Alteration of Serum 25(OH) Vitamin D, Vitamin D Binding Protein, and C-reactive Protein Levels in Acute Leukemia Patients

Liejun Jiang 1,*, Xiaomei Zhang 2,*, Yanyun Chen 1, Xiaocong Huo 3, Shuang Deng 1, Xiafang Yang 1, Yu Luo 1, Yanfang Luo 1, Xiaoxu Lu 1, Min Zhang 1, Huayi Huang 1, 4

* These authors contributed equally to this work

1 Department of Laboratory Medicine, The People’s Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, China
2 Department of Hematology, Changzhou First People’s Hospital (The Third Affiliated Hospital of Soochow University), Changzhou, Jiangsu, China
3 Department of Hematology, The People’s Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, China
4 Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY, USA

SUMMARY

Background: Acute leukemia is a common hematologic malignancy with poorly differentiated leukocytes. Alteration of circulating vitamin D (VD) and its carrier vitamin D binding protein (VDBP) have been reported in certain types of cancers and may play a role in the course of the disease. Understanding of the status of serum VD and VDBP, as well as the acute phase protein C-reactive protein (CRP) levels in pre- and post-treatment of acute leukemia patients, may be helpful in the management of acute leukemia.

Methods: Enzyme linked immunoabsorbent assay (ELISA), chemiluminescence immunoassay, and immunofluorescent assay were used to analyze the 25(OH) vitamin D (25(OH)D), VDBP, and CRP in the serum of a cohort of leukemia patients.

Results: Serum 25(OH)D levels in patients (pre- and post-treatment) were significantly lower than in control subjects. There was no significant difference in 25(OH)D levels between pre- and post-treatment. Serum VDBP level was raised in both pre- and post-treatment of acute leukemia patients, with that of pre-treatment being higher. The average serum VDBP was reduced in post-treatment; however, no significant difference was found. Elevated serum CRP levels in both pre- and post-treatment patient groups have been observed but were reduced significantly after treatment. Results also revealed that serum VDBP levels in acute myeloid leukemia patients were significantly higher than in acute lymphoid leukemia patients, while 25(OH)D levels in acute myeloid leukemia were significantly lower than in acute lymphoid leukemia. No significant difference between the serum CRP levels of acute myeloid leukemia and acute lymphoid leukemia was observed.

Conclusions: Serum 25(OH)D, VDBP, and CRP may be used together and could be potential indicators of the disease course of acute leukemia and assist in its management which merits further investigation.


KEY WORDS

acute leukemia, serum, vitamin D, vitamin D binding protein, C-reactive protein
INTRODUCTION

Leukemia is a common hematological neoplasm with a rising incidence rate worldwide, including in China [1]. It is categorized as either acute or chronic leukemia and further sub-typed into several categories, including myeloid and lymphoid neoplasms. For acute myeloid leukemia (AML), its characteristic is the accumulation of malignant and immature hematopoietic myeloid precursors which lack differentiation and have disordered proliferation [2]. Thus, differentiation induction of leukemic cells has been the focus of treatment of this malignancy, with the most common regimen used thus far being the all-trans retinoic acid [3,4]. For acute lymphoblastic leukemia (ALL), it is characterized by an excessive number of developing lymphoblasts which cause serious bone marrow failures and bone fractures [5-7]. Vitamin D (VD) is a group of fat-soluble secosteroids synthesized in skin with exposure to ultraviolet light from the sun; a small amount of VD can also be taken up from foods. Recent studies have found that VD has anti-cancer properties through an unclear mechanism [8]. VD and its analogs have been shown to exert differentiation induction on leukemic cells, specifically, 1α, 25-dihydroxyvitamin D3 (1,25(OH)2D3) could induce the differentiation of myeloid leukemia cells into granulocyte and macrophage lineages [2,3]. The circulating total and free 25-hydroxyvitamin D levels were found to be inversely associated with colorectal cancer risk in a certain population [9]. The study also found a positive association between circulating total 25-hydroxyvitamin D (25(OH)D) and survival rate in colorectal cancer patients [10]. Reports showed that 1α,25-dihydroxyvitamin D3 or its analogs had potency in differentiating AML cells in vitro and ex vivo, which led to early clinical trials in AML and myelodysplastic syndrome patients [2]. However, the efficacy and mechanism of VD on leukemia are still unclear.

Vitamin D binding protein (VDBP) belongs to the albumin gene family and is the carrier of VD and its metabolites in circulation to various target tissues [11]. Studies found that plasma VD concentration was significantly associated with that of VDBP [10]. In prostate cancer, higher circulating VDBP may be independently associated with decreased prostate cancer risk in certain populations, and this was independent of 25(OH)D status [12]. Since VD may have anticancer activities, VDBP may hence play a role in the biological function of VD. However, there is no clear indication that VDBP is a marker for leukemia in either diagnosis or therapeutic monitoring so far.

C-reactive protein (CRP) is an acute phase protein which is an indicator of the inflammation of various types of pathological statuses such as cancer, cardiovascular disease, rheumatoid arthritis, and pulmonary diseases [13]. CRP may reflect infection or a chronic inflammatory circumstance thus guiding the management of acute leukemia in clinical practice.

In this report, by using a chemiluminescence immunoassay, enzyme linked immunosorbent assay (ELISA), and immunofluorescent assay to analyze the serum concentrations of 25(OH) vitamin D, vitamin D binding protein, and C-reactive protein in acute leukemia patients. We showed the alterations to serum 25(OH)D, VDBP, and CRP levels in both AML and ALL patients, and their correlations were also described.

MATERIALS AND METHODS

Patients

A total of 57 cases of acute leukemia patients (39 cases of acute myeloid leukemia and 18 cases of acute lymphoblastic leukemia) were enrolled consecutively in this study from December 2015 to April 2017; 33 cases were male and 24 cases were female, with ages ranging from 2 to 81 years old (44.8 ± 23.1). All patients were complete hospitalized without outdoor activity during treatment. Patients were provided hospital diet from the department of food and nutrition without calcium and vitamin D fortifying. Patients were diagnosed based on WHO classifications of tumors of hematopoietic and lymphoid tissues [14]. Fifty-five routinely healthy individuals without known cancer or inflammatory disorders were selected as controls, with age and gender matched to that of the patient group (21 - 83 years old, 42.8 ± 15.0; 29 males and 26 females). These individuals were on regular diet without consuming calcium or vitamin D supplements prior to the blood drawing. The study was carried out with the permission of the Institute Review Board of the People’s Hospital of Guangxi Zhuang Autonomous Region on the use of human materials. All of the patients had paired pre-treatment and post-treatment blood samples. For acute promyelocytic leukemia, the ATRA (all-trans-retinoic acid) combined with arsenic trioxide regimen was used; for other AML, the DA (daunorubicin and cytarabine) regimen was applied; for ALL, the VDLP (vincristine, daunomycine, L-asparaginase, and prednisone) regimen was used in the management.

Blood sample collection

Fasting blood samples were obtained the day before chemotherapy (baseline) and during remission before discharge using the serum separation tubes (BD Healthcare, Franklin Lakes, NJ, USA). The blood samples were transported to laboratory within 30 minutes of collection and were centrifuged at 3,000 rpm at 4°C for 10 minutes. The serum was then collected into a 2 mL centrifuge tube and stored at -80°C until required.

Enzyme linked immunosorbent assay (ELISA)

The Quantikine ELISA Human Vitamin D BP Immunoassay (Catalog number DVBDBP0) kit was purchased from R & D Systems, Inc. (Minneapolis, MN, USA) and was used following the manufacturer’s instructions. In brief, all required reagents, working standards, and sample dilutions were prepared and stored properly.
prior to the assay sampling. A plate layout was prepared and 100 µL of Assay Diluent RD1-19 was added to each well. Then, 50 µL of standard, control, or sample was added to corresponding wells, and the plate was covered with adhesive strip and incubated for 1 hour at room temperature on a horizontal orbital microplate shaker. After incubation, all liquids in wells were aspirated, followed by addition of 400 µL of wash buffer into each well for washing four times. After the last wash, the plate was inverted and blotted against clean paper towels to drain the remaining wash buffer. This was then followed by addition of 200 µL of human vitamin D BP conjugate into each well and then covered with a new adhesive strip and incubated for 2 hours at room temperature on the shaker. The washing procedures mentioned above was then repeated, and 200 µL of substrate solution was subsequently added into each well and incubated for 30 minutes at room temperature while protected from light. Following this step, 50 µL of stop solution was then added to stop the color development, the plate was gently tapped to ensure thorough mixing, and finally, the OD 450 nm was measured immediately on a microplate reader.

**Chemiluminescence immunoassay**

The serum VD level was quantitatively measured by using a Roche cobas Elecsys Vitamin D total (25-Hydroxyvitamin D, REF 05894913 190) assay kit, using a Roche Cobas E601 electrochemiluminescence immunoassay system (Roche Diagnostics GmbH, Mannheim, Germany). All tests were performed according to the laboratory standard operating procedures.

**Immunofluorescent assay**

The serum high sensitive C-reactive protein (hsCRP) level was analyzed by the i-CHROMA hsCRP-All in one immunofluorescence assay on a John Star immunofluorometer (Boditech Med Inc., Chuncheon-si, Gangwon-do, Korea) following the instructions and procedures from the manufacturer.

**Statistical analysis**

The statistical analysis was performed using the SPSS version 18.0, of which the Independent-samples t-test, Paired-Samples t-test, and Pearson correlation were applied. A p-value of less than 0.05 was considered significant.

**RESULTS**

**Serum 25(OH)D and VDBP levels in pre- and post-treatment of acute leukemia patients and in control subjects**

In order to understand if there is any alteration of VD and VDBP levels in pre- and post-treatment of acute leukemia patients, the chemiluminescence immunoassay and an ELISA assay kit were used to analyze these target compounds. The analytical results showed that the serum 25(OH)D levels in patients (pre- and post-treatment) were significantly lower than in control subjects (p = 0.027, p = 0.006, Table 1). There was no significant difference in 25(OH)D levels between pre- and post-treatment (p = 0.691, Table 1). Furthermore, the VDBP level was significantly higher in both pre- and post-treatment acute leukemia patients than control subjects (p = 0.000 for both), with that in pre-treatment being higher (Table 1). Results also showed that although the average serum VDBP was reduced in post-treatment, it was not significant (p = 0.515).

**Serum CRP levels in pre- and post-treatment of acute leukemia patients and in control subjects**

The acute phase protein C-reactive protein was measured in the serum of pre- and post-treatment acute leukemia patients and compared with control subjects. The high sensitivity method was adopted to analyze this acute phase protein. Table 2 shows that both pre- and post-treatment patient groups had dramatically elevated CRP levels (p = 0.00 for both). After treatment, the serum CRP level was reduced significantly compared to that of pre-treatment (p = 0.042).

**The variations of serum 25(OH)D, VDBP, and CRP levels between AML and ALL patients**

Statistical analysis results revealed that serum 25(OH)D levels in AML were significantly lower than in ALL (48.79 ± 21.58 vs. 66.82 ± 28.80, p = 0.011). Meanwhile, VDBP levels in AML were significantly higher than in ALL patients (175.50 ± 70.52 vs. 134.17 ± 74.13, p = 0.048). There was no significant difference in 25(OH)D levels between pre- and post-treatment (p = 0.042).

**Correlation of serum VDBP and 25(OH)D levels**

In order to know the correlation between serum VDBP and 25(OH)D levels, Pearson’s correlation analysis was performed. The statistical results showed that the correlation between serum VDBP and 25(OH)D levels in this study was weak (r = -0.014, Table 4 and Figure 1).

**DISCUSSION**

Poor differentiation and disordered proliferation are the major characteristics of leukemia cells. Thus, strategies in inducing the differentiation of leukemic cells and inhibiting the malignancy are key intervention approaches in clinical management. VD and its analogs were found to exert differentiation properties and cancer cell growth inhibition in certain types of cancers including leukemia [5-10,15]. Circulating VD is carried by its carrier, the VDBP, which may also play a role in cancers [9,10,16]. CRP is an acute phase protein which reflects the inflammatory and infection statuses of the body [13]. In this study, alterations in 25(OH)D, VDBP, and CRP in AML and ALL were found.
Table 1. Serum vitamin D binding protein and 25(OH)D levels in pre- and post-treatment of acute leukemia patients and in control subjects.

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>VDBP ($\bar{x} \pm s$, ng/mL)</th>
<th>t</th>
<th>p</th>
<th>25(OH)D ($\bar{x} \pm s$, nmol/L)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Pre-tr vs. Controls</td>
<td>57/55</td>
<td>162.45 ± 73.61 vs. 95.37 ± 66.84</td>
<td>5.044</td>
<td>0.000</td>
<td>54.49 ± 25.29 vs. 63.82 ± 18.15</td>
<td>-2.237</td>
<td>0.027</td>
</tr>
<tr>
<td>* Post-tr vs. Controls</td>
<td>57/55</td>
<td>157.35 ± 66.70 vs. 95.37 ± 66.48</td>
<td>4.912</td>
<td>0.000</td>
<td>53.17 ± 22.19 vs. 63.82 ± 18.15</td>
<td>-2.775</td>
<td>0.006</td>
</tr>
<tr>
<td>Δ Pre-tr vs. Post-tr</td>
<td>57/57</td>
<td>162.45 ± 73.61 vs. 157.35 ± 66.70</td>
<td>0.655</td>
<td>0.515</td>
<td>54.49 ± 25.29 vs. 53.17 ± 22.19</td>
<td>0.399</td>
<td>0.691</td>
</tr>
</tbody>
</table>

* - Independent-samples t-test, Δ - Paired-Samples t-test, Pre-tr - Pre-treatment, Post-tr - Post-treatment.

Table 2. Serum C-reactive protein levels in pre- and post-treatment of acute leukemia patients and in control subjects.

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>CRP ($\bar{x} \pm s$, ng/mL)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Pre-tr vs. Controls</td>
<td>57/55</td>
<td>44.17 ± 60.02 vs. 0.81 ± 0.87</td>
<td>5.450</td>
<td>0.000</td>
</tr>
<tr>
<td>* Post-tr vs. Controls</td>
<td>57/55</td>
<td>25.15 ± 42.22 vs. 0.81 ± 0.87</td>
<td>4.352</td>
<td>0.000</td>
</tr>
<tr>
<td>Δ Pre-tr vs. Post-tr</td>
<td>57/57</td>
<td>44.17 ± 60.02 vs. 25.15 ± 42.22</td>
<td>2.080</td>
<td>0.042</td>
</tr>
</tbody>
</table>

* - Independent-samples t-test, Δ - Paired-Samples t-test, Pre-tr - Pre-treatment, Post-tr - Post-treatment.

Table 3. Comparison of serum vitamin D binding protein, 25(OH)D, and C-reactive protein levels between acute myeloid and acute lymphoid leukemia (Independent-samples t-test).

<table>
<thead>
<tr>
<th></th>
<th>Acute myeloid leukemia (n = 39)</th>
<th>Acute lymphoid leukemia (n = 18)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDBP ($\bar{x} \pm s$, ng/mL)</td>
<td>175.50 ± 70.52</td>
<td>134.17 ± 74.13</td>
<td>-2.108</td>
<td>0.048</td>
</tr>
<tr>
<td>25(OH)D ($\bar{x} \pm s$, nmol/L)</td>
<td>48.79 ± 21.58</td>
<td>66.82 ± 28.80</td>
<td>2.631</td>
<td>0.011</td>
</tr>
<tr>
<td>CRP ($\bar{x} \pm s$, ng/mL)</td>
<td>37.58 ± 52.32</td>
<td>58.37 ± 73.71</td>
<td>1.077</td>
<td>0.291</td>
</tr>
</tbody>
</table>

Table 4. Correlation of serum VDBP and 25(OH)D levels (Pearson’s correlation).

<table>
<thead>
<tr>
<th></th>
<th>VDBP</th>
<th>25(OH)D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation</td>
<td>1</td>
<td>-0.014</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.885</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>112</td>
<td>112</td>
</tr>
</tbody>
</table>

Alteration of serum VDBP and 25(OH)D levels in pre- and post-treatment of acute leukemia patients

Numerous studies have found that serum VD concentration was altered in many types of diseases including cancers [8-10]. In leukemia, studies found that VD had potency in inducing the differentiation of leukemia cells [2,17-20]. High serum levels of VD have been found to benefit the survival of AML patients after chemotherapy [21]. Reisi et al. reported that the serum VD levels were lower in childhood ALL patients than in control group, and that the VD level was associated with the efficacy of chemotherapy and also survival [22]. Our study found that serum 25(OH)D in AML and ALL patients (both pre- and post-treatment) were significantly reduced.
lower than in control subjects (p = 0.027, p = 0.006, Table 1). This indicates that a disordered consumption or metabolism of VD has occurred in acute leukemia, though the pathogenesis is still unclear; this phenomenon may be a consequence of the high demand and consumption in malignant cells, reduced synthesis of VDBP, low intakes of VD due to ailing, the lack of sunshine exposure during illness, or all of these factors combined.

VDBP is the carrier of VD in circulation. By using a monoclonal antibody coated human ELISA kit from R & D Systems Inc., to analyze the VDBP concentration in serum, we found that serum VDBP level was increased in both pre- and post-treatment acute leukemia patients (p = 0.000 for both), with that in pre-treatment being higher (Table 1). Results also showed that although the average serum VDBP was reduced in post-treatment, it was not significant compared to pre-treatment (p = 0.515). To our knowledge, the role of serum VDBP in leukemia is rarely reported. However, in regards to other types of cancers, Layne et al. reported that serum VDBP in prostate cancer was significantly and inversely associated with prostate cancer risk; in other words, higher serum VDBP correlated with a lower risk of prostate cancer [12]. Our results might reflect a certain human VDBP isoform detected in serum since there are three VDBP isoforms based on its genotypes, and different detection methods used could obtain different serum VDBP levels [23]. We were unable to follow up the treatment outcome of the patients due to various reasons and difficulties. High serum VDBP levels may be a compensatory mechanism for insufficient circulating VD levels or low production.

Elevated serum CRP levels in pre- and post-treatment of acute leukemia patients

CRP is an acute phase protein which is elevated in various types of inflammatory diseases caused by viral and bacterial infection, rheumatoid arthritis, and cancer [13]. Thus, CRP is used mainly as a marker for inflammation and infection.

Herishanu et al. reported that serum CRP in chronic lymphocytic leukemia was elevated and correlated with shorter survival rates and development of second cancers in patients [24]. The study also found that high serum CRP level was associated with bone marrow transplant-related mortality in AML and myelodysplastic syndromes [25]. Our study found that serum CRP was elevated in both pre- and post-treatment patient groups of acute leukemia (p = 0.00 for both). After treatment, CRP level was reduced significantly compared to pre-treatment (p = 0.042, Table 2). Our results indicated that inflammation and infection were the causes for the elevation of serum CRP during the disease course. Thus, CRP is an important indicator in the management of the infection and inflammation in acute leukemia. Interestingly, we found that serum VDBP levels in AML patients were significantly higher than in ALL pa-
CONCLUSION

Taken together, serum 25(OH)D, VDBP, and CRP together could be potential indicators of disease course and monitoring tools of therapy for acute leukemia patients which merit further study. However, the detailed mechanism is to be further elucidated.

Acknowledgment:
This study was supported by a grant from the France, 2017 (ISBN-13 9789283244943).

Declaration of Interest:
None declared.

References:


