage.

Results

We conducted a prospective observational study was carried out with a total number of 365 patients (males = 149, females = 216) with confirmed reports of low vitamin B₁₂ levels (<200pg/ml) during the period 1st Dec 2014 to 1st Dec 2016. These patients were included as per definite vitamin B₁₂ reporting and related lab investigations, diagnoses and follow ups. Results are summarized in Tables 1 to 5. Patient's ages ranged from 16 to 70 years in



females and 21 to 70 years in males and categorized in three age-groups as > 16 years < 40, > 40 and < 60, > 60 and < 70 years. Related lab tests done were Hb, MCV, Creatinine, vitamin B₁₂, Folic acid, LDH. Data was analyzed by grouping on the basis of age, gender and vitamin B₁₂ level. Gender and age distribution were noted to be males (total n = 149)- Group >16 <40 yrs, n = 35, 23.48%, Group >40 <60 yrs, n = 47, 31.54% and elderly Group >60 <70 yrs, n = 67, 44.96% and for females (total n = 216); Group >16 <40 yrs, n = 49, 22.6%, Group >40 <60 yrs, n = 86, 39.81%, and elderly Group >60 <70 yrs, n = 81, 37.50% (table 1 and 2). The study showed mean B₁₂ level in vitamin B₁₂ deficient individuals as 118.40 ± 42 pg/ml. Mean concentrations of other parameters are MCV = 85 fl (normal range 76-96fl), creatinine = 1.12 mg/dl (0.5-1.5 mg/dl), folic acid 5.15 ng/ml (2.72-16.1 ng/ml), LDH = 354 U/L (< 480 U/L), bilirubin = 0.45 mg/dl (< 1.0 mg/dl). Around 65.67% (n = 44) males patients and 81.48% (n = 66) female patients in elderly group of >60<70 yrs showed B₁₂ levels of < 150 pg/ml and subsequently deficiency was found to be prevalent in females than in male elderly patients (Table 3). Impaired memory (n = 35, 42.15% in males, n = 61, 44.20% in females) was more frequent clinical finding (Table 4), followed by dyspepsia, ataxia, anemia, drowsiness, weakness. Other clinical conditions were neuropsychiatric symptoms such as urinary or fecal incontinence, polyneuritis, paraesthesia (Table 4). Mean Hb was low (males = 12.48 gm/dl; range 13-18 gm/dl; females = 8.45 gm/dl; range 11.5-16.4 gm/dl), mean folic acid, LDH and bilirubin levels were normal. Various clinical conditions were also found co-existing with B₁₂ deficiency or outcome of prevailing insufficiency. More notable were dementia both in males (n = 23, 27.71%) and females (n = 45, 32.60%) followed by hypertension, diabetes, general neuronal dysfunction (Table 5).

Table 1: Gender wise distribution of Vitamin B₁₂ deficient group (n = 365)

Gender	Number of patients	Percentage
Males	149	40.82%
Females	216	59.17%

Table 2: Age wise (< 60 yrs, > 60 yrs) distribution of vitamin B₁₂ deficient categories (n = 365)

Gender	Number of patients	Percentage %
Males = 149		
Group >16 <40 yrs	35	23.48
Group >40 <60 yrs	47	31.54
Group >60 <70 yrs	67	44.96
Females = 216		
Group >16 <40 yrs	49	22.6
Group >40 <60 yrs	86	39.81
Group >60 <70 yrs	81	37.50

Table 3: Distribution of Vitamin B₁₂ deficiency in male and female patients (n = 365) according to B₁₂ status

Gender and age-groups & subgroups	Patients in Vitamin B ₁₂ deficiencies categories			
	Vitamin B ₁₂ 150-220 pg/ml		Vitamin B ₁₂ < 150 pg/ml	
	n	%	n	%
Males = 149				
Group >16 <40 yrs (N = 35)	25	71.42	10	28.57
Group >40 <60 yrs (N = 47)	18	38.29	29	61.70
Group >60 <70 yrs (N = 67)	23	34.32	44	65.67
			83	
Females = 216				
Group >16 <40 yrs (N = 49)	29	59.18	20	40.81
Group >40 <60 yrs (N = 86)	34	39.53	52	60.46
Group >60 <70 yrs (N = 81)	15	18.51	66	81.48
			138	

n/N = number of patients, % according to B₁₂ deficiency categories within age-groups



Table 4: Various clinical manifestations in Vitamin B₁₂ markedly deficient (< 150 pg/ml) individuals (males = 83, females = 138). Cumulative number of patients were also depicted as percent onset of respective clinical conditions. (Note: Multiple clinical condition existed in pateints)

Clinical Conditions	Cumulative Number of patients in all age groups >16 to < 40 yrs, > 40 to < 60 yrs and > 60 to < 70 yrs			
	Males (n = 83)		Females (n = 138)	
	n	%	n	%
Paraesthesia	22	26.50	46	33.33
Dyspepsia	26	31.32	52	37.68
Diarrhea	11	13.25	20	14.49
Vomiting	05	6.02	11	7.97
Impaired memory	35	42.16	61	44.20
Ataxia	21	25.30	66	47.82
Polyneuritis	18	21.68	57	41.30
Drowsiness	17	20.48	61	44.20
Excretion Inconsistencies	11	13.25	21	15.21
Weakness	19	22.89	48	34.78
Anemia	21	25.30	49	35.50

Table 5: Occurrence of different diseases in Vitamin B₁₂ markedly deficient (<150 pg/ml) individuals (Males = 83, females = 138). Cumulative number of patients were also depicted as percent onset of respective clinical conditions. (Note: Multiple clinical condition existed in pateints)

Clinical Conditions	Cumulative Number of patients in all age groups >16 to < 40 yrs, > 40 to < 60 yrs and > 60 to < 70 yrs			
	Males (n = 83)		Females (n = 138)	
	n	%	n	%
Dementia	23	27.71	45	32.60
Ischemic heart disease	11	13.25	10	7.24
Stroke	14	16.86	09	6.52
Hypertension	18	21.68	23	16.66
Diabetes	17	20.48	16	11.59
Parkinson's	09	10.84	12	8.69
General neurological disorders	15	18.07	29	21.01

Discussion

During past few years, it was well documented, after several studies executed on cohorts, prospective, linear, multi-centered, that diagnoses and management of sub-clinical and clinical vitamin B₁₂ deficiency is somewhat difficult to diagnose and intricate to manage due to multi-faced reasons and higher prevalence, respectively [1, 17-28]. Generally clinical studies carried out in last two decades in groups of both adults and elderly population of vitamin B₁₂ deficient individuals and reported several clinical conditions and diseases that are associated with vitamin B₁₂ deficiency [17-25]. In this regard, hematological disorder is noted to be primary cause of vitamin B₁₂ deficiency and its related sequelae are often severe and irreversible in the children, the majority of which, with clinical deficiency, may manifest into megaloblastic anemia [10]. In various studies reported earlier, 56% to 77% of patients had signs of macrocytosis or anemia [19, 29-32] with overt vitamin B₁₂-deficiency.



In our presented study, we have found that vitamin B₁₂ deficiency and its prevalence is directly proportional to age; which means elderly the patients was, lower would be its Vitamin B₁₂ levels. In addition females were found to be more B₁₂ deficient than males and more in the category of less than 150 pg/ml. In two previous studies by our group [4, 26], similar pattern of clinical manifestations were noted in B₁₂ deficient selected population. Thorough survey of literature showed that none of the individual symptom or in some cases cluster of symptoms have been linked to deficiency of vitamin B₁₂ (10), however it is evident that more persistent B₁₂ deficiency usually appears nearly at about 60 years of age and remains asymptomatic for many years [10, 33].

It was notified that Vitamin B₁₂ deficiency if remains untreated might induce severe repercussions such as causing vascular diseases inclusive of stroke, myocardial infarction and deep vein thrombosis [34, 35]. Furthermore, combined deficiency of vitamin B₁₂ and iron is noted in individuals aged 60 and 70, which is in agreement with our study as well [1, 34-37]. Numerous previous and recent studies notified several neurological disorders that results from Vitamin B₁₂ deficiency such as paresthesias (with or without objective signs of neuropathy), weakness, motor disturbances (including gait abnormalities), vision loss, and a wide range of cognitive and behavioral changes (e.g., dementia, hallucinations, psychosis, paranoia, depression, violent behavior, and personality changes) [6, 10, 26, 38]. Furthermore, tingling of the hands and feet is perhaps the most common neurologic complaint reported [10, 17, 19, 26, 39]. It has been suggested that patients with underlying B₁₂ deficiency may also develops neuropsychiatric sequelae such as combined sclerosis of spinal cord (classic finding), polyneuritis, ataxia and babinski's phenomenon (which is a frequent finding) and cerebral syndromes, urinary and fecal incontinence (rare) [5, 10]. Consequently, gastro-intestinal manifestations such as anorexia, flatulence, diarrhea, and constipation may also precede Vitamin B₁₂ deficiency [10, 18, 20, 26, 39, 40].

In past two decades, a number of comparative and analytical studies stated certain other nutritional or physiological factors that may facilitate or corroborate B₁₂ deficiency and its related conditions. Development of vascular complications, co-morbid with elevated levels of homocysteine (Hcy) was reported as few of those clinical findings that might influence B₁₂ and folic acid deficiency [10, 21, 26, 41]. Moreover, hyperhomocysteinemia as stated, increases the risk of developing a vascular occlusion [1, 21, 42], consequently facilitating the risk of coronary heart disease and ischemic stroke [41-44]. Additionally, vitamin B₁₂ deficiencies among infants and young children might proceed to induce clinical manifestations at a very later stage of life, which are mostly seen in older patients of B₁₂ deficiency, such as hematologic, neurologic, gastrointestinal, and cardiovascular consequences [22-25, 45, 46]. Subsequently, low or marginal vitamin B₁₂ status in pregnant women increases the risk of neural tube birth defects as well [47].

Conclusion

Current presented prospective observational study thus showed deficient vitamin B₁₂ levels in many of the selected population, mostly elders and females, their underlying clinical condition that might have caused probable B₁₂ deficiency and symptoms/outcome associated with vitamin B₁₂ deficiencies.

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