ORIGINAL ARTICLE

Serum Vitamin B12 Level and Dietary intake in Adult Atopic Dermatitis: A Case Control Study

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Abstract

Background

Vitamin B12 is a contributing factor in pruritus and peripheral nerve regeneration. Its role in atopic dermatitis (AD) is still unclear. This study aimed to compare vitamin B12 level between AD patients and healthy controls, determine its correlation with pruritus and AD severity, and evaluate dietary pattern with energy, macro and micronutrient intakes.

Methods

This was a case control study involving adult AD patients and age-, gender-, ethnicity- and body mass index-matched healthy controls. All adult patients who fulfilled UK Working Party AD diagnostic criteria were included. Exclusion criteria include patients on systemic agents, diseases known to affect B12 level and vegan diet. AD severity was determined using SCORing Atopic Dermatitis (SCORAD) index. Serum vitamin B12 level were measured. A three-day 24-hour dietary recall was collected and analyzed.

Results

A total of 42 AD patients and 42 controls were recruited. Mean SCORAD index was 39.2±16.6, and AD duration was 12.7 ± 8.1 years. Vitamin B12 was lower among AD (215.6 ± 110.2 pmol/L) versus control (295.1± 119.9 pmol/L), $p<0.01$ despite similar dietary B12 intake in both groups. There were no significant correlations between AD duration and severity with vitamin B12 level. Energy intake (kcal/day) was significantly lower in AD ($p=0.04$). There were no significant differences in proportion of main food groups consumed and other macronutrient and micronutrient intakes.

Conclusion

Serum vitamin B12 level was significantly lower in AD patients despite similar dietary pattern and nutrient intake with healthy controls. There were no correlations with AD severity or disease duration. Dietary pattern of AD patients should be routinely assessed to ensure adequate nutrition.

Key Words: C*obalamin, B12, Atopic dermatitis, Nutrition*

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Introduction

Atopic dermatitis (AD) is a common chronic inflammatory, pruritic skin disorder affecting up to 10% of the adult population.[1] Micronutrients may have an impact on atopic dermatitis as proper nutrition, especially vitamins, minerals,

and trace elements play an active role in immune health. The relationship between nutrition and atopic pathogenesis has been debated for many years.[2] Current knowledge is still not sufficient to discern the exact role of specific vitamins and trace minerals on AD.

Vitamin B12 (cobalamin) is a chemical compound with vitamin properties, that is mainly present in sufficient amount in animal-derived foods.[3] Self-imposed dietary restriction (i.e. red meat, egg, seafood) without consulting a doctor or dietitian is a common practice among AD patients.[4,5] The lack of supervision of these dietary modifications has been associated with risk of nutrient deficiency in both children and adults.[6-8] A local study in toddlers with AD[6] yielded a non-significant lower level of serum vitamin B12 among food restricted group. However, clinical manifestation of B12 deficiency from diminished intake or absorption may not manifest for several years after the depletion of body stores.[9] Furthermore, prevalence of vitamin B12 deficiency is difficult to assess since under-diagnosis is likely and subclinical disease is considered not uncommon.[10]

Vitamin B12 precursor has been shown to have a potent action as a nitric oxide scavenger in inflammatory conditions. The ability of vitamin B12 to regulate inflammatory cytokines suggests that it may have antioxidative properties.[11] Topical vitamin B12 has some efficacy in the treatment of atopic dermatitis.[12]

Hence, the aim of this case control study is to compare serum vitamin B12 level between AD patients and healthy volunteers. Correlations between serum B12 level with AD severity, disease duration and pruritus were determined. Dietary pattern, energy and other macro- and micronutrient intakes were also assessed.

Materials and Methods

A case control study was performed. Patients with AD attending Dermatology clinic Hospital Tengku Ampuan Afzan were screened and recruited from August 2020 until May 2021. Healthy age, gender, ethnicity and body mass index-matched volunteers constituted the control group. We included patients aged 18 years old and older who met the criteria for atopic dermatitis based on the UK Working Party Atopic Dermatitis diagnostic criteria.[13] Exclusion criteria were (i) patients on systemic agents which include azathioprine, methotrexate and cyclosporin (ii) patients with other pruritic conditions including urticaria, (iii) generalized hyperpigmentation, (iv) atrophic glossitis, (v) patients conditions known to affect vitamin B12 level which include pernicious anemia , hypochlorhydria due to atrophic gastritis, partial or total gastrectomy, bariatric surgery, ileal resection of >20 cm, malabsorptive disorders, short bowel syndrome, inflammation of the ileum – eg: Crohn's disease, celiac disease, chronic pancreatitis, small intestine bacterial overgrowth, Whipple's disease, ongoing/ previous history of gastric cancer/gastrectomy, (vi) patients on medications affecting vitamin B12 level which include B12 supplement, proton pump inhibitor, H2 receptor antagonist and metformin, (vii) patients on vegan diet, and (viii) pregnancy.

Demographic data of the participants and their body mass index (BMI; weight [kg]/height m^2) and waist circumference (cm) were recorded, as was the SCORing Atopic Dermatitis (SCORAD),[14] and Dermatology Life Quality Index (DLQI) score[15] for the cases. Severity in the SCORAD index is classified as mild (<25), moderate (25-50), and severe (>50). The maximum score is 103. All participants were asked to recall their 3-day dietary intake which consists of two weekdays and one weekend. Description include the type of food or beverage consumed and portion size and cooking methods. Portion size was determined using a common food guide (cups, bowls, tablespoon, teaspoons, glasses).

Participants are first asked about all the food they consumed within the last 24-hours, followed by a thorough probing whereby they detailed information for each food/beverage, for the

type, amount, any addition to the food/toppings, preparation methods including the type of oil/fat used is obtained. If the food was packaged, the brand name, as well as the amount consumed, was obtained. Finally, the record of foods and the amount consumed was reviewed, to provide the investigator a chance to clarify any unclear information.[16] To understand food preferences, main food groups consumed, meat (chicken, beef, and pork), seafood (fish, crustaceans, and mollusk), vegetables, egg, milk and peanut, cakes and sweets were examined.

Dietary intake was analyzed using Nutritionist Pro™ Software (Axxya Systems, the United States Department of Agriculture (USDA) Standard Reference Database, First DataBank, Inc., San Bruno, California) for macronutrients and micronutrients. The Nutritionist Pro™ software contains Malaysian Food composition databases as well as other international databases such as USDA Food Database, Canadian Food Database, and Mexican Food Database. Schofield's equation was used to determine the basal metabolic rate (BMR). The Energy Intake (EI): BMR ratio was used to identified under reporters. Classification of the EI: BMR ratio into under reporters (EI: BMR<1.2), plausible (EI: BMR 1.2-2.4), and over reporters (EI: BMR>2.4) were used.[17]

Venous blood was obtained for serum B12 levels. Assays for serum B12 level was performed using Access® Immunoassay System Model DxI 800 UniCel®, which use chemiluminescent paramagnetic microparticle immunoassay (assay range 133 – 675 pmol/L) from (Beckman Coulter, USA). Basic hematological profile was measured as well. These tests were conducted in the Pathology Department of Hospital Tengku Ampuan Afzan, Pahang.

This study was approved by Medical Research and Ethical Committee (MREC), Malaysia with research code NMRR-20-1768-55814. The sample size of this study was calculated using the statistical online software OpenEpi Version 3[18] based on the case-control study by Polat, M., et al.[19] Fourty two subjects in each group were needed to be able to reject the null hypothesis with a probability power of 0.9.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS Version 24.0, IBM Corp). Most continuous variables were normally distributed and were summarized as mean and standard deviation (SD). Qualitative data were expressed as percentages. The chi-square test and t test were used in analyzing differences between groups. The significance level was set at $p < 0.05$. Correlations between variables were tested with the Pearson correlation analysis. Significance was determined according to the two tailed alternative hypothesis, and results were deemed significant for p values < 0.05.

Results

A total 33 men and 31 women were enrolled in the study. The case and control groups were matched for age, sex, race and body mass index. The mean age of participants was 28.1 ± 7.2 years. Majority of the participants are female (60.7%) and Malays (86.9%). The mean BMI was similar with 25.3 ± 5.9 and 23.8 ±5.1 in the case and control groups, respectively, ($p = 0.2114$). There was significant difference in the proportion with comorbidities between AD patients (45.2%) and controls (16.7%), $p < 0.01$.

Table 3 shows there is lower energy intake among AD patients AD 1589.85 kcal±406.69 versus control 1755.61 kcal±331.12, ($p=0.04$). However despite trend of lower intake of protein, carbohydrate, and fat, these differences were not significant ($p=0.27$, $p=0.06$, $p=0.22$ respectively). No significant differences noted on intake of all other micronutrients.

Serum vitamin B12 levels were within the normal range of 133–675 pmol/L. In the case group, the mean serum vitamin B12 level was lower 215.6±110.2 pmol/L and in the control group it was 295.1±119.9 pg/ml, which was statistically significant (p=0.002179). The mean dietary B12 intake were not statistically difference in both group(AD 3.09 ± 2.07 mcg/

day versus control 3.55±1.78 mcg/day, *p=0.29*).

The mean eosinophil and platelet to lymphocyte ratio were significantly higher (AD 7.6 ±7.3 and 14.4%±7.5%, respectively) compared to control group (*p=0.0002953; p=0.02058* respectively). There is significant correlation of eosinophil with the SCORAD which showed that higher eosinophil percentage correlated with higher SCORAD index score (*p=0.009*, Pearson correlation *r=0.397*).

Mean duration of disease among AD patients were 12.7±8.1 years. The mean SCORAD index scores in the case group was 39.2±16.6. Pearson correlation analysis showed higher SCORAD correlated with higher DLQI (*r=0.457, p=0.002*). However there were no significant correlations between serum vitamin B12 level and disease severity (SCORAD) (*r=0.068, p=0.669*), VAS pruritus (*r=-0.021, p=0.896*), and duration of disease(*r=-0.111, p=0.486*).

Table 1. Demographic data

Demographic data	Total (n=84)		*p – value*
	Case (n=42) Mean±SD or n (%)	Control (n=42) Mean±SD or n (%)	
Age, in years[a]	28.0 ± 7.2	28.2 ± 7.1	0.94
Gender[b]			
Male	16 (38.1%)	17 (40.5%)	0.82
Female	26 (61.9%)	25 (59.5%)	
Race[b]			
Chinese	4 (9.5%)	5 (11.9%)	0.92
Indian	1 (2.4%)	1 (2.4%)	
Malay	37 (88.1%)	36 (85.7%)	
Comorbidities	19 (45.2%)	7 (16.7%)	<0.01
Bronchial asthma	1 0(23.8%)	0	
Allergic Rhinitis	4 (9.5%)	0	
Endometriosis	1 (2.3%)	0	
Hypertension	1 (2.3%)	0	
Acne	7 (16.7%)	7 (16.7%)	
Smoking	5 (11.9%)	5 (11.9%)	1.00
Alcohol	1 (2.4)	1 (2.4)	1.00

[a]Independent t test; [b]Chi Square test

Significant differences were noted among the proportion of AD subjects, there were no significant differences between proportion of food pattern intake among participants (Table 4). There was less proportion (5/42 subjects) among AD subjects who consumed nuts (which include peanut/ groundnut, almond , and cashew) however this was not statistically significant (11.9% in AD subjects versus 28.6% in control, *p=0.06*).

Table 2. Clinical characteristic and laboratory parameters

	Case (n=42) Mean ± SD or n (%)	Control (n=42) Mean ± SD or n (%)	*p value*
Clinical characteristicst[a]			
BMI[1], in kg/m2	25.3 ± 5.9	23.8 ± 5.1	0.21
Waist circumference, in cm	81.1 ± 10.7	79.7 ± 9.7	0.56
SCORAD[2]	39.2 ± 16.6	NA	NA
Severity			
Mild(<25)	11(26.2%)	NA	NA
Moderate (25-50)	20(47.6%)	NA	NA
Severe(>50)	11(26.2%)	NA	NA
VAS pruritus	6.1 ± 1.7	NA	NA
Duration of atopic dermatitis, in years	12.7 ± 8.1	NA	NA
DLQI score[3]	12.9 ± 6.2	NA	NA
Laboratory Parameter[a]			
Serum vitamin B12(pmol/L)	**215.6 ± 110.2**	**295.1 ± 119.9**	**<0.01**
Hemoglobin (g/dl)	13.9 ± 1.5	13.7 ± 1.6	0.55
PCV[4] (%)	41.6 ± 3.9	40.4 ± 4.1	0.16
MCV[5] (fL)	80.1 ± 9.0	81.6 ± 7.1	0.37
MCH[6] (pg)	26.8 ± 3.4	27.8 ± 2.7	0.13
WBC[7] (10^9/L)	9.6 ± 5.7	8.4 ± 4.3	0.31
Neutrophil (%)	57.4 ± 13.3	57.9 ± 9.5	0.84
Lymphocyte (%)	26.6 ± 10.2	30.6 ± 8.4	0.06
Eosinophil (%)	**7.6 ± 7.3**	**3.1 ± 2.6**	**<0.01**
Platelet (10^9/L)	330.3 ± 76.7	316.3 ± 70.7	0.39
Absolute neutrophil count(ANC)	5.2 ± 2.7	4.9 ± 2.8	0.61
Neutrophil to Lymphocyte Ratio (NLR)	2.7 ± 1.9	2.1 ± 1.0	0.10
Platelet to Lymphocyte Ratio (PLR)	**14.4 ± 7.5**	**11.2 ± 4.1**	**0.02**

[a]Independent t test; [b]Chi Square test; [1]Body Mass Index; [2]SCORing Atopic Dermatitis; [3]Dermatology Life Quality Index; [4]Packed Cell Volume; [5]Mean Corpuscular Volume; [6]Mean Corpuscular Hemoglobin; [7]White Blood Cell; NA: Not applicable; VAS: Visual Analogue Score

Table 3. Energy and nutrient intake

Nutrient intake per day	Total (n=84)[a]		*p – value*
	Case (n=42) Mean ± SD	Control (n=42) Mean ± SD	
BMR[1](kcal)	1513.50 ± 234.12	1496.48 ± 280.91	0.76
EI[2] (kcal)	**1589.85 ± 406.69**	**1755.61 ± 331.12**	**0.04**
EI: BMR Ratio	**1.07 ± 0.28**	**1.19 ± 0.23**	**0.02**
Protein (g)	67.94 ± 20.82	72.81 ± 19.45	0.27
Carbohydrate (g)	208.24 ± 64.78	234.16 ± 61.15	0.06
Fat (g)	53.38 ± 19.61	58.17 ± 15.85	0.22
SFA[3] (g)	11.53 ± 6.82	10.44 ± 5.61	0.42
MSFAT[4] (g)	11.70 ± 7.66	11.97 ± 7.62	0.87
PUFAT[5] (g)	9.09 ± 5.29	10.95 ± 7.25	0.18
Vitamin A (IU)	4582.65 ±2571.05	4700.60 ± 3340.15	0.86
Beta Carotene (µg)	954.87 ± 1238.95	1038.39 ±1642.26	0.79
Vitamin C (mg)	34.24 ± 34.48	34.16 ± 33.71	0.99
Vitamin D (IU)	45.85 ± 56.77	48.21 ± 75.46	0.88
Vitamin E (IU)	6.61 ± 3.94	7.82 ± 5.60	0.26
Thiamine (mg)	0.74 ±0.40	0.71 ± 0.30	0.72
Riboflavin (mg)	1.14 ± 0.56	1.07 ± 0.46	0.53
Niacin (mg)	14.43 ± 6.38	15.08 ± 7.33	0.66
Pyridoxine (mg)	0.93 ± 0.54	0.92 ± 0.54	0.93
Pantothenic acid (mg)	1.04 ± 0.78	1.11 ± 1.23	0.75
Folate (µg)	103.71 ± 65.00	100.35 ± 73.99	0.83
Vitamin B12 (µg)	3.09 ± 2.07	3.55 ± 1.78	0.29
Calcium (mg)	353.84 ± 198.35	372.54 ± 182.04	0.65
Phosphorus (mg)	926.17 ± 338.32	913.28 ± 254.88	0.84
Iron (mg)	19.46 ± 9.74	19.77 ± 10.71	0.89
Zinc (mg)	4.41 ± 1.80	4.42 ± 2.31	0.98
Copper (mg)	0.69 ± 0.40	0.64 ± 0.33	0.54
Magnesium (mg)	122.76 ± 57.69	118.79 ± 57.57	0.75
Total dietary fiber (g)	5.07 ± 3.75	3.89 ± 3.32	0.13

[a]Independent t test; [1]Basal Metabolic Rate; [2]Energy intake; [3]Saturated fatty acids; [4]Monounsaturated fat;
[5]Polyunsaturated fat

Table 4. Dietary pattern

Dietary Pattern	Total (n=84) [b]		*p – value*
	Case (n=42) n (%)	Control (n=42) n (%)	
Poultry	35 (83.3%)	39(92.9%)	0.18
Beef	10 (23.8%)	14 (33.3%)	0.33
Fish	26 (61.9%)	27 (64.3%)	0.82
Wheat	24 (57.1%)	28 (66.7%)	0.37
Dairy	5 (11.9%)	9 (21.4%)	0.24
Egg	24 (57.1%)	30 (71.4%)	0.17
Nuts	5 (11.9%)	12 (28.6%)	0.06
Shellfish	9 (21.4%)	12 (28.6%)	0.45
Vegetables	31 (73.8%)	28 (66.7%)	0.47
Fruits	9 (21.4%)	10 (23.8%)	0.79
Cakes & sweets	15 (35.7%)	15 (35.7%)	1.00

[b]Chi Square test

There were 32 (38.1%) under reporters (EI:BMR ratio <1.2) and 52 (61.9%) plausible reporters (EI:BMR ratio 1.2-2.4). There was no over reporters (EI: BMR ratio >2.4) among the participants (Table 5).

Table 5. Energy Intake - BMR ratio (EBR)

EI[1]:BMR[2] ratio (EBR)	Participants		Total (%)
	Case	Control	
EBR <1.2 (under reporters)	14	18	32 (38.1)
EBR 1.2-2.4 (Plausible)	28	24	52 (61.9)
EBR >2.4 (over reporters)	0	0	0

Energy intake; Basal Metabolic Rate

Discussion

Vitamin B12 supports myelinization and axonal transport, thus helping regeneration of peripheral nerve cells.[20] Its deficiency plays a role in the etiology of neuropathic itching by causing small-fiber neuropathy.[21] Anti-inflammatory and anti-oxidative properties of vitamin B12 are exerted by downregulation of pro-inflammatory cytokines and reduction of nitric oxide. Low vitamin B12 has been associated with chronic pruritic dermatoses including generalized pruritus[19] and chronic spontaneous urticaria.[22] Robust itch sensations, increased neuronal activity, and hyperinnervation are observed in AD skin.[23] Chesini and Caminati[24] described a patient with severe, refractory AD and low serum vitamin B12. Subsequent correction of his vitamin B12 level with oral supplementation resulted in significant AD improvement. Therefore, assessment of vitamin B12 level in patients with difficult-to-control atopic dermatitis was suggested.[24] Emerging data indicate that a topical vitamin B12 preparation may prevent atopic dermatitis flares due to its high affinity binding to nitric oxide produced by keratinocyte.[25,26]

Our findings showed significantly lower serum vitamin B12 level in AD patients compared to healthy controls despite similar dietary patterns and intake of macronutrients and other micronutrients. Mean serum vitamin B12 levels were within normal limits in both groups. We postulate that long standing utilization of tissue

vitamin B12 in maintaining and regenerating peripheral nerve cells in AD may have resulted in reduction in the level of serum vitamin B12. The historical lower normal value for serum vitamin B12 was 148 pmol/L[27] compared to our reference value of 133 pmol/L with overall coefficient of variation of performance of 5–15% between different assays and inter-method biases of plus 10% or minus 20% from the all-laboratory trimmed mean.[28] However, it is still unknown whether serum B12 correlated with its tissue level. Additionally, level that represent subclinical deficiency, i.e., a low serum vitamin B12 in the absence of clinical symptoms is unclear.[29,30] Raising the lower range to 258 ng/L has been considered, but the benefit of detecting subclinical deficiencies may be limited as there are few associated metabolic abnormalities[31] and a small number of subclinical deficiencies that progress to clinical deficiencies. Low-normal levels with potential effect on patients' health are often ignored by physicians and even genuinely low levels may be ignored if neuropathy or anaemia are not evident. Further testing using a functional test such as methylmalonic acid (MMA) assay is recommended for levels <221 pmol/L.[32]

Subclinical reduction in vitamin B12 due to subclinical malabsorption and increased utilization of proliferating tissue have been postulated to contribute to inflammation in AD (33). Vitamin B12 modulates inflammation cascade by suppressing production of T lymphocytes-derived cytokines, in particular interleukin-6 (IL-6), interferon-gamma (IFN-gamma), and interleukin-1 beta (IL-1 beta).[34] Inverse correlations between chronicity of AD and severity of pruritus with serum vitamin B12 level was demonstrated in our cohort, which supports this postulation. However, our study was not sufficiently powered to determine this relationship.

Dietary pattern and nutrient intake were estimated using 3 days diet recall in our study. Vitamin B12 was slight lower than the recommended nutrient intake (RNI) for Malaysia at 4 microgram per day.[35] This was not a contributing factor to the lower level of serum B12 in our AD cohort as the intake for both cases and control was similar. The validity of 24-hour dietary recall is dependent on the degree of accuracy in which respondents recall their food consumption. Under reporting of energy intake is defined as EI:BMR ratio below 1.2.[36] The prevalence of underreporting in our study was lower than Arumugam et al (77.4%)[37] and Zainuddin et al (61%).[17] Factors associated with underreporting include obesity, age, gender, social status and controlled eating habits.[38,39] Reporting bias may lead to a misinterpretation of the individual's nutritional state and may result in misleading associations between diet and disease.

Apart from that, serum eosinophils levels were also noted to be significantly higher in our cohort and the degree of severity also correlated with eosinophil level. This was consistent with previous studies.[40-42] We also notice a higher platelet to lymphocyte ratio which was in line with previous studies.[43-45] These simple indicators may serve as a potential adjunct markers of ongoing systemic inflammation in atopic dermatitis patients in the future.[43]

In our cohort, AD severity correlated well with the DLQI indicating its effect on patients' quality of life. This has to be emphasized in regards to aiming for optimal disease control to improve patients' daily activity and emotional burden. Patients with subclinical vitamin B12 deficiency are prone to symptoms of depression[46-48] and anxiety,[49] thus exacerbation or worsening of depressive symptoms may occur with a concomitant chronic disease like AD that significantly affects quality of life.

Limitation

Our study is limited by the possibility of recall bias with the 3-days dietary recall. Comprehensive evaluation of other biomarkers like homocysteine and methylmalonic acid were not possible due to unavailability of the assays at our centre. Other micronutrients level including vitamin D, zinc, folate and trace

mineral were beyond the scope of this study. Concurrent parasitic infestation was not tested in this study.

Conclusion

Serum vitamin B12 level was significantly lower in AD patients. There were no differences in dietary pattern, macronutrient and micronutrient intakes between patients with AD and healthy controls. Serum vitamin B12 level did not correlate with AD severity or disease duration. Although the role of vitamin B12 in AD is still unclear, dietary patterns of AD patients should be assessed to ensure adequate nutrient intake.

Conflict of Interest Declaration

The authors have no conflict of interest to declare.

Acknowledgement

We would like to thank the Director of General of Health Malaysia for his permission to publish this article.

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