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Serum Vitamin B12 Level in Children with Type 1 Diabetes

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ABSTRACT

Background: Type 1 Diabetes Mellitus is known to be associated with multiple co-morbidities. Vitamin B12 deficiency is a potential co- morbidity that might have been overlooked in these patients. The aim of this study is to evaluate the serum level of vitamin B 12 in a well-defined population of Type 1 diabetes.

Methods: The cross sectional study carried out on 40 patients with Type 1 Diabetes Mellitus visiting endocrinology unit in Kanti children's Hospital, Kathmandu, Nepal. Thirty healthy non diabetics were also selected. Serum C-peptide, vitamin B12, creatinine, blood glucose level and glycosylated hemoglobin were assessed in both groups. SPSS ver. 22 was used to analyze the data.

Results: The mean serum vitamin B12 level of the diabetic group was 206.92±82.18 pmol/L. Among the population 40.0% i.e. 16 out of 40 were found to be deficient and 37.5 % i.e. 15 out of 40 were subclinically deficient. Whereas the mean serum vitamin B12 level of the non diabetic group was 340.71±136.02pmol/L. Out of 30 only 2(6.7%) were deficient, 8 (26.75%) were found to be subclinically deficient. Significant difference was noticed in the mean serum level of vitaminB12 between these two groups.

Conclusions: This study shows the presence of low serum vitamin B12 levels in Type 1 Diabetics. The routine screening for this condition along with confirmatory test and detail clinical examination could benefit the patients with Type 1 diabetes.

Keywords: Autoimmune disease; Type 1 Diabetes mellitus; Vitamin B12.

INTRODUCTION

About 90% of Type 1 diabetes mellitus (T1DM) is due to autoimmune-mediated destruction of the beta cells in pancreas.[1-3] Being an autoimmune condition it is found to be associated with other autoimmune endocrine disorders like Grave's disease, Hashimoto's thyroiditis and Addison's disease.[4,5] Besides these systemic association, autoimmune diabetes also thought to affect nutritional status, interfering various vitamin level including Vitamin B 12.[6]

Vitamin B12 deficiency can result from nutritional deficiency, gastrointestinal causes, defective transport and malabsorption syndromes.[7] Malabsorption syndrome includes lack of intrinsic factor or loss of gastric parietal cells. In patients with Type 1 diabetes, presence of parietal cell antibodies (PCA) and antibodies to intrinsic factor have been demonstrated.[8,9] In addition concomitant presence of various autoimmune conditions, mentioned earlier might impair vitamin B 12 absorption in Type 1 diabetes.[6] This study aimed to evaluate the serum level of vitamin B 12 in a well-defined population of T1DM.

METHODS

A cross sectional study was performed in 40 patients diagnosed with Type 1 diabetes mellitus visiting outpatient department under endocrinology unit of Kanti Children's hospital Kathmandu, Nepal from April 2015 to January 2016. Thirty children with out diabetes and not under vitamin B12 supplement were selected from outpatient department of same hospital.

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Sample size was calculated from the prevalence of Type 1 diabetes mellitus in the Endocrine Department of hospital. 2.6%, with the confidence interval of 95% and margin of error of 5%. Ethical approval was received from Institute review board, Kanti Children's Hospital. Informed consent was taken from the parents or legally authorized representative. Questionnaire was developed and used to obtain the medical and dietary history. Patients with recent vitamin B12 administration, patients taking medicine like aspirin, anticonvulsants, colchicines, patients on vegetarian diet,and patients with renal insufficiency were excluded.

Fasting blood samples were collected for biochemical parameters. The blood samples were centrifuged at 4000 rpm for 10 min, aliquot extracted and stored at -20°C until analysis.

The serum concentrations of Glucose and Creatinine were determined using fully automated chemistry analyzer, BT 3000 (Biotechnica Instrument, Via Licenza, Rome). HbA_{1c} was measured by Nycocard (Axis-Shield, Oslo, Norway), an immunometric assay. Serum Vitamin B12 was measured using the fully automated enhanced chemiluminiscent immuno analyzer (Vitros ECi, Ortho Clinical Diagnostics,Buckinghamshire,England). The CVs of Vitamin B12 at level 1 and 3 were 1.8% and 2.1% respectively.Daily internal quality controls were run for all the above mentioned analytes. Serum C-peptide was measured using DRG ELISA (Enzyme Linked Immunosorbent Assay) kit (DRG-International, Springfield, USA).

The data was analyzed using Statistical Analysis Software SPSS (Statistical package for social sciences) version 22.0. Analyses included standard descriptive variable summaries, measures of distribution with frequency tables and quantitative variables expressed in terms of mean ± SD (Standard Deviation). Scale variables (Quantitative) were tested for statistical significance using Pearson's Correlation and values of "p" less than 0.05 were considered statistically significant. Mean values of study variables were also compared between and within categories using standard independent samples t-tests and Analysis of Variance (when comparing across more than two groups) and values of "p" less than 0.05 were considered significant measures of association between the variables.

RESULTS

The mean age of patients with T1D Mwas 10.44 ± 3.68 years. The age ranges from 4 years to 18 years. The mean age for the male patient was 10.6 ± 3.74 years and for the female patient was 10.26 ± 3.71 years. The study population included 21 male and 19 female. A total of 30 healthy non diabetics were also included in the study. Table 1 shows the characteristics of the study population.

Table1. Characteristics of the study population expressed as mean ± SD or median (range) as possible.

Variable	Type 1 Diabetic (n = 40)	Non-Diabetic (n = 30)	P-value
Duration of diabetes (years)	1.5 (0.083-8)	NA	NA
Age (years)	10.44 ± 3.68	9.4 ± 2.53	0.013
Body Mass Index (BMI) (kg/m^2)	16.43 ± 2.49	20.27 ± 2.23	0.60
Fasting blood glucose (mmol/L)	16.38±6.08	4.85±0.59	<0.001
HbA1c (mmol/mol)	96.83 ±26.09	29.9±4.36	<0.001
Creatinine (μmol/L)	70.74±15.03	64.55±15.0	0.61
C-peptide (nmol/L)	0.75±0.38	1.67±0.75	0.001
Vitamin B12 (pmol/L)	206.92±82.18	340.71 ± 136.02	<0.001

NA: Not Applicable,

Among total patients 19 (47.5%) were recently diagnosed and time of diagnosis was less than one year, 19 (47.5%) were between 1 year to 4 years and rest were diagnosed more than 4 years ago.The mean serum C-peptide of diabetic group was 0.75±0.38nmol/L and that of healthy group was 1.67±0.75nmol/L(Figure 1). The difference between the mean of two groups was statistically significant with P value< 0.005.The mean C-peptide level of those diagnosed before one year was 0.79 ± 0.39 nmol/L and those diagnosed for more than one year was 0.66 ± 0.33nmol/L. No significant difference was observed between the means of these two groups. Similarly, the mean difference in serum C-peptide level of those presented with diabetic ketoacidosis (DKA) and without DKA was also insignificant, P = .71.

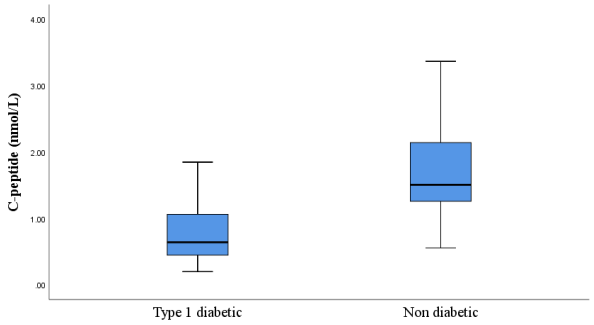

Figure1. Boxplot showing mean levels of serum C-peptide in Type 1 diabetic and Non diabetic group.

The mean serum vitamin B12 level of the diabetic was 206.92±82.18 pmol/L. Among the total cases 40.0% i.e. 16 out of 40 was found to be deficient and 37.5 % i.e. 15 out of 40 were subclinically deficient using the manufacturer's cut- off. Rest of population (22.5%) had normal vitamin B12 level. Whereas the mean serum vitamin B12 level of the healthy group was 340.71±136.02 pmol/L. Out of 30, 2 of them (6.7%) were deficient, 8 out of 30 (26.75) were found to be sub clinically deficient and 20 out of 30 (66.7%)had normal vitamin B12 level.(Figure 2)

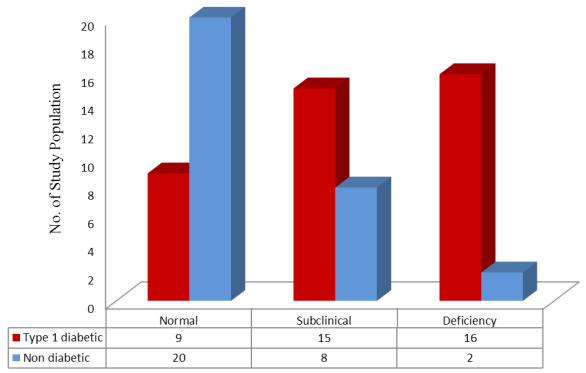

Figure 2. Vitamin B12 status in Type 1 Diabetic and Non-diabetic group.

When serum B12 levels were analyzed based on the published cut- off of 148 pmol/L (200 pg/ml), 22.5% were deficient, 57.5% were deficient subclinically and 20% had normal serum vitamin B12 in Type 1 diabetic.

Significant difference was noticed in the mean serum level of vitamin B12 between two groups (diabetic and non-diabetic), P=<0.001.(Figure 3) Furthermore significant difference was observed between diabetic and non-diabetic for each category of vitamin B12 level; X^2 (2, N = 70) = 19.01, p < .01.

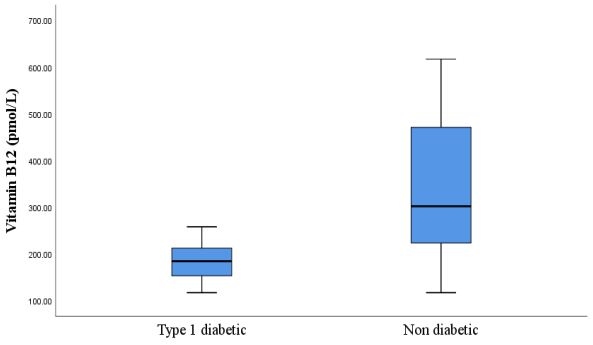

Figure3. Boxplot showing mean levels of serum Vitamin B12 in Type 1 diabetic and Non-diabetic.

There was no significant difference in vitamin B12 level between male (mean: 201.82± 67.59 pmol/L,) and female (mean: 212.54 ± 97.42 pmol/L), P = 0.83. Table 2 shows the correlation between vitamin B12 levels and age, BMI, duration of diabetes and diabetic control (HbA1c).

Table 2. Correlation of vitamin B12 levels with different variables

Variables	Pearson's Correlation Coefficient, r	P value
Vitamin B12 and Age	-0.18	0.25
Vitamin B12 and BMI	-0.5	0.75
Vitamin B12 and Duration of disease	0.13	0.42
Vitamin B12 and HbA1c	0.03	0.84

DISCUSSION

T1DM once considered as rare condition, now has significant impact in global health. Around 79,100 children under 15 years are estimated to develop T1DM annually worldwide.[10] Type 1 diabetes is diagnosed on the basis of clinical conditions and World Health Organization (WHO) criteria. No such laboratory tests are available to differentiate Type 1 diabetes from other types of diabetes. However there are various tests, which support the presence of Type 1 diabetes in an individual, one of them being C-peptide level assessment.[11] Being an autoimmune condition various antibodies are associated with Type 1 diabetes including GAD65, IA-2, IAA, ICA, and ZnT8. Presence of these autoantibodies in combination can diagnose Type 1 diabetes with high sensitivity and

specificity.[12] As these tests are expensive and not easily available in all laboratories,clinical symptoms and WHO criteria to diagnose Type 1 diabetes.

This study assessed the level of C-peptide in the patients diagnosed and treated as T1DM. The fact that most patients needed insulin shortly after their diagnoses, were not overweight and lacked a significant family history of diabetes in more than one generation make other diagnoses, such as Type2 diabetes and Maturity Onset Diabetes of the Young, unlikely. As the renal insufficiency can influence the serum C-peptide level,[13] this study measured the serum creatinine level in all patients, to rule out the bias in C-peptide level due to renal condition. The serum creatinine level was normal in all the patients, reflecting that alter in C-peptide level is not because of renal involvement.

In this study the mean level of C-peptide in patient with diabetes was 0.75±0.38 nmol/L. The study also evaluate C-peptide level in healthy group who fulfilled the criteria of normal fasting blood glucose and normal serum creatinine level, the mean level of C-peptide in this group was 1.67±0.75 nmol/L. The difference in the mean of two groups was found to be statistically significant. More likely the low mean C-peptide level in the diabetic compared to the non diabetic might be due to Type 1 diabetic status.

Multiple co-morbidities must be considered while dealing with Type 1 diabetes. Patient with Type 1 diabetes are at increased risk for other organ-specific autoimmune diseases, such as thyroid autoimmunity and Addison's disease. Graves' disease and hypothyroidism are so prevalent in Type 1 diabetes that routine testing of thyroid-stimulating hormone is common.[14,15] Concomitant presence of these autoimmune conditions with Type 1 diabetes increases the risk for impaired vitamin B12 absorption.[16] Besides,in autoimmune patients with Type 1 diabetes, presence of PCA and antibodies to intrinsic factor have been demonstrated.[8] These factors might be responsible for the low vitamin B12 level in Type 1 diabetes. So, Vitamin B12 deficiency might be potential co-morbidity that might have been overlooked. Defining the prevalence of low serum vitamin B12 levels in the diabetic population may help determine whether physicians should consider screening for vitamin B12 levels in patients with diabetes and carry out further evaluation. In addition, symptoms of vitamin B12 deficiency occur late. Vitamin B12 deficiency induced nerve damage may be confused with or may contribute to diabetic peripheral neuropathy. Identifying the correct etiology of neuropathy is crucial because simple vitamin B12 replacement may reverse the neurologic symptoms inappropriately attributed to hyperglycemia.[17]

Tests to measure and quantify serum vitamin B12 levels in the body are readily available and inexpensive. However, the screening test has some limitations and drawbacks. Themain drawback is that there is no universally accepted serum vitamin B12 cut-off to define deficiency although the value of <148 pmol/L is often used, and at this serum vitamin B12 level or below, metabolites like serum homocysteine, serum and urine methylmalonic acid (MMA), become elevated.[18] The World Health Organization has suggested use of this cut-off to define vitamin B12 deficiency.[19] When serum vitamin B12 results are normal but still the clinical suspicion of deficiency exists, additional 'confirmatory testing' may help to identify vitamin B12 deficiency. There is compensatory elevation of homocysteine and MMA levels preceding the drop in serum vitamin B12 deficiency and these are regarded as more sensitive indicators of vitamin B12 deficiency than just low serum vitamin B12 level. Elevated serum homocysteine and MMA level has a sensitivity of 95.9% and 98.4%, respectively to diagnose vitamin B12 deficiency.[20,21] As these tests are more expensive, not readily available, and reference intervals are not standardized,they are not used as the initial test to diagnose vitamin B12 deficiency.

That said, this study used serum vitamin B12 level as initial marker for vitamin B12 status and observed high prevalence of low serum vitamin B12 level with the mean of 206.92±82.18 pmol/L in diabetic group. Similar result was observed in the study conducted by Ann Sarah Koshy et al.[6] Study done by P. Perros et al. and Davis R. E et al.also concluded that the risk of developing pernicious anemia is very high in Type 1 Diabetes.[16, 22] Similarly William J. Riley et al.in their study enforced that young patients with T1DM are at risk for atrophic gastritis and cobalamin deficiency can initially be identified by screening for PCA.[23] Hunger-Battefeld W et al.observed that there was increase in parietal cell antibodies by 15.8% and pernicious anemia by 7.2% in T1DM.[24]

Healthy non diabetic group were also underwent vitamin B12 assay and the difference in the mean of two groups came out to be significant, indicating that the low vitamin B12 level in patient with Type 1 diabetic was not merely the attribute of population.This study also compared the serum vitamin B12 status in manufacture cut off value (176 pmol/L to 687 pmol/L as reference interval)and published meta-analysis cut

off value(<148 pmol/l as deficiency, 148 pmol/L to 258 pmol/L as subclinical deficiency). Using manufacture cut off value 40.0% i.e. 16 out of 40 of patients were found to be deficient and 37.5 % i.e. 15 out of 40 were subclinically deficient but using published cut- off value 22.5% were found deficient and 57.5% were deficient subclinically. The difference in the prevalence of low vitamin B12 levels due to different cut- off values used has been reported in many studies in the past.[25] As this study did not performed additional confirmatory tests the predictive value of these two cut off points could not be assessed.

Besides, there was no significant difference observed in vitamin B12 level in male and female. However study done by Gonzalez-Gross, M et al.showed that females had significantly higher Cobalamin.[26] This study could not find the relation between Vitamin B12 and BMI. But study done by Pinhas-Hamiel, O et al.indicated that obesity in children and adolescents was associated with an increased risk of low vitamin B12 concentration.[27] Same result was obtained by Baltaci, D et al.[28] Moreover this study did not show any correlation between vitamin B12 and age, duration of diabetes and diabetic control.

So far low level of vitamin B12 is estimated to affect 10 -15% of people over the age of 60.[29] As this study includes patient below 60 years, average age being 10 years, age cannot be considered as a risk factor for low level of vitamin B12. Gender bias was also ruled out since there was equal representation of male and female. Hence it appearsthat factors other than age, gender, diet, drugs, duration of diabetes, and diabetic control might play a role in insufficiency or deficiency of vitamin B12. A detailed history regarding malabsorption was not available and hence that needs to be addressed. Since the study did not evaluate the presence of various antibodies like PCA, intrinsic factor antibodies and genetic susceptibility, the role of autoimmune antibodies and genetic involvement for low vitamin B12 level cannot be ruled out.

CONCLUSIONS

This study demonstrated serum vitamin B12 deficiency in most of the children with Type 1 diabetes. The routine screening for this condition along with confirmatory tests and detail clinical examination could benefit the patients with Type 1 diabetes. However, further studies in a larger population using additional tests to investigate the actual cause of deficiency are must to strengthen this statement.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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