Original Article

Determination of serum vitamin D3 levels in patients with acute lymphoblastic leukemia

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Abstract

Background: Regarding the role of vitamin D3 as a steroid hormone in regulating the metabolism of minerals and bone homeostasis, its antitumor role has been confirmed in epidemiologic and empirical studies. Accordingly, this investigation conducted on acute lymphoblastic leukemia (ALL) patients to understand whether vitamin D3 has role in increasing the risk of getting ALL or not. **Materials and Methods:** In this study, 40 patients with acute lymphoblastic leukemia were included after confirmation of cytological tests in Hamedan, Qazvin and Tehran. Moreover, 40 healthy persons without any hematologic diseases in both themselves and their immediate relatives were selected. The level of vitamin D3 in blood serum of patients and control group was measured with Chemiluminscence method. **Results:** Ninety percent of the subjects in the experimental group and 75% of the control group suffered from severe vitamin D3 deficiency. 10% of the experimental group and 20% of the control group had a mild to moderate vitamin D3 deficiency and range from 10 - 19 ng / ml. Among ALL patients, none of them had the desired level (20- 50 ng / ml) of vitamin D3, while two persons in the control group had a desirable level of vitamin D3 with a range of 20- 50 ng / ml. It is found that there is a significant difference between mean of vitamin D3 level in acute lymphoblastic leukemia patients and healthy control group ($p < 0.05$). **Conclusion**: It seems that there is a correlation between the incidence of ALL and serum vitamin D3 level. However, additional studies are needed to understand more about the mechanisms of this.

Keywords: Vitamin D3, Acute lymphoblastic leukemia, Vitamin D3 deficiency, Cytological tests

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Introduction

Leukemia is the most prevalent cancer in childhood with 32% frequency among all childhood cancers. Annual incidence of leukemia was 3.7 and 4.9 per 100 000 population in Tabriz and Ardebil (1) . According to our knowledge there is no accurate incidence of ALL in Iran. The most common type of leukemia among children is acute lymphoblastic leukemia (ALL) which comprise 80% of childhood (2-5years) leukemia. However, ALL can affect adult as well but with far less frequency (about 20% of ALL). There are different subtypes of ALL according to cellular morphology, immunophenotype, gene expression and genetic abnormality which some of them are with poor and some with good prognosis (2-4).

Ionizing radiation, magnetic exposure, poisonous chemical substances, viral infection, and alcohol and tobacco consumption in parents has been

shown that can increase the risk of ALL in children. Moreover, it has been found that children with Down syndrome are 15 times more susceptible to affecting by ALL in comparison with healthy children. Splenomegaly, fever, bone pain, neutropenia and low hemoglobin level are the most common reported manifestations for affected patients (5-7).

Vitamin D3 is a steroid hormone, which has a very important role in calcium metabolism and phosphor and calcium homeostasis. Additionally, it has major role in cell differentiation, apoptosis and angiogenesis (8). Considering the important role of vitamin D3 in biological mechanisms in human body, specifically in bone homeostasis as steroid hormone, its anti-tumor activity has been confirmed in epidemiological and empirical studies (9). As an instance, individuals with undesirable level of vitamin D3 are more prone to cancer and have higher mortality due to breast cancer, colorectal and prostate cancer in comparison with person with normal level of vitamin D3 (10). Antitumor activity of vitamin D3 is through prevention of proliferation and invasion of tumor cells. Vitamin D3 inhibits BCL2 (B-cell lymphoma 2) expression by BOX induction and induction of apoptosis in chronic lymphoid leukemia (11). Vitamin D3 level below 20ng/ml consider as vitamin D3 deficiency and the level of 20 to 29 ng/ml and 30 ng/ml consider as insufficient and normal level of vitamin D3 respectively (12).

With respect to clinical importance of ALL and leading role of vitamin D3 in cancers, current investigation aim to evaluate association between ALL and vitamin D3 level.

Methods

Study population. In this case-control investigation, 40 ALL patients as experimental group and 40 healthy individuals as control group with the age mean of 9.3 years were included. ALL in patients had been confirmed by clinical findings, cellular morphology, and flow cytometry before in well-equipped clinical centers in Iran (Ali Asghar hospital of Tehran and Besat educational and therapeutic center of Hamedan province). Moreover, patients had not any other cancers beside ALL and any immediate relatives affected by cancer. Study subjects in control group were without any blood diseases in not only themselves but also in their immediate relatives. Additionally, those who were under chemotherapy, radiotherapy or immunotherapy were excluded from this study. Written Patients consent form was given to all study subjects and was filled by them before beginning any procedures. Furthermore, some demographic characteristics including age and place of residence was questioned from study subjects.

Laboratory Measurements. Initially, 5 ml vein blood sample without anticoagulant were collected from all the study subjects. Immediately, samples were sent to laboratory for serum separation and further procedures and were kept in -20° C refrigerator until subsequent use.To evaluate vitamin D3 level, chemiluminscence assay performed on each sample using DiaSorin LIAISON 25 OH vitamin D total assay kit according to the kit protocol and by LIAISON immunoanalyzer.

Statistical Analysis. Data were collected and analyzed by IBM SPSS 22. P value calculated by t-test and considered as significant when it was less than 0.05.

Results

This study was carried out on 40 healthy and 40 ALL patients. The age mean among patients and control subjects was similar (Table 1). The mean age of patients group was 9.30 ± 2.24 year as compared to that of control group 9.37 ± 1.95 year (mean ± SD). There was no significant differences in age between control group and patients group.

Table 1. The age status of the study subjects. Forty ALL patients and 40 healthy control were included

	Age mean	SD	Minimum	Maximum
Patients group	9.3	2.24	6	15
Control group	9.375	1.95	6	15

SD, standard deviation

Regard to place of residence, percentage of those who reside in villa was higher in both control and experimental group (Table 2). In patients group

52.5% and 47.5% housing in villa and apartment respectively. Moreover, higher number 60% in control group was housing in villa and 40% residence in apartment.

Table 2. Place of residence distribution among study subjects. (40 patients with ALL versus to 40 healthy control).

	Place of residence by percentage	
	Villa	Apartment
Patients group	52.5	47.5
Control group	60	40

Vitamin D3 level was measured using chemiluminescence analyzer and it was found that 90 percent (36 out of 40) of experimental group and 75 percent (30 out of 40) suffer from sever vitamin D3 deficiency. Moreover, some individuals in experimental and control group suffer from mild to moderate vitamin D3 deficiency (10-19 ng/dl) , Furthermore, it was observed that 10 % in control (n=4) and 20% (n=8) in patients group, respectively (Figure 1). In the control group, two person (5%) found to have desirable level of vitamin D3 (20-50 ng/ml. But none of the patients group had desirable level (20-50 ng/ml vitamin) of vitamin D3 (Figure 1).

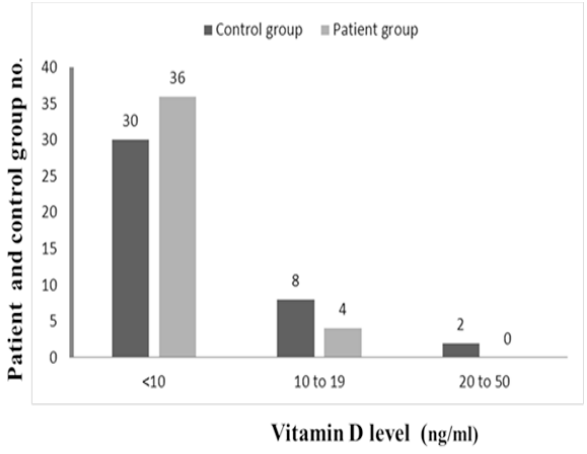

Figure 1: Comparison of vitamin level between control ( ) and patient groups ( ). Numbers of controls and patients are shown on top of the columns.

The mean of vitamin D3 level in control group was significantly higher than patents group due to existence of some persons with normal level of vitamin D3 (Table 3). The mean vitamin D3 level was 4.15 ng/dl ± 3.59 (mean ± SD) as compared to control group. The mean of vitamin D3 level in control group was 7.35 ± 7.95 ng/dl (mean ± SD; min 3, max 30). The Statistical analysis was performed on mean of vitamin D3 level in the control and the experimental group and it is found that there was a significant difference between mean of vitamin D3 level in them ($p < 0.05$). Accordingly. There was an inverse relationship between vitamin D3 level and acute lymphoblastic leukemia risk.

Table 3. Vitamin D_3 level in control and experimental group. Forty ALL patients and 40 healthy control were included in Vitamin D3 level determination

	Mean	SD	Maximum	Minimum
Patients group	4.15	3.59	3	17
Control group	7.35	7.95	3	36

SD, standard deviation

Discussion

This case control investigation conducted on ALL patients and healthy persons to find out if vitamin D3 has role in increasing the risk of getting ALL. ALL is the most frequent kind of leukemia in children. Indeed, it includes 80% of childhood (2-5 years) leukemia though can affect adult as well (13). It is indicated that those with undesirable level of vitamin D3 are susceptible to cancer and have higher mortality due to breast cancer, colorectal and prostate cancer in comparison with person with normal level of vitamin D3 (10). Recent studies have shown that vitamin D3 inhibits BCL2 expression by BOX induction and induction of apoptosis in chronic lymphoid leukemia (11). Furthermore, active form of vitamin D3 can make change in apoptosis and regulator of cell cycle like P21 and P27 that lead to

prevention of cells proliferation (14).

The current study, shown that there is a significant difference between mean of vitamin D3 serum concentrations level of control and ALL patients ($p < 0.05$). In a study carried out on 2562 volunteers in Washington, Garland et al. indicated that those with serum vitamin D3 concentration of more than 20 ng/ml run less risk of colon cancer (15). Another investigation conducted on 105 patients affected by blood cancer including ALL, dysplasia and acute and chronic lymphoid disorders in 2011. They found that there is a significant association between response to treatment and the level of vitamin D3 of below 25 ng/ml (16).

Shnafeit et al. in 2010 in a study on 390 patients affected by chronic lymphoblastic leukemia found that 30.5% (119 patients) have low level of vitamin D3 (17). Additionally, in 2013 Arshi nazi et al. in a case control study measured vitamin D3 level in 30 patients with acute myeloblastic leukemia (AML) and 56 patients with ALL with the age mean of 24. They found the mean of vitamin D3 in AML patients was 17 ng/ml and in ALL patients was 10.52 which these finding corroborate our results (18).

In ALL steroid resistance and hypovitaminosis D are reported that both associated with a poor prognosis Glucocorticoids (GC) are currently use as a treatment protocols in T-cell acute lymphoblastic leukemia and its effect is mainly through the induction of apoptosis (19). Pistor, Met.al has demonstrated that 1,25-OH2 vitamin D3 upregulates the GC receptor and increases GC induced apoptosis (19).

Other investigation has reveals that three quarters of the patients have total vitamin D intake below the RDI (Recommended dietary intakes) 400 IU/day (20) Vitamin D, through its hormonal metabolite 1a,25-dihydroxyvitamin D3, is also a key factor influencing bone mass accretion (20).

The presented study should only be interpreted as a pilot investigation in which vitamin D3 concentrations was determined in ALL patients. Furthermore, our study clearly argues for additional research, for example, to investigate the effect of vitamin D3 on the therapeutic efficacy of glucocorticoids and methotrexate in both B- and T-ALL patients and it might has clinical implications for therapeutic resistant ALL.

Conclusion

There is possible association between initiation of acute leukemia and vitamin D3 deficiency. Accordingly, it is important to carry out some other investigations in different locations and more extended population to make it clearer and prescribe vitamin D3 to deficient people to prevent acute leukemia. Vitamin D3 deficiency might also has a role in treatment of ALL.

Conflicts of Interest

There is no conflict of interest among authors.

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