Investigation of blood levels of zinc, vitamin B\(_{12}\) and folate in patients with haematological malignancy

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Abstract

Objectives: Zinc, vitamin B\(_{12}\) (VB\(_{12}\)) and folate have important roles in haematopoiesis. We assessed deficiencies in zinc, VB\(_{12}\) and folate levels in patients with primary haematological malignancy before the administration of cancer chemotherapy.

Methods: We retrospectively reviewed serum levels of zinc, VB\(_{12}\) and folate in 37 patients with haematological malignancy.

Results: Of the 37 study patients, 27 had been diagnosed with malignant lymphoma, 8 with leukaemia and 2 with multiple myeloma. The percentages of patients with sufficient, low and deficient levels of zinc were 18.9%, 59.5% and 21.6%, respectively. VB\(_{12}\) levels were sufficient for more than 80.0% of patients. The percentages of patients with sufficient, low and deficient levels of folate were 51.4%, 43.2% and 5.4%, respectively.

Conclusions: Zinc and folate levels tended to be deficient in patients with haematological malignancy.

Keywords: Haematological malignancy, Zinc, Vitamin B\(_{12}\), Folate

Introduction

Numerous anticancer drugs induce cytotoxicity, which often causes myelosuppression. This effect is particularly noticeable in patients undergoing cancer chemotherapy for haematological malignancy as the onset of severe myelosuppression can make continuing the cancer chemotherapy difficult.

Zinc is a cofactor for more than 200 enzymes within the body and is a necessary element of transcriptional gene regulation, cellular differentiation and proliferation, and of the catalytic activity of carbonic anhydrase,\(^1\) an enzyme found in high concentrations in red blood cells. Prasad et al. reported several zinc deficiency disorders accompanied by anaemia in infants who consumed food with high concentrations of phytic acid.\(^2\) Xia et al. reported a high rate of erythrocyte osmotic fragility in rats fed a low-zinc diet,\(^3\) and Idei et al. reported a positive correlation between serum levels of zinc and haemoglobin (irrespective of sex) in an analysis of individuals who attended health screenings for people aged 40 years and older.\(^4\) These reports demonstrate that zinc has a profound connection with erythropoiesis. In contrast, a study by King et al. revealed that zinc deficiency leads to the downregulation of lymphocyte precursors in mice.\(^5\) In addition, a trial by Barnett et al. reported elevated serum zinc levels and peripheral T-cell count in zinc-deficient nursing home patients over the age of 65 years who had received 30-mg zinc/day for 3 months.\(^6\) These results suggest that zinc may influence the maturation of white blood cells.

Vitamin B\(_{12}\) (VB\(_{12}\)) and folate are essential for DNA synthesis and play important roles in the maturation of red blood cells.\(^7\) As the deficiency of either VB\(_{12}\) or folate leads to the onset of megaloblastic anaemia,\(^8\) both appear indispensable for red blood cell production.

Given that myelosuppression is frequently reported in patients undergoing cancer chemotherapy, it is extremely important to determine the levels of zinc, VB\(_{12}\) and folate in these patients prior to treatment. Therefore, we formulated a hypothesis to determine whether normal levels of these factors could reduce the incidence of delayed recovery and exacerbate myelosuppression caused by cancer chemotherapy. However, the levels of zinc, VB\(_{12}\) and folate are rarely measured in routine clinical care. Therefore, as part of our preliminary analysis, we conducted a survey to determine their serum levels in patients with primary haematological malignancy before the administration of cancer chemotherapy.

Methods

Subjects

Patients with primary haematological malignancy, whose serum levels of zinc, VB\(_{12}\) and folate had been measured prior to undergoing initial cancer chemotherapy, were included in this study. Patients were admitted to the Division of Haematology at Fujita Health University Hospital between January 2014 and December 2014. Patients who used drugs containing zinc, VB\(_{12}\) or folate were excluded from the study, as were patients who had undergone gastric and/or ileocecal resection (which could potentially interfere with the absorption of VB\(_{12}\)).
Investigation

This study was a retrospective survey of information gathered from patients using electronic health records from the Fujita Health University Hospital. Patient background information consisted of sex, age, type of malignancy and staging. Based on the results of tests carried out prior to initiating chemotherapy, we investigated white blood cell, neutrophil, platelet, red blood cell and reticulocyte counts, as well as the levels of haemoglobin, iron, zinc, VB₁₂, and folate.

Assessment

The reference standards for zinc, VB₁₂ and folate levels were defined as follows. Based on facility reference values and previous reports, normal serum zinc levels were defined as greater than or equal to 80 μg/dL, with low levels between 60 and 80 μg/dL and deficient levels below 60 μg/dL; normal serum VB₁₂ levels were defined as greater than or equal to 200 pg/mL, with low levels between 180 and 200 pg/mL and deficient levels below 180 pg/mL; and normal folate levels were defined as greater than or equal to 6 ng/mL, with low levels between 3 and 6 ng/mL and deficient levels below 3 ng/mL.

Serum zinc levels were measured by a BM6010 analyser (JEOL Ltd., Tokyo, Japan) using 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino) phenol, a chelate compound of zinc. Serum levels of VB₁₂ and folate were determined using an electro-chemiluminescence immunoassay (ECLI A) using a cobas8000 system (Roche Diagnostics K.K., Tokyo, Japan).

Statistical analysis

To identify variables exhibiting a normal distribution, the Kolmogorov–Smirnov adjustment test was used. Variables exhibiting a normal distribution were expressed as means ± standard deviations. Variables not exhibiting a normal distribution were expressed as medians with interquartile ranges. To compare values between two groups, the unpaired Student’s t-test was used for normally distributed variables, whereas the Mann–Whitney U test was used for variables not exhibiting a normal distribution. Pearson's correlation coefficient was used to study the correlation between age and concentration of zinc or folate. Spearman’s rank correlation coefficient was used to study the correlation between age and the concentration of VB₁₂. The χ² test was used for ratio comparisons between groups. The Statistical Package for the Social Sciences (SPSS), version 22.0 (IBM Corporation, Armonk, NY, USA) was used for all statistical analyses. P values lower than 0.05 were considered statistically significant.

Statement of ethics

This study was conducted in compliance with the Ethical Guidelines for Clinical Research, with approval from the Ethical Review Board for Epidemiology/Clinical Research at Fujita Health University Hospital.

Results

Patients

Of 250 patients with primary haematological malignancy admitted to the Division of Hematology at Fujita Health University Hospital between January 2014 and December 2014, those who had missing data, were taking zinc, VB₁₂ or folate, or had undergone a gastric or ileocecal resection were excluded from the study. Of the remaining 37 patients used for analysis (Figure 1), 21 were men and no significant differences were observed between the sexes (P=0.41). The mean age was 62.5±13.7 years. There were 27 cases of malignant lymphoma, 8 cases of leukaemia and 2 cases of multiple myeloma. Patient

Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>All (n=37)</th>
<th>Malignant Lymphoma (n=27)</th>
<th>Leukaemia (n=8)</th>
<th>Multiple Myeloma (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>62.5±13.7</td>
<td>63.3±13.1</td>
<td>60.9±17.7</td>
<td>52, 65</td>
<td></td>
</tr>
<tr>
<td>Sex (male, female)</td>
<td>21, 16</td>
<td>15, 12</td>
<td>5, 3</td>
<td>1, 1</td>
</tr>
<tr>
<td>Stage (%)</td>
<td>I</td>
<td>14.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>11.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>14.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>51.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory Data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucocytes (×10⁹/μL)</td>
<td>6.1 (4.2–8.6)</td>
<td>5.5 (4.4–8.5)</td>
<td>5.5 (3.2–15.0)</td>
<td>6.2, 6.4</td>
</tr>
<tr>
<td>Neutrophils (×10⁹/μL)</td>
<td>3.3 (2.2–5.0)</td>
<td>3.6 (2.7–6.0)</td>
<td>0.4 (0.2–1.7)</td>
<td>5.0, 3.8</td>
</tr>
<tr>
<td>Platelets (×10⁹/μL)</td>
<td>19.7±11.3</td>
<td>22.0±11.3</td>
<td>11.2±8.6</td>
<td>25.6, 20.8</td>
</tr>
<tr>
<td>Erythrocytes (×10⁹/μL)</td>
<td>4.1 (3.4–4.3)</td>
<td>4.2 (4.1–4.5)</td>
<td>2.5 (2.5–2.6)</td>
<td>2.2, 3.7</td>
</tr>
<tr>
<td>Reticulocytes (×10⁹/μL)</td>
<td>5.7±2.7</td>
<td>6.5±2.4</td>
<td>3.8±2.8</td>
<td>2.2, 3.7</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>204 (92–475)a</td>
<td>124 (81–295)b</td>
<td>487 (433–708)c</td>
<td>865, 364</td>
</tr>
<tr>
<td>Serum iron (μg/mL)</td>
<td>100.3±60.1</td>
<td>79.3±44.8</td>
<td>170.4±60.0</td>
<td>132.0, 75.0</td>
</tr>
</tbody>
</table>

Data loss: a: 8; b: 6; and c: 2.

Figure 1  Study flow chart. VB₁₂: vitamin B₁₂.
background and laboratory data according to type of malignancy are shown in Table 1.

Assessment of serum values
Serum levels of zinc, VB_{12} and folate were normal in 18.9%, over 80% and approximately 50% of patients, respectively (Table 2).

<table>
<thead>
<tr>
<th>Classification</th>
<th>Serum zinc</th>
<th>%</th>
<th>Serum vitamin B_{12}</th>
<th>Normal</th>
<th>Low</th>
<th>Deficient</th>
<th>Serum folate</th>
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<td>Low</td>
<td>59.5</td>
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<td>Normal</td>
<td>83.8</td>
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Relationship with type of malignancy
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leukaemia; the two patients with multiple myeloma had zinc levels of 65.0 μg/dL and 73.0 μg/dL. Serum VB_{12} levels were 385.0 pg/mL (232.0–546.0 pg/mL) in patients with malignant lymphoma and 542.5 pg/mL (219.5–819.3 pg/mL) in those with leukaemia; the two patients with multiple myeloma had serum VB_{12} levels of 201 pg/mL and 224 pg/mL. Serum folate levels were 6.7±2.4 ng/mL in patients with malignant lymphoma and 5.2±1.6 ng/mL in those with leukaemia; the two patients with multiple myeloma had serum folate levels of 5.5 ng/mL and 6.5 ng/mL (Figure 2). No factors were identified that revealed significant differences between malignant lymphoma and leukaemia (data not shown).

Relationship with sex
Serum levels of zinc, VB_{12} and folate were normal in 18.9%, over 80% and approximately 50% of patients, respectively (Table 2).

<table>
<thead>
<tr>
<th>Classification</th>
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<th>%</th>
<th>Serum vitamin B_{12}</th>
<th>Normal</th>
<th>Low</th>
<th>Deficient</th>
<th>Serum folate</th>
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Figure 2 Relationship between type of malignancy and serum zinc, vitamin B_{12} and folate levels. A: Malignant lymphoma. B: Leukaemia. C: Multiple myeloma. Zinc and folate are expressed as means±standard deviations. Vitamin B_{12} is expressed as medians with interquartile ranges. Dotted lines show cut-off values between sufficient and potentially deficient values. Chain lines show cut-off values between potentially deficient and deficient values.

Figure 3 Relationship between sex and serum levels of zinc, vitamin B_{12} and folate. Circles: Malignant lymphoma. Triangles: Leukaemia. Squares: Multiple myeloma. Zinc and folate were expressed as means±standard deviations. Vitamin B_{12} was expressed as medians with interquartile ranges. Dotted lines show cut-off values between sufficient and potentially deficient values. Chain lines show cut-off values between potentially deficient and deficient values.
Relationship with age

No correlation was observed between serum zinc level and age ($r=-0.26, P=0.13$) or between serum folate level and age ($r=-0.023, P=0.89$). However, a correlation was observed between serum VB₁₂ level and age ($r=0.34, P=0.039$).

Discussion

We investigated the serum levels of zinc, VB₁₂ and folate in patients with primary haematological malignancies prior to the administration of cancer chemotherapy. Serum zinc and folate levels were low in this patient population, with fewer than 20% of patients exhibiting normal zinc concentrations. In contrast, serum VB₁₂ levels were high. A comparison between malignant lymphoma and leukaemia patients did not reveal any differences, and no disparities were observed according to differences among the particular malignancy. Comparisons for multiple myeloma patients were difficult because there were only two cases. The results of this survey may prove valuable for taking preventive measures against increasingly severe myelosuppression and delayed recovery during cancer chemotherapy for haematological malignancies. Not only does zinc have a profound connection to the formation of red blood cells, but it may also influence the maturation of white blood cells. Folate is also essential for the formation of red blood cells. As such, under conditions where serum zinc and folate levels are deficient, severe myelosuppression after cancer chemotherapy for haematological malignancy is to be expected.

Measures to prevent the development of myelosuppression include the administration of granulocyte colony-stimulating factor for neutropenia, erythropoietin or red blood cell transfusions for anaemia and thrombopoietin receptor agonists or platelet transfusions for thrombocytopenia. Furthermore, infectious disease may accompany neutropenia and blood transfusions. While certain drug formulations are expensive, zinc, VB₁₂ and folate supplements are low-cost. Therefore, taking countermeasures prior to the initiation of cancer chemotherapy appears to be effective, not only to prevent a poor prognosis but also for economic reasons.

There are many reasons why patients with primary haematological malignancy may have low serum levels of zinc and folate. One is that, because the patients in this study were older individuals (mean age of 62.5 years), and many had progressive cancer, a decline in dietary intake could have contributed to low levels of zinc and folate. Cachexia, also known as wasting syndrome, is a form of malnutrition that accompanies progressive cancer and is considered a causative factor of reduced food intake. Because zinc and folate are imperative for cell growth, these nutrients might have been depleted by the proliferating tumour cells. However, serum VB₁₂ levels were high in this patient group. Normal VB₁₂ levels are between 3 and 5 ng and, as the minimum recommended daily amount is only 0.1 μg, we consider VB₁₂ deficiency unlikely. However, a metabolic hallmark of chronic myelogenous leukaemia is a large increase in serum VB₁₂ levels. Therefore, it is necessary to assess serum VB₁₂ levels in cases of haematological malignancy.

Correlations between serum levels of zinc, VB₁₂ or folate with sex and age were also studied. Serum folate levels tended to be lower in men than in women. This finding was consistent with the results of a study of healthy middle-aged and older adults. As most of the patients in this study were middle-aged or elderly, we believe that attention should be paid to low serum folate levels among older male patients with haematological malignancies. No correlation was observed between age and serum zinc or folate levels. Thus, it is possible that sex had a greater influence than age on the levels of these nutrients.

There were some limitations to this study. As it was retrospective in design, the study lacked a comparative control group. Another limitation was the small number of patients in whom serum levels of zinc, VB₁₂ and folate were measured because these parameters are rarely measured in routine clinical care. Therefore, a prospective study is required to verify our hypothesis. However, as a preliminary study, the results may be sufficient to provide a foundation for further research.

The results of this study add to existing knowledge on the serum levels of zinc, VB₁₂ and folate in patients with primary haematological malignancies. We plan to conduct a prospective study in patients with primary haematological malignancies prior to cancer chemotherapy and assess any resulting myelosuppression to explore the impact of the levels of these nutrients on treatment efficacy.

Conflicts of interest

The authors declare that they have no conflicts of interest. We have full control of all primary data and we agree to allow the journal to review the data if requested.

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References


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