

Original Article**EFFECT OF VITAMIN B12 DEFICIENCY ON THYROID FUNCTION TESTS AMONG ADULTS VISITING TERTIARY HEALTH CARE CENTRE IN EASTERN NEPAL*****Nisha Ghimire,¹ Soumitra Mukhopadhyay¹, Rekha Limbu², Prabesh Kumar Chaudhary³, Surendra Prasad Shah⁴, Pawan Kumar Lal Das⁵**¹Department of Physiology, Nobel Medical College, Biratnagar; ²Department of Basic and Clinical Physiology, B.P Koirala Institute of Health Sciences Dharan; ³Department of Pathology, Nobel Medical College, Biratnagar; ⁴Department of Internal Medicine, Nobel Medical college Biratnagar; ⁵Department of Physiology, B&C Medical College Teaching Hospital and Research Centre Birtamod, Jhapa, Nepal**Submitted: 7th – October- 2024, Revised: 26th November- 2024, Accepted: 10th – December-2024****DOI: <https://doi.org/10.3126/mjen.v3i2.73048>****ABSTRACT****Background**

Vitamin B12 deficiency is commonly observed in hypothyroidism and autoimmune thyroiditis. Low vitamin B12 can disrupt thyroid function. Though the prevalence of this deficiency has been documented in various regions of Nepal, studies in Eastern Nepal are limited. The objective of this study was to assess the percentage distribution of vitamin B12 deficiency among adults visiting the tertiary health care centre in Eastern Nepal and to find out the effect of vitamin B12 deficiency on thyroid function test.

Methods

This study, conducted at Nobel Medical College from January 2022 to December 2023, was cross-sectional in design and included 379 patients. Subjects underwent general health check-ups, including Vitamin B12 levels and thyroid function tests. Descriptive analysis was performed, and Pearson's correlation assessed the relationship between vitamin B12 and thyroid hormones. Independent t-tests compared hormone levels between groups. P value < 0.05 was considered as statistically significant.

Results

The vitamin B12 status distribution was as follows: 62.5% normal (>300pg/ml), 18.2% borderline deficient (200-300pg/ml), and 19.3% deficient (<200pg/ml). Prevalence was higher among females. Group II subjects (vitamin B12 <300pg/dl) had significantly lower mean free triiodothyronine (FT₃) levels (2.82±0.742 vs. 3.14±0.49 pg/mL, p=0.003) and higher mean thyroid stimulating hormone (TSH) levels (4.69±3.56 vs. 2.55±1.47 mIU/L, p<0.001) compared to group I (B12>300pg/ml). Vitamin B12 showed a negative correlation with TSH (p=0.013).

Conclusion

The study indicates a decrease in FT3 levels and an increase in TSH levels among those with vitamin B12 deficiency or borderline cases.

Keywords: Thyroid hormones, Thyroid stimulating hormone, Thyroxine, Triiodothyronine, Vitamin B12

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INTRODUCTION

Vitamin B12 deficiency is a widespread concern globally. It is caused by poor diet, absorption problems, or other health conditions.¹ Vitamin B12 is also known as cobalamin, and it is important to maintain normal neurological function, red blood cell production, and DNA synthesis. Humans cannot produce vitamin B12 in their body and daily intake is necessary. Animal proteins or fortified cereals should be included in diet to maintain normal vitamin B12 level.² Serum vitamin B12 levels is classified as normal (above 300 pg/mL), borderline deficiency (200 - 300 pg/mL) and deficiency categories (below 200 pg/mL).³

Studies have highlighted increased prevalence of vitamin B12 deficiency among different demographic groups. In India, study indicates 75% prevalence of vitamin B12 deficiency among young and middle-aged individuals.⁴ Similarly, in Kathmandu, Nepal study conducted among adult and elderly patients, 33.5% were deficient and 27.9% were borderline deficient.⁵

Furthermore, evidence have suggested association between vitamin B12 deficiency and thyroid disorders. Patients with hypothyroidism had lower vitamin B12 levels than healthy participants.^{6,7} Shulhai AM et al. suggested that nutrition influences thyroid function through the diet–gut–thyroid axis, where dietary changes affect gut bacteria, leading to nutrient deficiencies like vitamin B12. These deficiencies can impact thyroid health through immune responses, nutrient absorption, and genetic changes.⁸ Krishnamurthy HK and colleagues found that low vitamin B12, along with other nutrient deficiencies significantly affected thyroid function.⁹ Kacharava et al. identified that patients with vitamin B12 deficiency had significantly higher mean values of anti-TPO (thyroid peroxidase) antibodies in autoimmune thyroid disorders compared to controls.^{7,10} However some studies indicate that the data is insufficient and more studies should be conducted to prove relation between Vitamin B12 deficiency and thyroid disorder.^{11,12}

Though prevalence of vitamin B12 deficiency has been documented in various regions of Nepal, study done in Eastern Nepal is limited. So, a cross-sectional study was conducted at tertiary health care centre with the general objective to find out the effect of vitamin B12 deficiency on thyroid function test. Our main objectives were to determine the percent distribution of vitamin B12 deficiency and to compare and correlate thyroid function test in normal, borderline vitamin B12 deficiency and vitamin B12 deficiency patients.

METHODS

The study was conducted between January 2022 to December 2023 after obtaining ethical clearance from Institutional research committee. The study included 379 subjects who visited the Nobel Medical College outpatient department (O.P.D.) for general health check-up, which also included the determination of vitamin B12 levels and thyroid function parameters. Subjects were 18 to 60 years of age, and they were further divided into three age groups. Subjects who reported any chronic illnesses or malabsorption syndrome were excluded from the study. After taking consent, patients were selected for the study. The laboratory estimation of Vitamin B12 was done by chemiluminescence method. Free triiodothyronine (FT₃), thyroxine (FT₄), and thyroid stimulating hormone (TSH) were also estimated using chemiluminescence immunoassay method. (Siemens Advia Centaur XP).

Sample size calculation was done using following formula. Taking Prevalence 33.5%⁵, Sample size $n = (Z1 - \alpha/2)^2 \cdot (p \times q) / d^2 = 345$, considering 10% drop-out, $n = 345 + 34 = 379$, where n = Desired sample size, $Z1 - \alpha/2 = 1.96$ (At 95% CI or 5% level of significance), P = Expected prevalence or based on previous research, $q = 1 - P$, d = Margin of error or precision = 5%. Statistical analysis- Data were analysed using Microsoft Excel and Statistical Packages for the Social Sciences version 16. A descriptive analysis was performed, and the findings were expressed as percentages. This analysis focused on key factors such as age, gender distribution, and the prevalence of vitamin B12 deficiency. Gender was categorized as either male or female.

Subjects were divided into three groups based on their vitamin B12 levels: those with normal levels (above 300 pg/mL), those with borderline-deficient levels (200 to 300 pg/mL), and those with deficient levels (below 200 pg/mL). Age was grouped into three categories: young adults (ages 18-39), middle-aged individuals (ages 40-59), and the elderly (ages 60 and older).⁵

The normal reference range of our Laboratory for FT₃ is 2.5-4.16pg/ml, FT₄ -0.89-1.76ng/dl and TSH is 0.34-5.12 uIU/ml. To compare the differences of mean thyroid hormones level (FT₃, FT₄ and TSH) with vitamin B12 level, the subjects were categorized into two groups: group II (vitamin B12 <300pg/ml) consisting of 142 participants from both the borderline vitamin B12 deficient and vitamin B12 deficit categories (69 + 73 = 142) (Table 1). From the 237 subjects in group I (vitamin B12 > 300pg/ml), we randomly selected 142 using computer-based randomization (shuffling in excel). So, we took 142 subjects from each group.

RESULTS

In our study involving 379 subjects, numbers of females were more compared to males. Among the participants, 18.2% had borderline deficiency of vitamin B12, whereas 19.3% were found to be vitamin B12 deficient. The highest prevalence of deficiency was noted in the 40-59 age group (Table 1). A higher prevalence of vitamin B12 deficiency was noted among females across all age groups compared to males (Table 2), with the 40-59 age group being the most affected. Among individuals classified as borderline deficient, slightly higher counts were found in females than in males (Table 2).

A significant decrease in mean FT₃ levels and an increase in mean TSH levels were seen in group II (vitamin B12 < 300 pg/ml) compared to group I (vitamin B12 > 300 pg/ml), while no significant difference was noted in FT₄ levels (Table 3). Furthermore, it was found that vitamin B12 was significantly negatively correlated with TSH, weakly negatively correlated with FT₄, and weakly positively correlated with FT₃ (Table 4).

Table 1: Percent distribution of subjects (n=379) based on gender, age group, and Vitamin B12 levels

Category	Gender (n=379) (%)	Age (n=379) (%)	Vitamin B12 level (n=379) (%)
Female	227 (59.9%)		
Male	152 (40.1%)		
Normal			237 (62.5%)
Borderline			69 (18.2%)
Deficiency			73 (19.3%)
18-39		108 (28.5%)	
40-59		177 (46.7%)	
Above 60		94 (24.8%)	

Table 2: Subgroup analysis and percent distribution of vitamin B12 status by gender and age group among study participants 40 mini

Vitamin B12 Status n=379	Gender	18-39 yrs	40-59 yrs	Above 60 yrs	Total (n) (%)
Normal n=237 (62.5%)	Female	41 (17.3%)	66 (27.8%)	31 (13.1%)	138 (58.2%)
	Male	21 (8.8%)	51 (21.5%)	27 (11.4%)	99 (41.8%)
Borderline deficiency n=69 (18.2%)	Female	15 (21.7%)	19 (27.5%)	11 (15.9%)	45 (65.2%)
	Male	6 (8.7%)	10 (14.5%)	8 (11.6%)	24 (34.8%)
Deficiency n=73 (19.3%)	Female	14 (19.2%)	18 (24.7%)	12 (16.4%)	44 (60.3%)
	Male	11 (15.1%)	13 (17.8%)	5 (6.8%)	29 (39.7%)

Table 3: Comparison of FT₃, FT₄ and TSH between group I (vitamin B12>300pg/ml) and group II (vitamin B12<300pg/ml)

Thyroid Hormones	Group I vitamin B12 level>300pg/ml Mean ± SD n=142	Group II Vitamin B12 <300pg/ml Mean ± SD n=142	p-value
FT ₃ pg/mL	3.14±0.496	2.82±0.742	0.003*
FT ₄ ng/DI	1.45±0.645	1.71±2.648	0.207
TSH uIU/L	2.55±1.479	4.69±3.569	<0.001*

Table 4: Pearson's Correlation of vitamin B12 level with FT₃, FT₄ and TSH

Vit B12 n=379	Correlation coefficient	p -value
FT ₃	.163	.076
FT ₄	-.103	.263
TSH	-.227*	.013*

FT₃- free triiodothyronine, FT₄-free thyroxine, TSH – thyroid stimulating hormone

Pearson's correlations *, p<0.05

DISCUSSION

The objective of this study was to assess the percent-age distribution of vitamin B12 deficiency among male, female and various age groups of subjects in eastern part of Nepal, examine the effect of vitamin B12 deficiency on thyroid function test.

Among the 379 participants, a higher proportion were female compared to male. The analysis of vitamin B12 levels revealed that most of the subjects had normal vitamin B12 level, whereas 18.2% were found to having borderline deficiency, and 19.3% were suffering from deficiency (Table-1). The age distribution indicated that the majority of subjects were in the 40-59 age range, followed by the 18-39 age group, with the smallest proportion in the 60 and older category. It clearly exhibited that female had higher prevalence of vitamin B12 deficiency compared to males in all age groups. (Table-1,2)

Our study revealed slightly lower percentage of vitamin B12 deficiency compared to previous studies conducted in Nepal by Gyawali P et al and Paudel P et al.^{5,13} This might be because we had not done categorization of the population based on nutritional intake in our study. The study conducted by Paudel P¹³ et al comprised only strict vegetarians. Since they did not include animal products in their diets, their study exhibited a higher prevalence of vitamin B12 deficiency compared to our study. However, our findings

are consistent with result of study conducted in Eastern Nepal in 2016. This study showed prevalence of vitamin B12 deficiency to be 21%¹⁴ which is comparable to result of our study (19.3%). Our results demonstrated a higher prevalence of vitamin B12 deficiency among females which is identical to the study done in Eastern Nepal.¹⁴ However it is slightly different from studies conducted in other areas of Nepal.^{5,13} The reasons might be linked to dietary habits or other factors which need to be determined. Furthermore, in our result the highest proportion of deficiency was observed among individuals aged 40-59. This is consistent with other studies.¹⁴ It is also mentioned that the reason may be due to factors such as atrophic gastritis and resulting in malabsorption.¹⁵ When thyroid hormone levels were compared between group I (Vitamin B12 >300pg/ml) and group II (vitamin B12 <300), the mean FT₃ levels were lower and the mean TSH levels were significantly higher in group II. But FT₄ levels not affected significantly. (Table 3). This is also consistent with our correlation study (Table 4) where B12 was significantly positively correlated with TSH but there was weak positive correlation with FT₄ and negative correlation with FT₃. It signifies that as vitamin B12 level decreases there is increase in TSH, FT₄, and decrease in FT₃. Levels similar type of result was shown by Luo S et al also indicated increased TSH, increased T₄ but decreased T₃ in subacute combined spinal cord degeneration patients whose main causative agent is vitamin B12 deficiency. They suggested it reflects the decline in tissue-specific deiodinase activity which will not convert FT₄ to FT₃. When FT₃ level decrease it

cause increase in TSH by negative feedback mechanism.¹⁶ However, even the trend in our study showed increase TSH, increase FT₄ and decrease FT₃, the increase in FT₄ was not significant in our study. Also, correlation of vitamin B12 was significant with TSH but not with FT₄ and FT₃. So, more study should be conducted in large number of vitamins B12 deficiency and borderline vitamin B12 deficiency groups to confirm status of FT₄, FT₃ and TSH in vitamin B12 deficiency patients. If the results observed are comparable to our results, vitamin B12 examination should be considered in the assessment and management of thyroid disorders.

The limitation of our study was that it lacked dietary habit information, and we could not assess TPO antibodies.

CONCLUSION

A higher percentage of vitamin B12 deficiency was observed among females and individuals aged 40-59. The decrease in FT₃ levels and increase in TSH levels among those with vitamin B12 deficiency or borderline cases and negative correlation of vitamin B12 with TSH suggest association of vitamin B12 deficiency with abnormal thyroid functions.

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Conflict of interests: None

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