

Lower serum B12 vitamin concentrations are not accompanied by hyperhomocysteinaemia in chronic spontaneous urticaria

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Summary

Background. Lower serum vitamin B12 concentrations have been observed in patients with chronic spontaneous urticaria (CSU). It is known that vitamin B12 deficiency is closely related to hyperhomocysteinaemia, which is associated with a proinflammatory state.

Aim. To assess the relationship between vitamin B12 status and concentrations of homocysteine (Hcy) with acute phase response in patients with CSU.

Methods. Circulating concentrations of vitamin B12, Hcy and C-reactive protein (CRP) were measured in 42 patients with CSU of varying severity, and compared with 19 healthy controls (HCs).

Results. Significantly lower concentrations of vitamin B12 and higher concentrations of CRP were observed in the serum of the patients with CSU compared with HCs ($P < 0.01$ and $P < 0.001$, respectively). However, there were no significant differences in plasma Hcy concentrations between the investigated groups. In addition, no correlations were found between the concentrations of vitamin B12, Hcy and CRP.

Conclusions. Lower values of vitamin B12 concentration in patients with CSU were not associated with higher Hcy concentrations, suggesting that such patients do not have functional vitamin B12 deficiency.

Introduction

Various changes in the levels of immune/inflammatory, coagulatory/fibrinolytic, hormonal and metabolic markers have been reported in patients with chronic spontaneous urticaria (CSU).^{1–9} Vitamin B12 deficiency is one of the most important determinants of hyperhomocysteinaemia, which is related to the proinflammatory state and results in an increased risk for atherosclerosis.^{10,11}

Measurement of serum levels of homocysteine (Hcy) may provide important new insights into the

mechanism by which vitamin B12 deficiency leads to different patterns and manifestations of the disease.¹² For patients with decreased serum vitamin B12 concentrations, measurement of Hcy and/or methylmalonic acid (MMA) concentrations is the most sensitive test of vitamin B12 deficiency.¹³ However, other factors can also affect Hcy concentration, including vitamins B2, B6 and B9 (folate) levels, as well as liver and renal disease, white cell count, methotrexate and antibiotics.^{11–13} It is known that lower serum vitamin B12 concentrations are found in patients with chronic urticaria.^{14,15}

We wanted to know whether the decrease in vitamin B12 concentration and systemic inflammatory state contributes to higher plasma Hcy concentration in patients with CSU. Therefore, the circulating concentrations of vitamin B12, Hcy and C-reactive protein (CRP) were measured in patients with CSU of varying severity and compared with those of healthy controls.

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Methods

The ethics committee of the Medical University of Silesia approved the study, and written informed consent was obtained from all participants.

Participants

We enrolled 42 patients with active CSU (29 women, 13 men; mean $\pm$ SD age 39 ± 9.0 years), with median disease duration of 3.0 years.

In all cases, any known causes of CSU were ruled out by appropriate investigations. Each patient underwent the following tests: routine laboratory tests (full blood count, urine analysis, liver function, erythrocyte sedimentation rate, CRP, serum glucose, and creatinine), stool testing (for parasites), hepatitis serology, antinuclear and anti-thyroid microsomal antibody [including anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG)] tests, thyroid function tests, chest radiography and abdominal ultrasonography. In addition, patients had dental, gynaecological, and ear, nose and throat consultations, and autologous serum skin test (ASST)¹ was performed.

The urticaria activity score (UAS) was estimated over 4 days. On the day of blood sampling, it was graded as follows: mild (0–8), moderate (9–16) or severe (17–24). The study comprised 23 patients with mild and 19 patients with moderate to severe urticaria symptoms.

None of the subjects had taken any oral corticosteroids within 5 weeks or antihistamines within at previous 5 days or any other drugs (including vitamin B12) before the study.

The control group consisted of 19 healthy individuals (12 women, 7 men) of comparable age (40.3 ± 8.0 years) and body mass index (BMI).

Blood collection

Blood samples were taken from the elbow veins of participants after fasting (approximately 8 h after the most recent meal). For Hcy assay the blood was collected into tripotassium EDTA Vacutainer Tubes (lavender/purple top), and kept on ice; the plasma was harvested within 30 min. The frozen samples were stored at -85°C until analysis.

Automated assay of C-reactive protein, vitamin B12 and homocysteine

Concentrations of serum CRP (immunoturbidimetric assay), plasma Hcy (enzymatic assay) (both Roche

Diagnostics, Mannheim, Germany) and vitamin B12 (electrochemiluminescence immunoassay-ECLIA) were measured on an automated system (Roche/Hitachi cobas c; Roche Diagnostics). Normal laboratory ranges were < 5.0 mg/L, 200–900 pg/mL and 5–15 $\mu\text{mol/L}$, respectively.

Statistical analysis

Results are expressed as median and interquartile range. The data were not distributed normally, thus nonparametric tests were used. The Kruskal–Wallis variance analysis was used to screen differences between the groups, while the Mann–Whitney *U*-test was used to compare data between the patient groups and the healthy population. Spearman rank test was used for correlations. $P < 0.05$ was considered statistically significant.

Results

Differences between groups

There were no significant differences between the moderate to severe CSU, mild CSU and HC groups for Hb (median 13.4 vs. 13.7 vs. 13.9 g/dL, respectively), MCV (87.7 vs. 85.6 vs. 87.2 fL, respectively) and BMI (24.9 vs. 24.61 vs. 24.17, respectively) ($P > 0.05$ for all). All values of Hb and MCV were within the normal range, and BMI was < 28 in all cases.

Serum vitamin B12 concentration

Serum vitamin B12 concentrations were significantly lower in patients with CSU than in HCs (median 275.5 vs. 364 pg/mL, $P < 0.01$ (Fig. 1). In addition, there were significant differences in serum vitamin B12 concentration between patients with CSU and HCs (median 277 vs. 364 pg/mL for mild CSU vs. HCs, $P < 0.05$; 247 vs. 364 pg/mL for mild moderate–severe CSU vs. HCs, $P < 0.01$) (Fig. 1). There was no significant difference between the two CSU groups ($P > 0.05$) (Fig. 1).

B12 values that were slightly below the lower limit were found in 1/23 (4%) of the mild CSU group and 5/19 (26%) of the moderate–severe CSU group.

No significant differences in vitamin B12 concentration were observed between ASST-positive and ASST-negative patients with CSU (selected according to similar UAS) (median 289 vs. 275 pg/mL, respectively, $P > 0.05$).

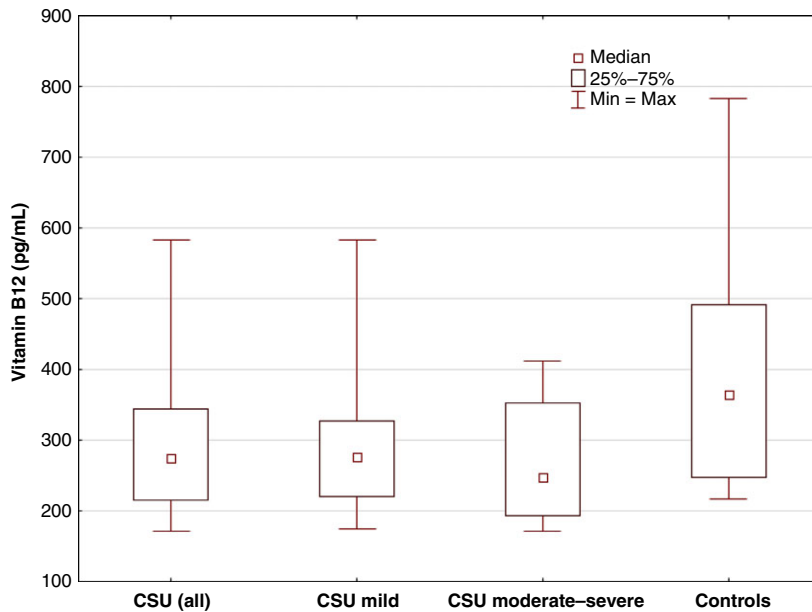


Figure 1 Serum vitamin B12 concentration in healthy controls (HCs) and patients with chronic spontaneous urticaria (CSU) with different disease activities. CSU (all) vs. HC, $P < 0.01$; CSU moderate-severe (m-s) vs. HC, $P < 0.01$; CSU mild vs. HC, $P < 0.05$; CSU m-s vs. CSU mild, $P > 0.05$.

Plasma homocysteine concentration

There were no significant differences in plasma Hcy concentration between the groups (Fig. 2). However, in 2/23 (8.5%) of the mild CSU group and 2/19 (10.5%) of the moderate/severe CSU group, Hcy values were beyond the upper limit. Higher Hcy values were associated with a lower vitamin B12 concentration in one patient with CSU.

Serum C-reactive protein concentration

Serum CRP concentrations were significantly higher in patients with CSU (as a whole) vs. HCs (median 2.85 vs. 0.5 mg/L, $P < 0.001$). There were significant differences in serum CRP concentration between the mild CSU, moderate-severe CSU and HC groups (median 1.2 vs. 0.5 mg/L for the mild CSU vs. HC groups, $P < 0.001$; and 10.3 vs. 0.5 mg/L for the moderate-severe CSU vs. HC groups, $P < 0.001$).

No significant differences in serum CRP concentrations were observed between the ASST-positive and ASST-negative patients with CSU (selected according to similar UAS).

Correlation between serum C-reactive protein, vitamin B12 and homocysteine concentrations

Significant positive correlations were found between concentrations of vitamin B12 and CRP in the HC group ($r = 0.67$, $P < 0.01$) but not in the patients with CSU ($r = -0.13$, $P = 0.41$). There were no

significant correlations between concentrations of CRP and Hcy in the HCs ($r = 0.17$, $P = 0.49$) or the patients with CSU ($r = 0.08$, $P = 0.63$), and there were no significant correlations between concentrations of vitamin B12 and Hcy in the HCs ($r = -0.01$, $P = 0.97$) or the patients with CSU ($r = 0.28$, $P = 0.07$).

Discussion

To our knowledge, there have been only two reports regarding vitamin B12 status in chronic urticaria.^{14,15} Lower serum vitamin B12 concentrations, accompanied by higher frequency of antigastric parietal cell antibodies in patients with CSU, suggest an autoimmune aetiology for vitamin B12 deficiency.¹⁴ Similar to the previous study,¹⁴ our cohort of patients with CSU had decreased serum vitamin B12 concentration compared with the HCs. In contrast to Mete *et al.*,¹⁴ we divided our patients with CSU according to disease severity/activity. There were significant differences in serum vitamin B12 concentrations between patients with CSU with mild and those with moderate-severe symptoms vs. HCs. However, concentrations of this vitamin did not differ significantly between the two CSU groups.

B12 values were slightly below the lower limit in 1/23 (4%) of the mild CSU group and 5/19 (26%) of the moderate-severe groups. Similarly, the previous study¹⁴ showed that about one-third of patients with CSU had serum vitamin B12 concentrations below the

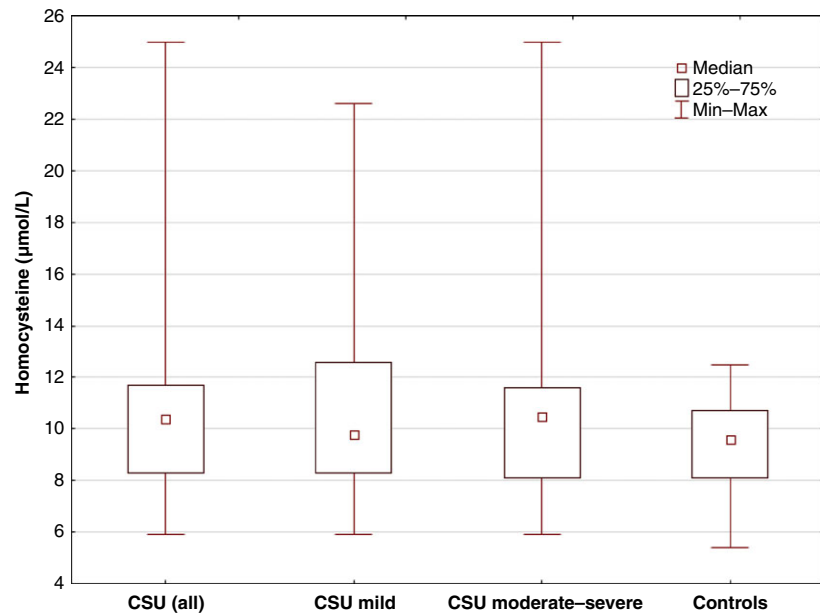


Figure 2 Plasma homocysteine concentration in healthy controls and patients with chronic spontaneous urticaria (CSU) with different disease activities. There were no significant differences in plasma homocysteine concentration between all investigated groups ($P > 0.05$).

normal reference range. The reason for lower B12 concentrations in some patients with CSU is unknown, and might suggest an autoimmune aetiology in B12 deficiency. Mete *et al.* observed higher frequencies of serological markers of autoimmunity (anti-thyroid and antigastric parietal cell antibodies) in patients with low B12 concentrations, but none of the patients with low vitamin B12 concentrations showed clinical evidence of B12 deficiency.¹⁴

One limitation of our study is the lack of data regarding the intrinsic factor or the parietal antibodies. However, anti-TPO was evaluated and showed that all values were < 35 IU/mL, while all values of anti-TG were within the normal range.

We then investigated if there were any differences in serum B12 concentrations between patients with negative and positive ASST after identifying the so-called autoreactive CSU. In our study, ASST-positive and ASST-negative patients with CSU had similar serum vitamin B12 concentrations, and all had negative results for serological markers of thyroid autoimmunity. Our results indicate that CSU may be accompanied by lower serum B12 concentrations regardless of ASST response (autoreactive origin). However, isolated low vitamin B12 concentrations are not synonymous with its true deficiency.^{16,17} Decreased vitamin B12 concentrations accompanied by elevation of Hcy and MMA are the most sensitive and specific indicators of functional vitamin B12 deficiency.^{10,18,19}

It seems important that many patients with low serum vitamin B12 concentrations are not vitamin B12-deficient.^{10,11} Vitamin B12 deficiency is relatively common, but the great majority of cases have sub-clinical vitamin B12 deficiency, which has no known clinical expression.¹³ Our study found no increase in plasma Hcy concentrations in patients with low vitamin B12 concentrations, which argues against functional deficiency. Chronic and systemic low-grade inflammation as determined by serum CRP is significantly increased in patients with more severe active CSU.²⁰ As expected, CRP, which is a biomarker of inflammation associated with CSU, was generally elevated in our patients with CSU. This observation may reflect vitamin B12 utilization in the presence of an underlying inflammatory process. Interestingly, it has been suggested that inflammation may lower folate and vitamin B12 status in patients with Behçet disease.²¹ However, our findings point to the lack of correlation between vitamin B12 and CRP, which suggests that vitamin B12 levels may not be a simple reflection of inflammation.

Conclusions

Lower values of vitamin B12 in patients with CSU were not associated with higher Hcy concentrations, which seems to argue against functional vitamin B12 deficiency in such patients. It may be important in future studies to determine the reasons underlying decreased serum vitamin B12 concentrations in CSU.

Acknowledgements

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What's already known about this topic?

- Lower serum vitamin B12 concentration has been observed in patients with CSU.
- It is known that vitamin B12 deficiency is closely related to hyperhomocysteinaemia, which is associated with a proinflammatory state.

What does this study add?

- Lower values of vitamin B12 concentration in CSU were not associated with higher Hcy concentrations, which seems to argue against functional vitamin B12 deficiency.
- CRP, a biomarker of inflammation associated with CSU, was generally elevated in patients with CSU.
- This observation may reflect vitamin B12 utilization in the presence of an underlying inflammatory process.

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