Title: A prospective analysis of micronutrient status in quiescent inflammatory bowel disease

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Keywords

Micronutrients, vitamins, minerals, trace elements, inflammatory bowel disease, nutrition

Abstract

Background and Aims

ESPEN guidelines advocate patients with inflammatory bowel disease (IBD) have their micronutrient levels checked regularly. This study described the micronutrient status of patients with quiescent IBD and explore whether biochemical micronutrient deficiencies related to time to subsequent disease relapse.

Methods

Sixteen micronutrients were measured prospectively in blood of patients with IBD in clinical remission [Harvey Bradshaw Index (HBI) ≤4 in Crohn's disease (CD) and a partial Mayo score <2 in ulcerative colitis (UC)]. Patients were followed prospectively using the electronic patient records. The ability of micronutrient status to predict time to relapse was tested with survival analysis and Cox regression.

Results

Ninety-three patients were enrolled; Fifty (54%) were also in biochemical remission defined as a normal faecal calprotectin (<250µg/g), C-reactive protein (<10 mg/L) and serum albumin (>35 g/L). Deficiencies in vitamin D were identified in 27(29%), zinc in 15(16%), vitamin B6 in 13(14%), vitamin C in 12(13%) and vitamin B12 in 10(11%). Fewer participants had low serum folate 7(8%), ferritin 8(9%), copper 4(4%), magnesium 4(4%) and plasma selenium 3(3%). Zinc deficiency was predictive of a shorter time to subsequent relapse (HR: 6.9; 95%CI [1.9 to 26], p=0.008); in sub analysis of those with CD this effect was even more profound (p=0.001).

Conclusion

We identified biochemical deficiencies for several micronutrients among adults with IBD clinically in remission. We have also highlighted a significant association between zinc deficiency and time to subsequent disease relapse in patients with CD which needs further investigation.

Introduction

Patients with inflammatory bowel disease (IBD) may be at risk of malnutrition[1]. The European Society of Clinical Nutrition and Metabolism (ESPEN) recommend regular screening for micronutrient deficiencies in this population[2]. This is rarely done in routine practice due to the limited availability of specialised laboratory facilities to undertake such analyses and the dearth of data to suggest that clinical presentations of micronutrient deficiencies are common, despite low biochemical levels often being reported[1]. The acute phase response perturbs plasma concentrations of certain vitamins and trace elements in active disease so it is often unclear the extent to which biochemical deficiencies reflect body store status or whether these are an epiphenomenon of the systemic inflammatory response (SIR)[3, 4].

In our tertiary referral unit, we introduced the ESPEN recommendations for micronutrient screening in clinical practice for patients with quiescent disease. Here, we present the prevalence of biochemical deficiencies for 16 micronutrients in adult patients with IBD with quiescent disease and explore their ability to predict a subsequent disease relapse.

Materials and Methods

Study population

We prospectively audited the micronutrient status of adult patients attending the Glasgow Royal Infirmary from September 2017 until July 2018. Participants eligible were in clinical remission, [Harvey Bradshaw Index ≤4 in Crohn's disease (CD) and a partial Mayo score <2 in ulcerative colitis (UC)]. Clinical data collected included Montreal classification of IBD phenotype and current medication. Plasma vitamin A, vitamin C, vitamin E/cholesterol ratio, vitamin D, vitamin K/triglyceride ratio, copper, selenium and zinc; serum folate, vitamin B12, ferritin and magnesium; whole blood vitamin B1 and manganese and erythrocyte selenium, vitamin B2 and vitamin B6 were analysed. Serum albumin, C-reactive protein (CRP) and faecal calprotectin (FC) were analysed. Patients were followed prospectively using their electronic patient records. No treatment modifications or supplementation were made based on abnormal micronutrient results in the absence of clinical symptoms during the study period. Time to relapse was defined as the need for escalation of IBD-related medication, hospitalization or surgery for IBD. As an audit of good clinical practice the requirement for ethics approval was waived[5].

Micronutrient analysis.

Micronutrients were assayed at the Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory, the national accredited service, as explained previously[6]. Plasma vitamin A (retinol) and E (α-tocopherol) were determined by high-performance liquid chromatography (HPLC). Plasma vitamin E was corrected for plasma total cholesterol to adjust for any variations in plasma lipids. Plasma Vitamin K (phylloquinone) was assessed using liquid chromatography tandem mass spectrometry and corrected for triglyceride levels. Vitamin B1 (thiamin diphosphate) was measured in whole blood using HPLC with post-column ferricyanide derivatization and fluorometric detection. Vitamin B2 (flavin adenine dinucleotide) was measured in erythrocytes with isocratic HPLC with a reversed-phase C18 column and

fluorescence detection. Vitamin B6 (pyridoxyl phosphate) concentrations in red cells was measured by HPLC using precolumn semicarbazide derivatization and fluorescence detection. Vitamin B2 and B6 concentrations in red cells were adjusted to haemoglobin. Vitamin C status was measured in plasma on a C18 reversed-phase analytical column (with electrochemical detection). Vitamin D status was assessed by measuring 25-hydroxy vitamin D by liquid chromatography-tandem mass spectrometry. Inductively coupled plasma mass spectrometry (Agilent Technologies, Cheadle, UK) was used to measure plasma zinc, copper, selenium, erythrocyte selenium, whole blood manganese using germanium and scandium as an internal standard. All methods were tested and calibrated against certified reference material. The between batch coefficient of variation of all methods described above was <10%.

Statistical analysis

Relationships between micronutrient with CRP, serum albumin and FC were explored with Spearman rank correlations. Predictors of micronutrient status (i.e. normal or deficient) were tested with linear and logistic regression analysis. The predictors explored were CRP, serum albumin, age, disease type, Montreal disease phenotype and behavior, use of micronutrient supplements, disease duration, gender, BMI and recent weight loss. The ability of micronutrient deficiencies to predict time to subsequent relapse was tested with Kaplan-Meier survival analysis and Cox regression accounting for confounders.

Results

Participants

Ninety-three patients were assessed [N=59 with CD, N=34 with UC/IBD unclassified (Table 1)]. Faecal calprotectin samples were returned by 75 patients (81%). There were 6 (6.5%) patients with serum albumin < 35 g/litre, 10 (11%) with CRP >10 mg/litre and 14 (15%) with FC >250μg/g of stool. Of those

with a raised FC, the majority had CD (11/14, 79%). Fifty (54%) patients had normal FC, CRP and serum albumin and were classified as in "biochemical" remission.

Prevalence of biochemical deficiencies

There were deficiencies of vitamin D in 27 (29%), zinc in 15 (17%), vitamin B6 in 13 (14%), vitamin C in 12 (13%) and vitamin B12 in 10 (11%) patients. Seven (8%) patients had low serum folate, 8 (9%) low ferritin, 4 (4%) low copper, 4 (4%) low magnesium and 3 (3%) with low plasma selenium. For the remaining micronutrients (vitamin B1, vitamin B2, vitamin E, vitamin K, manganese and RBC selenium) biochemical deficiencies were uncommon (Figure 1). There were no significant differences in micronutrient status between patients with CD and UC (Figure 1).

Among those in biochemical remission, vitamin D deficiency was identified in 16 people (32%), low vitamin B6 in 8 (16%), zinc in 7(14%), ferritin in 6 (12%), vitamin B12 in 5 (10%) and vitamin C in 5 (10%). Four patients were deficient in folate (8%), 3 (6%) low in copper and 2 (4%) low in vitamin A. Deficiencies of plasma magnesium, selenium, vitamin E, vitamin B2 and vitamin K were seen for single patients. No deficiencies were noted with RBC selenium and Vitamin B1 (Figure 1).

Correlations between micronutrients with albumin, CRP and FC

Significant correlations were observed between albumin and vitamin B2 (rho = 0.24; p=0.018), vitamin A (rho = 0.20; p=0.061), vitamin C (rho = 0.20; p=0.054), plasma selenium (rho = 0.28; p=0.007), zinc (rho =0.28; p=0.007) and serum folate (rho=-0.33; p=0.002). Likewise, CRP levels were statistically significantly correlated with vitamin B2 (rho =0.20; p=0.054), plasma selenium (rho = -0.28; p=0.006), RBC selenium (rho = -0.25; p= 0.015), copper (rho =0.41; p<0.001) ferritin (rho = 0.27; p=0.010) and folate (rho = 0.25; p=0.019). FC levels correlated significantly with magnesium (rho= -0.26, p=0.044), copper (rho= 0.37, p=0.003), ferritin (rho= -0.27, p=0.027) and manganese (rho= 0.26, p=0.04)

Predictors of micronutrient deficiencies

We explored possible predictors for the micronutrient deficiencies that were encountered in more than 8% of patients (vitamins B6, B12, vitamin C, vitamin D, ferritin, zinc, folate) and for high copper levels. In patients with CD with a stricturing phenotype, deficiency of B6 was more common (p=0.001) than in patients with an inflammatory phenotype. Young age (p=0.031), low albumin (p=0.018) and low BMI (p<0.0001) were predictive of zinc deficiency and high CRP was predictive of high copper levels (p=0.001). Seven patients received folic acid supplementation, 4 received B12 and 2 iron; none of which was associated with micronutrient status.

Micronutrient status and prediction of time to relapse

There was no association between micronutrient status and the duration of remission prior to micronutrient assessment. In survival analysis, zinc deficiency was predictive of a shorter time to subsequent relapse (HR: 6.9; 95%CI [1.9 to 26], p=0.008) (Figure 2a). When sub-analysis was performed in the subset of patients with CD only (Figure 2b), this association became even more profound (p=0.001). Neither CRP nor FC levels were predictive of time to relapse and a non-significant association (p=0.059) was seen for low albumin levels. The association between zinc deficiency and time to subsequent relapse remained statistically significant (HR: 9.5; 95%CI [2.2 to 42], p=0.0029) even after adjusting for plasma CRP (HR: 1; p=0.775) and FC (HR: 1; p=0.133). Subset analysis in patients in biochemical remission was not possible due to the very small numbers of patients with micronutrient deficiencies and lack of statistical power.

Discussion

Despite the absence of overt clinical manifestations of deficiencies, a few patients with quiescent IBD had low biochemical levels for vitamin D, vitamin B6, vitamin B12, vitamin C and zinc. We have also identified a strong relationship between biochemical zinc deficiency and time to next relapse, particularly in CD.

We have previously demonstrated the marked effect SIR can have on circulating plasma micronutrient levels. This can affect almost all micronutrients measured in plasma including vitamin D by approximately 35%, zinc by 25% and vitamin C by 75%[3, 4, 7]. Despite this long-standing evidence, plasma micronutrient concentrations are often not interpreted in the context of raised markers of inflammation in clinical settings. Sikora and colleagues, assessed micronutrient levels in newly diagnosed paediatric patients, and they noted zinc deficiency but also low albumin, low haemoglobin and high erythrocyte sedimentation rate (ESR) indicating active inflammation[8]. We would suggest that these observations were reflective of active disease and an epiphenomenon rather than indicating true micronutrient deficiencies. This is in accordance with our observations that certain micronutrients correlated either negatively or positively with CRP and serum albumin; both established positive and negative acute phase reactants respectively. However, when we looked at the patients in biochemical remission only, several of these deficiencies persisted. These can be true deficiencies, or these findings may be explained by the fact that the inflammatory markers used here are insensitive marker of active disease and mucosal healing in IBD.

This research adds to that of Siva et al, who looked at zinc deficiency and clinical outcomes in IBD[9]. They demonstrated that in patients with both UC and particularly in CD, those with low zinc concentrations also had a lower albumin and higher CRP. When these variables were controlled for, zinc deficiency remained an independent predictor of a CD-related hospitalization, complication or operation compared to those with normal levels. The significant relationship between zinc deficiency and time to

relapse in quiescent Crohn's disease is interesting and may have implications for clinical practice either as a prognostic marker of disease relapse but also as potential therapeutic target. Although low levels of zinc are found among patients with IBD, clinical manifestations are much rarer. This might be because low zinc level is a sensitive marker for disease activity in patients with subclinically active mucosal disease. Alternatively, as zinc is essential for cell growth and intestinal epithelial homeostasis patients[10] with a deficiency are more likely to suffer worsening mucosal inflammation. In two prospective cohorts of women, intake of zinc was inversely associated with risk of CD but not UC[11] further corroborating on the observations of the current study.

We did unexpectedly show that FC was not predictive of a relapse in patients with IBD despite ourselves[12] demonstrating this previously. However only 12 patients with CD had elevated FC at inclusion, and it is likely that this study lacked power to demonstrate this.

The ESPEN guidelines[2] recommend that patients in clinical remission should have their micronutrients screened with a view to supplementation but remark that inflammation may affect these results. We have shown that almost half of our cohort identified as clinically in remission were not in 'biochemical' remission making the identification of such patients challenging. It is also unclear whether supplementation in the presence of active disease would improve body stores. Santucci et al found that 37% of patients with zinc deficiency remained deficient and 15% had developed a new zinc deficiency despite supplementation[13].

In conclusion, we have identified several biochemical micronutrient deficiencies among an IBD cohort clinically and biochemically in remission. The potential for zinc levels to provide prognostic information in IBD course or comprise therapeutic target to maintain disease remission warrant further investigation within well-controlled trials.

Statement of authorship

MJM collected the data and produced the first draft; SD and CT collected the data and performed the statistical analysis; DT, FS and AC co-ordinated the laboratory analysis and contributed to the data interpretation; KG conceived the idea proposed the study, supervised the statistical analysis and edited the first draft; DRG proposed the study design, supervised data collection and contributed to the data interpretation and edited the final draft

Conflict of interests

KG received research grants and speakers' fees from Nestle Health Sciences and Nutricia-Danone outside of this work; The rest of the authors have no conficts of interest to declare.

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Figure legends

Figure 1: Prevalence of vitamin biochemical deficiencies among all patients, by IBD type and within those in biochemical remission

Figure 2: Panel A: Survival analysis displaying time to relapse in those deficient in zinc compared to those with sufficient levels (all patients with IBD), **Panel B:** Survival analysis displaying time to relapse in those CD patients deficient in zinc compared to those with sufficient levels (patients with CD)

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