Evaluation of Serum Vitamin B12 Levels and Its Correlation with Anti-Thyroperoxidase Antibody in Patients with Autoimmune Thyroid Disorders

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Received: 4 July 2013 / Accepted: 15 January 2014 / Published online: 6 February 2014
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Abstract Vitamin B12 deficiency has been reported in patients with Autoimmune thyroid disorders. However there is limited data on exact prevalence of low B12 and its correlation with anti-thyroperoxidase antibody (anti-TPO) levels in these patients. The aim of our study was to estimate serum vitamin B12 levels in autoimmune thyroid disorders and to correlate B12 levels with anti-TPO. 350 patients were selected by convenient sampling. Vitamin B12 levels and thyroid parameters were estimated using fully automated chemiluminescence method on Access 2. Results of our study shows that using the manufacturer's cut-off of 145 pg/mL, the prevalence of low serum vitamin B12 was found to be 45.50 %. Higher prevalence (55 %) was seen based on the published cut-off of 200 pg/mL. The study however did not demonstrate any significant correlation between vitamin B12 levels and anti-TPO ($r = -0.11$ and p value of 0.30).

Keywords Vitamin B12 · Autoimmunity · Auto antibodies · Autoimmune thyroid disorders (AITD) · Anti-thyroperoxidase (anti-TPO)

Introduction

Autoimmune thyroid diseases (AITD) is a group of thyroid disorders in which antibodies are produced against components of the thyroid gland. The diagnosis can be confirmed by the presence of characteristic auto antibodies such as anti-thyroperoxidase antibodies (anti-TPO) [1].

Association of vitamin B12 deficiency in AITD patients have been demonstrated in previous studies [2–7]. Presence of parietal cell antibodies (PCA) and antibodies to intrinsic factor have been demonstrated in patients with AITD [2–4]. These factors could contribute to vitamin B12 deficiency in these patients. In addition the dietary habits which vary from one population to another could also contribute to the deficiency. The clinical signs of B12 deficiency vary. Hematologic and neurologic abnormalities may coexist, may be present independently or may be very subtle and it is possible that serum B12 deficiency may be the first sign in otherwise asymptomatic patients [8].

B12 deficiency in the elderly population has been reported [9]. Study by Ness Aabram et al. [4] found a prevalence of 25 % B12 deficiency in AITD. Higher prevalence rates (40 %) have been reported by Jabbar et al. [5] and by Kankonkar et al. [6]. A recent article by Lippi et al. [7] however did not demonstrate any increase in B12 deficiency in thyroid disorders and therefore does not support the routine screening for B12 deficiency in sub clinical disturbances of thyroid function, although the utility of the same in overt thyroid dysfunction has not been ruled out. Due to these conflicting reports there is a need to evaluate the vitamin B12 levels in AITD in our population and also to evaluate if there is any association between B12 and anti-TPO levels.

This study has been undertaken to test the hypothesis that there is a high prevalence of vitamin B12 deficiency in

Electronic supplementary material The online version of this article (doi:10.1007/s12291-014-0418-4) contains supplementary material, which is available to authorized users.

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AITD in South Indian population attending a tertiary care hospital. The Primary objective of the study is to estimate serum vitamin B12 levels in patients with autoimmune thyroid disorders and to correlate B12 values with anti-TPO levels. The secondary objectives are to evaluate the influence of TSH, age and duration of disease on serum B12 levels. If the study shows a considerable proportion of AITD with vitamin B12 deficiency in this population then it would be worthwhile to consider regular screening and supplementation of vitamin B12.

Methodology

Study Design. Cross sectional study.

Setting. The study was conducted over a period of two years from 2008 to 2010 in the department of Biochemistry at St John's Medical College Hospital. The cases were sampled from those attending the Endocrine outpatient department of St John's Medical College Hospital.

Sampling. Convenient sampling.

Participants. Patients who are diagnosed with AITD were selected as the cases. They were selected by convenient sampling based on the inclusion/exclusion criteria. The diagnosis of AITD was based on history, clinical evaluation and estimation of thyrotropin (TSH), total and free thyroxine (T4 and FT4). The autoimmune nature was ascertained by the presence of anti-thyroperoxidase (TPO) antibodies.

Inclusion Exclusion Criteria. Strict Vegetarians and patients on drugs known to interfere with vitamin B12 absorption such as phenytoin, dihydrofolate reductase inhibitors etc. were excluded from the study. Subjects with history suggestive of malabsorption syndromes, previous gastrectomy were also excluded from the study. Subjects older than 65 years were excluded as elderly subjects are known to have B12 deficiency.

Bias. Diet may be a confounding factor in our study as a detailed dietary history was not available.

Operational Definitions. AITD is diagnosed based on history, clinical evaluation and laboratory findings.

Vitamin B12 levels were evaluated based on the manufacturer's recommendations as follows [10]:

- Normal range: 180–914 pg/mL (133–675 pmol/L).
- Indeterminate range: 145–180 pg/mL (107–133 pmol/L).
- Deficient range: <145 pg/mL (107 pmol/L).

Vitamin B12 levels were also evaluated based on a published meta-analysis report which considers a value less than 148 pmol/L (200 pg/mL) as deficient [11].

Sample Size. The sample size was calculated using the n-Master software program based on the previous published

values. The estimated sample size was 389. This ensured a power of 80 %, an effect size of five, at an alpha error of 5.0 %.

Study Variables. The main outcome variable in our study is serum vitamin B12. The other variables are serum TSH, T4, FT4, anti-TPO, age and duration of AITD. The laboratory reference range for TSH is 0.34–4.1 μ IU/mL, T4 is 6.09–12.23 μ g/dL and FT4 is 0.61–1.12 ng/dL. Anti-TPO value more than 50 U/mL was considered positive.

Data Sources/Measurements. The parameters were estimated in the fasting samples of AITD patients which are received by the laboratory for estimation of thyroid profile. Samples were collected for study purpose after informed consent from the patients. All parameters were estimated by fully automated chemiluminescence system Access 2 from Beckman and Coulter using dedicated reagents from the same manufacturer (closed random access system). The vitamin B12 is estimated by competitive-binding immunoassay while TPO antibody assay is a sequential two-step immunoassay sandwich assay. The chemiluminescent substrate Lumi-Phos 530 is used for the reaction and light generated by the reaction is measured with a luminometer. The analyte concentration in the sample is determined by means of a stored, multi-point calibration curve [10, 12].

Internal Validity. The parameters were calibrated using calibrators with traceability to certified reference materials. Internal quality control samples from Bio rad were run to monitor the precision and accuracy of the assays. In addition, the lab also participated in regular interlaboratory and External Quality Assurance Programmes.

The institutional ethical committee clearance was taken before conducting the study.

Statistical Methods. Data are expressed using descriptive statistics such as mean and SD for continuous variables and number and percentage for categorical variables. Mean B12 levels in the population has been evaluated by the one sample *t* test. Comparison of vitamin B12 between males and females was carried out using independent sample *t* test for two groups. Correlations between variables are expressed using Pearson's Correlation coefficient. All statistical analysis were carried out by using SPSS version 16. A *p* value <0.05 was considered as significant.

Results

Table 1 shows the characteristics of the study population. The estimated sample size was 389. However the final sample size achieved was 350 (100 males and 250 females) due to loss of tests in calibrations and repeats. The average age of patients was 32.2 years and average duration of thyroid disorder was 2.4 years. The mean vitamin B12 was

Table 1 Characteristics of study population

Characteristics	No of subjects
Total number of subjects	350
Male:female	100:250
Duration of AITD (years)	2.4 ± 0.3
Age (years)	32.2 ± 11.3
anti-TPO (U/mL)	642 ± 310

Table 2 Prevalence of low B12 based on different cut off

Cut off for vitamin B12	Manufacturer's cut off		Based on meta-analysis report
	Deficient <145 pg/mL (107 pmol/L)	Indeterminate 145–180 pg/mL (107–133 pmol/mL)	Deficient <200 pg/mL (<148 pmol/mL)
Prevalence of low vitamin B12	27.5 %	17 %	55.5 %

Table 3 Comparison of serum vitamin B12 levels in the males and females

Particulars	Mean B12 (pg/mL)	SD	SE of mean
Males (<i>N</i> = 100)	185.2	85.9	12.03
Females (<i>N</i> = 250)	207.9	92.2	9.65

p value: 0.12

Table 4 Correlation of vitamin B12 levels with different variables

Variables	<i>N</i>	Pearson's Correlation coefficient	<i>p</i> value
B12 and age	350	−0.17	0.08
B12 and duration of AITD	350	−0.10	0.20
B12 and Anti-TPO	350	−0.11	0.30

204.6 pg/mL, Standard deviation of 89.5, and the 95 % confidence interval (CI) being 188.9–220.4.

Table 2 shows the prevalence of low B12 based on different cut offs. Using the manufacturer's cut-off, the prevalence of low serum vitamin B12 was found to be 45.50 % with 95 % confidence interval (CI) of 17.07 and 58.04 % and a *p* value of 0.02. Out of this, 27.5 % had values in the deficient range while 17 % were in the indeterminate range. The remaining 56.5 % had values within the normal range. However when serum B12 levels were analyzed based on the published cut-off of 148 pmol/L (200 pg/mL), 55 % had low values. In general 85 % of the patients had values towards the lower end (<300 pg/

mL). Comparison of vitamin B12 levels in males and females showed no significant difference in the mean B12 levels (Table 3).

Table 4 shows the correlation between vitamin B12 levels and age, duration of AITD and anti-TPO. There was no correlation between B12 and anti-TPO (*r* = −0.11), B12 and duration of AITD (*r* = −0.10) or B12 and age (*r* = −0.17) with a *p* value of 0.08, 0.20 and 0.30 respectively.

Discussion

Autoimmune thyroid disorders are frequently treated by primary care physicians who must be able to manage both the disease and its multiple co-morbidities. Pernicious anemia is associated with autoimmune thyroid disorders and vitamin B12 deficiency is a potential co-morbidity that is often overlooked, despite the fact many AITD patients are at risk for this specific disorder. In addition, symptoms of B12 deficiency occur late. Clinical signs of B12 deficiency may be subtle and missed if not detected by laboratory evaluation of vitamin B12. B12 deficiency-induced nerve damage may contribute to peripheral neuropathy. Identifying the correct etiology of neuropathy is crucial because simple vitamin B12 replacement may reverse neurologic symptoms [8, 9, 11]. Thus the evaluation of B12 levels in the AITD population may help determine whether primary care physicians should consider screening for B12 levels in AITD patients.

The mean B12 value obtained in our study was very low 204.6 pg/mL 45.5 % had values below the lower limit of reference range (<178 pg/mL). 55 % had values below 200 pg/mL and 85 % of the subjects had vitamin B12 values less than 300 pg/mL Consistent with other reports the deficiency was similar in males and females [4].

Our study shows that the prevalence of low serum B12 in AITD was dependent on the cut-off used: 45.50 % using laboratory cut-off value and 55 % using published cut-off of 148 pmol/L. The difference in the prevalence of low B12 levels due to different cut-off values used has been reported in many studies in the past [11].

Correlation analysis did not show any correlation of B12 with anti-TPO levels (*r* = −0.11, *p* value >0.05). There was no correlation of B12 with age, duration of AITD and thyroid profile also. These findings are similar to reports by Ness Aabramof [4].

Vitamin B12 deficiency is estimated to affect 10–15 % of people over the age of 60, and the laboratory diagnosis is usually based on low serum vitamin B12 levels or elevated serum methylmalonic acid and homocysteine [9, 13, 14]. The average age of our patients was 32.2 years and hence age cannot be considered as a risk factor in our study. The

sample consisted of patients from various regions of Karnataka attending the Endocrine outpatient department of St John's Medical College Hospital and following different dietary habits. Thus, it appears that factors other than age, duration of disease and anti-TPO levels may play a role in B12 deficiency.

The lack of a gold standard complicates the diagnostic evaluations. Since, serum B12 assays and other biomarkers such as MMA and holotranscobalamin lack sufficient sensitivity and specificity when used alone, a combination of markers along with clinical evaluation is preferred to define the prevalence of cobalamin deficiency. However, markers such as MMA are expensive and not readily available in all laboratories. Hence serum cobalamin estimation continues to be used to assess vitamin B12 deficiency.

Limitations of the Study

The study did not evaluate other markers such as MMA and holotranscobalamin. Since a complete dietary evaluation was not carried out, the role of diet in B12 deficiency cannot be ruled out. The study did not evaluate for evidence of megaloblastic anemia or neuropathic disease. Therefore, the clinical significance of metabolically confirmed B12 deficiency in our patient group is unknown. Finally, the B12-deficient patients identified in our study were not followed for treatment effect with supplemental vitamin B12 or evaluated for methylmalonic acid levels. Such follow-up would have helped confirm the diagnosis of vitamin B12 deficiency and evaluate the beneficial effects of B12 supplementation.

Summary and Conclusion

Our study demonstrates the presence of low serum B12 levels in AITD. The B12 levels did not correlate with anti-TPO, age or duration of the disease. These findings merit further research on a larger population using additional markers to investigate into the cause of deficiency, the

factors involved, and benefit of B12 supplementation in these patients.

Acknowledgments The authors would like to thank the Research Society, St John's Medical College Hospital for providing the grant for the study.

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