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ORIGINAL ARTICLE

Assessment of the humoral immunity against diphtheria, tetanus, and hepatitis B among children with acute lymphocytic leukemia

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ABSTRACT

Background: Approximately all systemic therapies for childhood affect the immune system. The behavior of the immune system in leukemia patients following chemotherapy is not yet clearly defined. The probability of vaccination failure and the need for revaccination remain challenging for these patients.
Aim: To evaluate the humoral immunity against diphtheria, tetanus, and hepatitis B in children with acute lymphocytic leukemia (ALL) immediately and 6 months after chemotherapy.
Materials and Methods: In the present prospective cohort study, 21 patients with ALL referred to Mofid Children's Hospital were studied immediately and 6 months after chemotherapy. Serum samples were collected from patients, and the levels of immunoglobulins (IgG, IgM, IgE, and IgA) antibodies against diphtheria, tetanus, and hepatitis B were determined using specific enzyme-linked immunosorbent assay kits. The obtained data were analyzed using Statistical Package for Social Sciences 21 software.
Results: A total of 13 males and 8 females with an average age of 8.6 ± 2.5 years were included in the present study. Six months after chemotherapy, the mean level of IgG, IgM, IgE, and IgA displayed an increase of 563.1 units in IgG, 11 units in IgM, 11.3 units in IgE, and 5 units in IgA levels. Moreover, data revealed that 6 months after chemotherapy, the mean level of *IgG antibodies* displayed an increase of 7.09, 3.43, and 1.03 units against hepatitis B, diphtheria, and tetanus, respectively. A significant relationship was found between the antibody level against diphtheria and the age group of the patients ($p = 0.003$).
Conclusion: Humoral immune status was boosted after 6 months of chemotherapy, though all patients had some extent of lasting immune dysfunction. We indicate that survivors of childhood cancer have ongoing humoral immunological defects and may remain at risk for infectious complications after completion of therapy.
Relevance for Patients: The present study indicated that systemic therapies for pediatrics with leukemia affect the immune system. Pediatrics with leukemia may remain at risk for infectious complications after completion of therapy.

1. Introduction

Cancer is the second leading cause of death in children under 15, and leukemia is the most common type of cancer in this age group [1,2]. In leukemia patients, the humoral and cellular immune systems are impaired, and the patients are immunocompromised [3,4]. Various types of leukemia affect bone marrow (BM) cells and the immune system. For example, in chronic lymphocytic leukemia, mature B lymphocytes accumulate in

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lymphoid organs, and the BM [5,6]. In addition, immune system dysfunction is an inevitable side effect of chemotherapy, which is used in the treatment of leukemia.

Chemotherapy uses potent cytotoxic and immune-suppressing agents to eliminate cancerous cells, but these drugs also affect normal BM cells, leading to a reduction in blood cells [7] and defects in the humoral immune system. The abnormality in the immune system after chemotherapy in leukemia patients lasts for a general period of 6–12 months after stopping the treatment [8]; however, long-term abnormalities have also been reported [9], and B lymphocytes are mainly sensitive to this side effect of chemotherapy [10,11]. Therefore, these patients appear to be highly susceptible to infection, even diseases they have been vaccinated for, such as diphtheria, tetanus, or hepatitis B.

Post-chemotherapy immune system dysfunction has been reported, but previous findings are not consistent [12,13]. The probability of vaccination failure and the need for revaccination remain challenging for these patients [14]. In this regard, the behavior of the immune system in leukemia patients following chemotherapy is not yet clearly defined. In the present study, we aimed to evaluate the humoral immunity against diphtheria, tetanus, and hepatitis B in pediatrics with leukemia immediately and 6 months after chemotherapy.

2. Materials and Methods

The present prospective cohort study included 21 children (13 males and 8 females) with acute lymphocytic leukemia (ALL). The patients were assessed at 1 and 6 months after therapy termination. The treatment process of all enrolled patients was performed based on the Acute Lymphoblastic Leukaemia: BFM 20001 Schema protocol [15]. Briefly, all patients were treated with a combination of vincristine sulfate, adriamycin, and methotrexate.

Patients with a history of stem cell transplantation or primary immune deficiency disease were excluded from this study. All participants provided written informed consent, and the Institutional Review Board approved the protocol and consent forms at Shahid Beheshti University of Medical Sciences (approval number: IRSBMU.MSP.REC.1399.473).

The following data were collected immediately after and 6 months after the end of the chemotherapy: quantitative level of total immunoglobulins (IgG, IgM, IgE, and IgA), IgG antibody levels against diphtheria, tetanus, and hepatitis B, white blood cell (WBC) number, and neutrophil and lymphocyte percentages.

Serum samples were screened for IgG antibodies against diphtheria and tetanus using the commercial Human Diphtheria Antibody enzyme-linked immunosorbent assay (ELISA) Kit and Tetanus Toxoid IgG ELISA Kit, respectively (MyBioSource, United States of America [USA]). Furthermore, a hepatitis B antibody rapid test kit was used to identify the IgG antibody against the hepatitis B virus. All ELISA reactions were performed according to the manufacturer's instructions.

The patients were categorized into two groups based on age: group 1: ≤8 years old; group 2: >8 years old. The relationship between the immune system-related factors and the age group and gender of the patients was evaluated.

Data were analyzed using Statistical Package for Social Sciences (SPSS) V19 software (SPSS Inc., USA). The Kolmogorov–Smirnov test evaluated the normal distribution of continuous variables. Parametric data are expressed as mean ± standard deviation. The Chi-square test and Spearman correlation were used to analyze data. For all analyses, a $p < 0.05$ is considered significant.

3. Results

3.1. Total IgA, IgE, IgG, and IgM

The mean age of the patients was 8.6 ± 2.5 years old. In general, 6 months after chemotherapy, the mean level of IgG, IgM, IgE, and IgA exhibited an increase of 563.1 units in IgG level, 11 units in IgM, 11.3 units in IgE, and 5 units in IgA levels (Table 1). Changes in study variables 6 months after the completion of chemotherapy are presented in Table 2. Our analyses revealed that the IgM, IgE, and IgA levels were significantly increased after 6 months of chemotherapy compared to the immediate time after chemotherapy ($p = 0.001$), but the increment in IgG level was not significant ($p = 0.336$). Moreover, no statistically significant relationship was observed between the level of these IgG, IgM, IgE, and IgA and gender (Table 3).

3.2. IgG antibody levels against diphtheria, tetanus, and hepatitis B

In total, 6 months after chemotherapy, the mean level of *IgG antibodies* exhibited an increase of 3.43 and 1.03 units

Table 1. Changes in antibody levels

Antibody	Antibody levels (unit)		p-value
	Immediately after chemotherapy	6 months after chemotherapy	
IgG	665.6±509.9	1228.7±1815.2	0.366
IgM	46.9±56.9	57.9±44.3	<0.001
IgE	29.3±80.5	40.6±77.7	<0.001
IgA	84.9±85.4	89.9±69.3	<0.001

Note: Antibody levels are presented by mean±standard deviation.

Table 2. Changes in study variables 6 months after the completion of chemotherapy

Variables	Changes in variables		
	Increase, *n* (%)	Constant, *n* (%)	Decrease, *n* (%)
Serum levels of immunoglobulins			
IgG	10 (47.6)	0 (0)	11 (52.4)
IgM	9 (42.85)	3 (14.3)	9 (42.85)
IgE	12 (57.1)	3 (14.3)	6 (28.6)
IgA	8 (38)	3 (14.3)	10 (47.6)
Antibody titer against diphtheria	11 (52.4)	4 (19)	6 (28.6)
Antibody titer against tetanus	3 (14.3)	5 (23.8)	13 (61.9)
Antibody titer against hepatitis B	1 (4.8)	5 (23.8)	15 (71.4)
WBC count	16 (76.2)	1 (4.8)	4 (19)
Neutrophil count	12 (57.1)	0 (0)	9 (42.8)
Lymphocyte count	10 (47.6)	0 (0)	11 (52.4)

Abbreviations: *n*: Number of patients; WBC: White blood cell.

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against diphtheria and tetanus, respectively. In contrast, results indicated that the mean IgG antibody level against hepatitis B increased by 7.09 units compared to the immediate time after cessation of therapy (Table 3).

Our raw data analyses revealed that 6 months after chemotherapy cessation, one patient had the highest changes in IgG antibody levels against diphtheria (from 0.05 to 71), two patients had the highest changes in IgG antibody levels against tetanus (from 0.35 to 22.9; from 0.27 to >5), and four patients had the highest changes in IgG antibody levels against hepatitis B (from 12 to 6; from 14 to 9.35; from 1 to >200; from 15 to 10).

Our analyses revealed that the changes in antibody levels against diphtheria, tetanus, or hepatitis B were not significant after 6 months compared to the immediate period following chemotherapy cessation (p=0.157, 0.179, and 0.249, respectively; Table 3). No significant relationship was found between the antibody level against diphtheria, tetanus, or hepatitis B and the patient's gender (Table 3). In contrast, a significant relationship was found between the antibody level against diphtheria and the age group of the patients (p = 0.003; Table 3).

3.3. Levels of immune system-related factors

Six months after the completion of chemotherapy, the average levels of WBCs and neutrophils increased by 1121 n/mm^3 and 1.77%, respectively. The average level of lymphocytes decreased by 0.01% after 6 months following chemotherapy cessation. The relationship between the levels of WBCs, neutrophils, and lymphocytes within the age group was not significant (p > 0.05). Changes in WBC number were significant (p = 0.001), but neutrophil (p = 0.516) and lymphocyte (p = 0.262) levels did not change significantly after 6 months compared to the immediate period following chemotherapy cessation (Table 4). Six months following chemotherapy cessation, all patients enrolled in the analysis responded well to the treatment.

Table 3. Changes in antibody levels against diphtheria, tetanus, and hepatitis B

Parameter	Antibody levels		
	Immediately after chemotherapy	6 months after chemotherapy	p-value
Anti-diphtheria (unit)	0.25±0.46	3.68±15.43	0.157
Male	0.22±0.28	6.67±19.62	0.123
Female	0.30±0.68	0.44±0.83	0.358
p	0.383	0.138	
Age (years)			
≤8 (*n*=10)	0.24±0.26	3.65±15.76	0.244
>8 (*n*=11)	0.27±0.35	0.98±0.43	0.003*
Anti-tetanus (unit)	1.15±1.18	2.18±4.94	0.179
Male	1.04±1.08	1.26±1.71	0.349
Female	1.34±1.38	3.37±7.42	0.229
p	0.306	0.234	
Age (years)			
≤8 (*n*=10)	0.98±1.32	1.6±1.43	0.163
>8 (*n*=11)	1.29±1.15	2.98±8.4	0.258
Anti-hepatitis B (unit)	10.76±16.75	17.85±44.61	0.249
Male	12.37±21.09	10.39±19.99	0.404
Female	8.13±5.21	30.10±69.12	0.192
p	0.252	0.239	
Age (years)			
≤8 (*n*=10)	10.87±18.88	13.46±43.61	0.432
>8 (*n*=11)	9.97±7.23	16.64±23.54	0.189

Note: Antibody levels are presented as mean±standard deviation. *p<0.05 indicates statistical significance.

4. Discussion

The present study aimed to investigate the humoral immunity against diphtheria, tetanus, and hepatitis B in children with leukemia both immediately after chemotherapy and 6 months later. The mean of IgM, IgE, and IgA IgG, the total number of WBC significantly increased after 6 months; the mean level of antibodies against diphtheria, tetanus, and hepatitis B, as well as the lymphocyte and neutrophil levels, did not change significantly after 6 months in comparison to the immediate period following chemotherapy cessation.

Immunosuppression is a significant side effect of many antineoplastic drugs. The rebuilding of the immune system can differ depending on the nature of the disease, drug type and dosage, and the patient's age [16]. In a cross-sectional study performed by Ek *et al*. [17], involving 31 children with ALL, it was revealed that the levels of IgG and IgM increased, whereas the level of IgA decreased at 6 months compared to 1 month following treatment cessation; contrary to our findings, these changes were not significant. An increase in IgG, IgM, IgE, and IgA levels after treatment cessation indicates immune system restoration, as demonstrated in various studies [8].

The findings of our study revealed that the levels of antibodies against hepatitis B and tetanus, IgG, IgM, IgE, IgA, and WBCs were not significantly different in the two age groups of patients (≤8 or >8). In line with our findings, Williams *et al*. studied 116 ALL patients with a median age of 6 years (range: 2–17 years) at intervals of 6 months after chemotherapy up to 18 months; no significant relationship was found between age and immune system reconstruction [18]. Our study was limited only to leukemia, and no categorization was performed based on the type of leukemia.

Our study investigated the relationship between gender, antibodies against diphtheria, tetanus, and hepatitis B, and IgG, IgM, IgE, and IgA levels 6 months after treatment cessation. In

Table 4. Changes in white blood cell (WBC), neutrophils, and lymphocyte levels

Parameter	Leukocyte count		p-value
	Immediately after chemotherapy	6 months after chemotherapy	
WBC (*n*/mm^3)	5439±1619	6560±2089	0.001
Neutrophil (%)	49.19±14.93	50.96±8.84	0.516
Lymphocyte (%)	42.07±14.08	42.08±10.50	0.262

Note: WBC, neutrophil, and lymphocyte levels are presented as mean±standard deviation.

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addition, Williams *et al*. revealed that there is no relationship between gender and the recovery rate of the immune system after chemotherapy cessation [18]. They also reported that the WBC level did not change significantly with increased time after treatment cessation.

The present study displayed increased levels of WBC, lymphocytes, and neutrophils 6 months after treatment cessation. Several studies confirmed this finding [8,18]. Perkins *et al*. investigated the immune system status immediately and 6 months after treatment cessation in 20 patients with ALL and acute myeloid leukemia; it was reported that the number of WBCs decreased at both times, but this decrease is compensated to some extent after 6 months following treatment cessation [8]. In a study by Kosmidis *et al*., it was also reported that children experienced significant neutropenia during the first few months of treatment, but this is less common during maintenance chemotherapy [19]. However, lymphopenia with low levels of B and T cells is common and has been reported to persist for up to 6 months after treatment [14,19].

In this study, the average antibody level against diphtheria and tetanus increased 6 months after treatment, whereas the average antibody level against hepatitis B decreased. Although these changes were not significant, the immune system restoration resulted in increases in WBCs and IgG, IgM, IgE, and IgA in this study and previous studies [18,20], and an increase in antibody levels against diphtheria and tetanus was also expected. The decrease in antibodies against hepatitis requires further investigation; it may also return to the average level in a more extended period, as studies have reported that some changes in immune system reconstruction occur in the long term [19]. Collectively, we revealed improvement of the humoral immune system to some extent after 6 months of chemotherapy, but similar to Perkins *et al*., some degree of defects in the immune system were also reported [8].

One of the limitations of this research is that the sample patient population was small, and a study with a more significant population is warranted. In addition, this study did not separate the different types of leukemia, and it is recommended that future studies distinguish between these types for a more detailed investigation.

5. Conclusion

The findings of this study indicate that the status of the humoral immune system exhibits slight improvement 6 months after the end of chemotherapy compared to the initial assessment. In addition, there is no difference between age and the condition of the humoral immune system in the patients 6 months after chemotherapy treatment. We indicated that survivors of childhood cancer have ongoing humoral immunological defects and may remain at risk for infectious complications after completion of therapy.

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Conflicts of Interest

The authors declare that they have no competing interests.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Pediatric Congenital Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (IRSBMU.MSP.REC.1399.473). A questionnaire was designed for each child, and written informed consent was acquired from their parents during the course of sample collection.

Consent for Publication

We explained the aims of the present study to children and their parents. A questionnaire was planned for each of the included children and written informed consent was obtained from all children and their parents during the study.

Availability of Data

All data generated or analyzed during this study are included in this published article.

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