International Journal of Children and Adolescents

Original Article
IJCA, Vol. 1, No. 1, Jul, 2015.14-19.

Humoral immunity state in survivors of pediatric acute lymphoblastic leukemia

Gholamreza Bahoush ***(*corresponding author)***: *Associate Professor in Pediatric Hematologist Oncologist, Oncopathology research center, Ali Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran. bahoush04@yahoo.com*
Ebrahim Kalantar: *Medical Laboratory Technologist, PhD of Medical Genetics, Department of Immunology, Allied Health Sciences Faculty, Iran University of Medical Sciences, Tehran, Iran.*
Ahmadreza Shamshiri: *Epidemiologist, Dental Research Center, Assistant professor in School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.*

Received: 25 Jan 2015 Accepted: 29 May 2015

Abstract

Background and Objective: Since there are very few studies on the immunodeficiency state of the Iranian survivors of pediatric acute lymphoblastic leukemia (ALL), we conducted this study to determine the prevalence of humoral defects in children with ALL at least one year after completion of chemotherapy.

Methods: In this study, antibody titers for mumps, rubeola, rubella, tetanus and diphtheria toxoids, pertussis and poliovirus were measured in 28 survivor of childhood ALL and 29 normal children. Also, immunoglobulins titers for all participants were evaluated.

Results: In spite of normal serum immunoglobulin levels in all participants, the percentage of children with ALL who had protective titers was markedly lower than that anticipated for immunized controls ($p<0.001$). The rate of protective titers for mumps, rubeola, rubella, tetanus and diphtheria toxoids, pertussis and poliovirus were 7.1, 50, 25, 35.7, 10.7, 21.4, and 10.7 percent in patients and 93.1, 93.1, 100, 96.6, 86.2, 82.8, and 100 percent in controls, respectively.

Conclusion: The prevalence of humoral immune defects was high among the survivors of pediatric ALL. It appears that these survivors are at risk of developing these bacterial and viral infections and therefore have to be re-vaccinated as required.

Keywords: Immunodeficiency, Pediatric ALL survivors, Humoral immunity

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy among children, accounting for more than 35% of them (1). Considering the therapeutic advances in recent years, approximately 80% of children with ALL would stay in the first remission for 5 years or more after diagnosis. Infections are the most significant cause of morbidity and mortality during the treatment and convalescent phases of the disease. Resolution of any immune defect in children who are disease-free occurs within 6 months to 1 year (2). After that period, the risk of infectious complications is thought to be equal to that of the general population (2). However, the long-term risk of serious infections is unknown (2). There are several studies on the immune status of children with acute leukemia. Studies in children with ALL have shown that after chemotherapy, both humoral and cellular immune responses are depressed and the rate of recovery varies based on individual protocols (2-18). In addition, antibody responses to prior immunizations may be negatively affected. Also, there is a CDC guideline for revaccination of these patients (3,4,16). However, very few studies have evaluated immunodeficiency in Iranian survivors of pediatric ALL (12). On the other hand, the CDC guideline was not implemented for these survivors in our center before starting the study and serious diseases due to these specific viral/bacterial infections were never reported.

Therefore, the aims of this study were to determine the prevalence of defective humoral responses to common bacterial and viral vaccines in a cohort of survivors of ALL and to compare the results with the data of the control subjects as a normal population in Iran.

Methods

We performed a file review on children with precursor T- or precursor B-cell ALL who had been identified, treated and observed at Ali-Asghar Children's Hospital in Tehran. A multiparameter flow cytometric analysis was used to classify these patients for phenotyping. The children had been treated with either the modified BFM (for B-cell ALL), the NY1 (for T-cell ALL) and interfant-99 (for infant ALL) protocols. The patients were included in the study if their treatment were completed at least 1 year before entering the study and were considered to be clinically in remission. Exclusion criteria were: 1) Children with known primary or secondary immunodeficiency, and 2) any child who was known not to be up-to-date with their immunizations at the time of diagnosis. After approval of the project by the Oncopathology Research Center and the Ethics Committee of Tehran University of Medical Sciences, the study was started.

On clinical evaluation, the history of immunizations and serious infections was obtained. The dates of birth, ALL diagnosis, and completion of chemotherapy and the genotype and phenotype of the ALL were also recorded. All immunizations that were given to each child before, during, and after the diagnosis of ALL were also documented.

Humoral Immunity Evaluation: Antibody titers for mumps, rubeola, rubella, tetanus toxoid, diphtheria toxoid, and pertussis were measured with enzyme-linked immunosorbent assay (ELIZA) technique using Ridascreen® of R-biopharm company and antibody against all types of polio (1,2 and 3) were measured with the DEMEDITEC Polio Antibody ELIZA Test Kit in one commercial reference laboratory.

The humoral response to each vaccine was classified as: protective (when the level of the specific antibody to the given microbial agent was equal or greater than the reference value) or non-protective (when the level of the specific antibody to the given microbial agent was less than the reference values or when the level was reported to be indeterminate).

None of our patients described an increased incidence of unusual or serious infections. Of course, every patient with low CD20 level in the end of therapy received IVIg (5gr) at once. Also, all enrolled patients received monthly IVIg (2.5-5 gr) in fall and winter during the therapy.

As for the control group, 29 healthy children were selected from among those who referred to the laboratory center; they were examined for suspected immunodeficiency and were found to have normal quantities of immunoglobulins. Moreover, their past medical history was not consistent with an immune defect considering the similar age range as our ALL study population. Also, they had received appropriate immunized immunization. Titer measurement was performed with the same protocols in one laboratory for both groups.

*Statistical procedures***:** The frequency of protective titers in the study groups was reported as count and percent. Antibodies titers were summarized as geometric mean and 95% confidence interval. The categorical variables were analyzed with Chi-square or Fischer's exact tests and continuous variables were analyzed with t-test. Due to skewed distribution of antibodies titers, logarithmic transformation was applied. Considering the limited number of the patients with protective titers, the Mann-Whitney test was used to compare continuous variables between patients with or without the protective titers of routine vaccines. P values less than 0.05 were considered significant.

Results

Twenty eight children were treated at the time of initial diagnosis of ALL; 17 children with precursor B-cell type were treated with the conventional BFM protocol, and 6 others with the same type were treated with the BFM-IC 2002 protocol. Two children with T-cell ALL were treated with the NY1 protocol. Two infant patients were treated using the CCG protocol for infant ALL while the interfant-99 protocol was used to treat others. Thirteen percent of the children were younger than 2 years and 20% were older than 6 years at the time of diagnosis. The chemotherapy regimen lasted for a mean of 3 years (95% CI, 2.5-3.5). The median interval time between the end of chemotherapy and the assessment of antibody titer was 14 months (12-74).

Antibody titers to the seven mentioned diseases were measured for all enrolled patients. Table 1 shows qualitative comparison of protective frequencies against common vaccine-preventable infectious diseases between cases and controls. Table 2 shows quantitative comparison of cases and controls for serum IgG levels of routine vaccines as the geometric mean and 95% CI for each vaccine. The percent of children with ALL with protective titers ranged from 7.1% to 50% which

Table1. Comparison of protective frequencies against common vaccine-preventable infectious diseases between case and control groups.

	Case (n=28)	Control (n=29)	p value
Tetanus	10 (35.7%)	28 (96.6%)	<0.001
Diphtheria	3 (10.7%)	25 (86.2%)	<0.001
Pertussis	6 (21.4%)	24 (82.8%)	<0.001
Polio	3(10.7%)	29 (100%)	<0.001
Rubella	7 (25%)	29 (100%)	<0.001
Measles	14 (50%)	27 (93.1%)	<0.001
Mumps	2 (7.1%)	27 (93.1%)	<0.001

Table 2. Quantitative comparison between case and control subjects for serum IgG level of the routines vaccines.

Vaccine	Geometric mean	95% confidence interval of the difference of means		p value
		Lower	Upper	
Tetanus	0.33	0.21	0.54	<0.001
Diphtheria	0.21	0.11	0.39	<0.001
Pertussis	0.44	0.31	0.61	<0.001
Polio	0.03	0.02	0.04	<0.001
Rubella	0.02	0.01	0.05	<0.001
Measles	0.39	0.21	0.72	<0.001
Mumps	0.07	0.03	0.14	<0.001

was markedly lower than the percentage of immunized controls (range, 82.8-100%) anticipated after complete vaccination. Of 28 children who had titers drawn to 7 different vaccines, many had multiple non-protective responses to vaccines. Most of these children (62%) had 2 or more non-protective titers.

We compared age at ALL onset between children with protective versus non-protective titers to each of the 7 agents. The mean age at the time of diagnosis was about 3.9 years (95% CI, 3.0-4.7 years). Among these vaccines, only a protective level of pertussis antibody was significantly associated with older age at diagnosis (Table 3). This association was not seen for the remaining 6 vaccines.

Also, we compared age at the time of titer analysis with protective versus non-protective titer to any of the 7 agents. The mean age of all enrolled patients at the time of titer assessment was 8.6 years (95% CI, 7.5-9.7 years). Similarly, only the protective level of pertussis showed a significant correlation with age at the time of titer analysis (Table 3). However, for all other titers, age at the time of titer assessment was not correlated with a protective titer. Thus, only for pertussis, older children with ALL were significantly more likely to maintain protective antibody responses than younger children.

Comparison of the number of months after completion of chemotherapy and titer results for these patients showed that only protective response of pertussis had a significant correlation with the period between completion of chemotherapy and time of titer analysis (Table 3). Thus, if the time interval between the end of chemotherapy and titer analysis of pertussis was more prolonged, the number of cases with a protective titer against pertussis was increased.

Discussion

Similar to previous studies, this study indicated that a large number of these children persistently failed to maintain protective antibody responses to viral or bacterial vaccines or both. The important point was that protective antibody responses to all seven vaccines were markedly decreased in all enrolled patients as compared to controls. Brodtman and his colleagues reported the same finding in their study on 100 children with ALL one year after completion of chemotherapy. They also found that the chemotherapy protocol used did not affect the ability of these children to express protective antibody responses (2). In addition, T-, B-, and NK-cell numbers and proliferative responses to mitogens were all normal (2). Tilburg et al. published a systematic review and reported significant decreased protective antibody and vaccine responses in survivors of ALL therapy (19). However, most patients responded to re-vaccination and they suggested that T-memory cells were preserved in spite of prolonged chemotherapy (8, 19). In another study on patients treated for ALL, Calaminus et al. notified increased positive skin test responses without significant increasing antibody titers following re-vaccination. Moreover, adequate responses were observed to diphtheria and tetanus boosters

Table 3. Correlation of children's age, interval between end of therapy and study enrollment and titer results of pertussis.

	Non-protective titer n (22)	Protective titer n (6)	p value
	Median (min-max)	Median (min-max)	
Age (yr) at diagnosis	3 (0.6-8)	6.5 (4.5-8)	0.01
Age (yr) at the time of the study	8 (5-17)	11.5 (7-23)	0.005
Time interval (mo) between the end of chemotherapy and titer analysis	14 (12-27)	50.5 (41-74)	0.008

given during maintenance therapy (7,10,20). In addition, demonstration of serologic responses to hepatitis B vaccines given during maintenance therapy was reported (7). These findings also suggested that cellular immunity might be normal, although the absence of detectable antibodies to routine vaccines were not detectable. The frequent finding of non-protective antibody titers among patients treated for ALL indicates that children with ALL are more likely to be at risk of serious infections after the end of chemotherapy.

Although we did not have any data on the profile of antibody responses to routine vaccines before starting chemotherapy, it appears that our results could not be affected by this defect for several reasons: 1) Almost all control subjects developed protective titers to routine vaccines; 2) About 95% of all enrolled patients received the first booster dose of all studied vaccines before starting chemotherapy while only 20% of them received the second booster doses. Therefore, it can be assumed that most of the enrolled subjects had protective titers to these infections.

There was no significant correlation between the ability of these affected children to maintain protective antibody responses to routine vaccination (except pertussis) and the age at the time of diagnosis and age at the time of titer assessment. Also, the comparison between this ability and the time interval of titer assessment from the end of chemotherapy was only significant for pertussis. This may suggest that children who are older at the time of ALL diagnosis and at their titer assessment are more likely to express protective antibody titers than younger children. Furthermore, a longer period between the end of chemotherapy and the first titer analysis was only associated with the ability of these children to express protective antibodies for pertussis. Considering the contact with community acquired pertussis infection, we assumed that this finding was a bias in this study; however, another study with more statistical power may be need to have statistically significant results for other considered infections (2).

Different studies have proposed different explanations for the humoral deficit in including the existence of T-cell abnormalities before therapy and its persistency long after treatment completion. Furthermore, abnormalities in cell-mediated immunity can exist at the time of diagnosis in children with cancer. However, most children with malignancy have a normal T-cell count and it appears that immune function is generally normal at presentation (2).

There is a major question regarding the low frequency of acquiring severe life-threatening infection as a consequence of this humoral defect among the survivors of childhood ALL. One main reason can be short-interval monitoring of these children and early treatment of any suspected infection by pediatricians and/or hematologists/oncologists. Another reason may be maintenance of herd immunity in response to routine vaccination schedules in spite of non-protective titers to these infections. Furthermore, IVIg, as prophylaxis, was given to all patients for possible serious infections. Thus, it is probable that IVIg prophylaxis prevented the development of serious infections in these children.

There were no reports about developing considered infections in this study among all enrolled survivors. Therefore, routine re-vaccination of all survivors of pediatric ALL may be questionable. According to a report from Winick, routine assessment of antibody titers before re-vaccination may not be warranted given the associated cost, requirement for an additional visit and needle stick, absence of serologic correlates of protection for some vaccines (e.g., pertussis), and difficulty of having some antibody levels measured (e.g., polio) (13). Chisholm et al. recommended boosters against diphtheria, tetanus, pertussis, polio, H. influenzae, hepatitis B, measles, mumps, rubella, and pneumococcus after the completion of therapy for all patients in the UK (5,13). Those treated with chemotherapy are vaccinated at least 6 months after the completion of therapy which is similar to the last CDC guideline for re-vaccination of patients with cancer

after ending chemotherapy (15, 16). However, this guideline may not be suitable for high risk group of childhood ALL (6, 18).

Our study should alert pediatricians and hematologist/ oncologists to examine the humoral immune function 1 year after successful completion of chemotherapy in children with ALL. Brodtman et al. reported fluctuations in protective antibody titers of 100 children with ALL enrolled in their study after repeated re-vaccination. They showed that children who demonstrated deficient responses to common pediatric vaccines should be re-evaluated, and re-vaccinated when indicated, to ensure that these children maintained protective antibody responses. They suggested that a limited course of IVIg replacement, followed by re-evaluation of children's antibody responses should be considered in children who persistently show multiple non-protective titers (2).

Considering the very low prevalence and incidence of these infections which are routinely prevented through the national vaccination program, we suggest that revaccination be performed in cases with a deficient response to common pediatric vaccines. However, larger studies with more statistical power are required to further evaluate this recommendation. Until then, it appears that these survivors are at risk of developing these bacterial and viral infections and therefore have to be re-vaccinated, as required.

Acknowledgment

The authors are grateful to Dr. Mohsen Asadi-Lari for advice on statistical analysis and critical review of the manuscript. In addition, the authors are also grateful to Research Director of Tehran University of Medical Sciences for financial support of this study.

Conflicts of interest: None declared.

References

1. Allen UD. Immunizations for children with cancer. Pediatr Blood Cancer. 2007; 49:1102–1108.

2. Brodtman DH, Rosenthal DW, Render A, Lanzkowsky P, Bonagura VR. Immunodeficiency in children with acute lymphoblastic leukemia after completion of modern aggressive chemotherapeutic regimens. J Pediatr. 2005; 146:651–654.

3. Calaminus G, Hense B, Laws HJ, Groeger M, MacKenzie CR, Göbel U. Diphtheria (D) and Tetanus (T) antibody values in children with acute lymphoblastic leukemia (ALL) after treatment according to Co-ALL 05/92. Klin Pediatr. 2007; 219:355–360.

4. Committee on Infectious Diseases of the American Academy of Pediatrics. Active and passive immunization. In: Pickering LK, Baker CJ, Long SS, McMillan JA, eds. Red Book1: 2006

Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2006. pp. 71–87.

5. Chisholm J. Reimmunization after therapy for childhood cancer. Clin Infect Dis. 2007; 44:643–645.

6. Torben Ek, Mellander L, Hahn-Zoric M, Abrahamsson J. Intensive treatment for childhood acute lymphoblastic leukemia reduces immune responses to diphtheria, tetanus, and hemophilus influenza type b. Pediatr Hematol Oncol. 2004;26:727–734.

7. Ercan TE, Soycan LY, Apak H, Celkan, T, Ozkan A, Akdenizli E, et al. Antibody titers and immune response to diphtheria-tetanus-pertussis and measles-mumpsrubella vaccination in children treated for acute lymphoblastic leukemia. Pediatr Hematol Oncol. 2005; 27:273–277.

8. Esser MT, Marchese RD, Kierstead LS, Tussey LG, Wang F, Chirmule N, et al. Memory T cells and vaccines. Vaccine. 2003; 21:419-30.

9. Feldman S, Andrew M, Norris M, Iyer R. Decline in rates of seropositivity for measles, mumps and rubella antibodies among previously immunized children treated for acute leukemia. Clin Infect Dis. 1998; 27:388–390.

10. Wai Tsoi C F, Fan Leung T, Kay Sheung Chan P, Lee V, Kong Shing M, Wai Chik K, et al. Humoral immune response after post-chemotherapy booster diphtheria–tetanus–pertussis vaccine in pediatric oncology patients. Pediatr Blood Cancer. 2009; 52:248–253.

11. Kosmidis S, Baka M, Bouhoutsou D, Doganis D, Kallergi C, Douladiris N, et al. Longitudinal assessment of immunological status and rate of immune recovery following treatment in children with ALL. Pediatr Blood Cancer. 2008; 50: 528–532.

12. Mashhadi MA, Khazaei HA, Narouie B, Niazi AA, Moazzami K, Khademi R, et al. Abnormal immunoglobulin levels in Iranian patients with hematologic malignancies. Shiraz E-Med J.2009;10(3).

13. Winick N. Is Immunization necessary after therapy for acute lymphoblastic leukemia (ALL) has been completed? Commentary on Zengin et al. Pediatr Blood Cancer. 2009; 53:922–923.

14. Nilsson A, DeMilito A, Engstrom P, Nordin M, Narita M, Grillner L, et al. Current chemotherapy protocols for childhood acute lymphoblastic leukemia induce loss of humoral immunity to viral vaccinations antigens. Pediatrics. 2002; 109:1–6.

15. Patel SR, Ortin M, Cohen BJ, Borrow R, Irving D, Sheldon J, et al. Revaccination of children after completion of standard chemotherapy for acute leukemia. Clin Infect Dis. 2007; 44:635–642.

16. Pizzo PA, Poplack DG. Principles and Practice of Pediatric Oncology, Sixth Ed, 2011;pp. 1231-1233.

17. Reinhardt D, Houliara K, Pekrun A, Lakomek M, Krone B. Impact of conventional chemotherapy on levels of antibodies against vaccine-preventable diseases in children treated with cancer. Scand J Infect Dis.

2003; 35:851–857.

18. Torben Ek, Mellander L, Andersson B, Abrahamsson J. Immune reconstruction after childhood acute lymphoblastic leukaemia is most severely affected in high risk group. Pediatr Blood Cancer. 2005; 44:461–468.

19. Tilburg CM van, Sanders EAM, Rovers MM, Wolfs TFW, Beirings MB. Loss of antibodies and response to (re-) vaccination in children after treatment for acute lymphoblastic leukemia: a systematic review. Leukemia.2006; 20, 1717-1727.

20. Zengin E, and Sarper N. Humoral immunity to diphtheria, tetanus, measles, and hemophilus influenzae type b in children with acute lymphoblastic leukemia and response to re-vaccination. Pediatr Blood Cancer. 2009; 53:967–972.