Pediatr Blood Cancer 2010;54:716–720

Immunogenicity of Trivalent Influenza Vaccine in Children With Acute Lymphoblastic Leukemia During Maintenance Therapy

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Purpose. The aim of this study was to assess the immune response of children with acute lymphoblastic leukemia (ALL) to influenza vaccine and to compare it with healthy controls. ***Procedure.*** Thirty-two children aged 1–18 years with ALL on maintenance therapy and 30 healthy sibling controls were enrolled in the study. All children were vaccinated with trivalent inactivated influenza vaccine. Hemagglutinin-inhibition (HI) antibody titers were determined in sera of both patient and control groups just before and 4 weeks after vaccination. The ability of each group to mount a protective ($\geq$40) and/or fourfold titer was measured. ***Results.*** The protective response for virus subunits among patients and healthy controls were 43.4% versus 88% for H1N1 ($P = 0.04$), 63.3% versus 80% for H3N2 antigens ($P = 0.06$), and 26% versus 73% for B antigen ($P = 0.001$). Responses for H1N1 and B subunits were significantly lower in patients than controls. In the patient group, the significant response to each virus was demonstrated in the analysis of pre- and post-vaccination geometric mean titer (GMT) ($P = 0.001$). The percentage of patients and controls with fourfold increase in HI titers were 56.2% versus 80% for H1N1 ($P = 0.04$), 40.6% versus 53.3% for H3N2 ($P = 0.31$), and 59.4% versus 83.3% for B ($P = 0.038$). Immune responses for H1N1 and B subunits were significantly lower in patients than controls. ***Conclusions.*** Influenza vaccine is tolerated well in ALL patients with acceptable but limited immune response compared to healthy controls. These findings support the recommendation for annual influenza vaccination in children with ALL. Pediatr Blood Cancer 2010;54:716–720.

Key words: ALL; infection in immunocompromised hosts; immunology; pediatric hematology/oncology; vaccines

INTRODUCTION

Although influenza infection is often a mild illness, it can be life threatening in immunosuppressed patients [1]. This is an important issue in the case of patients with cancer who have a higher risk of serious influenza virus infection than healthy subjects. It is thus suggested that children with cancer may be more susceptible to complications of influenza [1–3].

Influenza infection can cause morbidity and mortality and high risk of interruption or delaying chemotherapy in patients with cancer [4]. To prevent such complications, vaccination is found to be the primary strategy. The Advisory Committee on Immunization Practice (ACIP) of the Centers for Disease Control and Prevention (CDC), American Academy of Pediatrics (AAP) have recommended annual vaccination against influenza, including children and immunosuppressed people [5,6]. Also, the British Royal College of Pediatrics and Child Health (RCPCH) have recommended that influenza vaccine should be given annually in influenza seasons to all patients receiving chemotherapy, and for those still within six months of completion of chemotherapy [7].

Although patients with cancer may benefit from influenza vaccine, there have been conflicting data concerning the immune response to influenza vaccination in patients with cancer [8–19].

It is worth noting that about 65% of pediatric oncologists, questioned in a recent study [20], routinely recommended yearly influenza vaccination for children with cancer, while others did not, because they thought that current influenza vaccine might not be immunogenic in children being treated for cancer.

Influenza vaccines must be updated annually for the vaccine antigens to match those of the circulating strains as influenza viruses mutate frequently [21]. To evaluate the injectable inactivated trivalent influenza vaccines, serum antibody responses measured by the hemagglutination inhibition (HI) assay are found to be the standard method [22].

Because of conflicting data concerning the immune response to influenza vaccine in patients with cancer and the need for constant evaluation of influenza vaccines, this study was undertaken to provide further insight into immune response after the trivalent inactivated influenza vaccine in children with hematologic cancer.

PATIENTS AND METHODS

The present controlled clinical trial was conducted from October 2007 to February 2008 at the Hematology/Oncology Center of Bahrami Children's Hospital in Tehran, Iran.

Study Design

Children aged 1–18 years with acute lymphoblastic leukemia (ALL) in first remission and receiving maintenance therapy were enrolled in this study. All patients were being treated, using the Children's Oncology Group (COG) ALL protocols. Treatment involved 4 weeks of induction chemotherapy followed by 8 weeks of consolidation and enterim maintenance. All patients then received one cycle of re-induction (delayed intensification). Only those patients who had completed their delayed intensification at least

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Conflict of interest: nothing to declare.

Grant sponsor: Tehran University of Medical Sciences.

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Received 15 June 2009; Accepted 7 December 2009

DOI 10.1002/pbc.22421
Published online March 2010 in Wiley InterScience (www.interscience.wiley.com)

3 months earlier were eligible for enrollment. Maintenance therapy involved daily oral 6 mercaptopurine and weekly oral methotrexate and monthly pulse of oral corticosteroid and intravenous vincristine. The exclusion criteria included previous history of influenza vaccination, egg or egg products allergy, previous history of relapse, history of receiving any plasma products or immunoglobulins in 90 days prior to or during 30 days after vaccination, absolute neutrophil count (ANC) <1000/μl or lymphopenia <1,000/μl at the time of vaccination, receiving other vaccine during the study, history of receiving myeloablative therapy and hematopoietic stem cell transplant (HSCT).

Thirty healthy siblings aged 1–18 years were eligible for enrollment and served as healthy controls. In all procedures taken in this study, we adopted the ACIP guideline of influenza vaccination. For convenience, all immunizations were scheduled to coincide with clinic visits for monthly pulse chemotherapy. Ethical approval was obtained from Tehran University of Medical Sciences ethics committee. Also, written consent was given by parents or guardian of each child enrolled into the study.

Vaccine and Schedule

All children were vaccinated with trivalent inactivated influenza vaccine (Influvac) licensed in Solvay Pharmaceuticals (B. V. Netherlands) for 2007–2008 season. The vaccine was stored between 2 and 8°C. They contained influenza surface antigen–surface hemagglutinin antigen (HA) from each of the strains: A/Solomon Islands 3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004-like strain per 0.5 cc dose. This vaccine complies with the WHO recommendation (northern hemisphere). The H1N1 antigen in the vaccine was different from influenza A/Mexico/2008/H1N1.

All children received the vaccine subcutaneously according to the following age-dependent. For children <36 months age, the vaccine was given as two doses of 0.25 ml, 3–4 weeks apart; for children 36 months–13 years age, two doses of 0.5 ml, 3–4 weeks apart; and for children >13 years age, one dose of 0.5 ml. Previously vaccinated children excluded from the study.

Sample Collection

After informed consent, a 10 ml blood sample was obtained from each participant via peripheral vein before immunization and 4 weeks after vaccination. Blood centrifuged to separate serum. The sera were immediately freezed and stored at −20°C until laboratory determination of HI antibody titers to the three antigens (H1N1, H3N2, and B) included in the vaccine. Any paired sera (pre- and post-vaccination) thawed once and tested on the same day using identical reagents.

Adverse Reactions Records

Prospective evaluation of any adverse effect of vaccine was obtained either by parental diary or telephone follow-up by study personnel. Parents were requested to record any local or systemic reactions for 5 days after vaccination.

Serological Analysis

Antibody levels were determined by serum HI. Susceptibility was defined as pre-vaccination HI antibody titer <40. Protective response was defined as achieving HI antibody titer ≥40 following vaccination. The titer represents the level at which approximately 50% of individuals will be protected [23,24]. Sero-response was considered as a fourfold or greater rise in HI antibody titer in children who had titers ≥40 before vaccination or a rise from <10 to ≥40 in those who were sero-negative.

Statistical Analysis

Data analyses were performed using the Statistical Package for Social Sciences (SPSS version 16-0). Log transformation performed for immune responses and results were measured as geometric mean titers (GMT). Before and after immunization (paired samples) comparisons were performed using the Wilcoxon-signed ranks test, and comparisons between two groups (unpaired samples) were performed using the Mann–Whitney *U*-test and three-way comparisons performed by the Kruskal–Wallis test. Comparisons of proportions were analyzed by Fisher's exact test in all independent cases and by the McNemar test in all dependent comparisons. Statistical significance was considered as $P < 0.05$.

RESULTS

Study Population

Thirty-two patients with ALL on maintenance therapy (male/female = 21/11) with mean age of 10.65 ± 4.1 years (median 10, range 1–18 years) and mean time on maintenance therapy of 14.5 ± 10.4 months (median 11 months), and 30 healthy siblings (male/female = 16/14) with mean age of 10.8 ± 4.2 years (median 11.5, range 1–18 years) completed the study. No significant difference was found among the proportion of gender and age distribution in the two groups.

Susceptibility

Prior to vaccination, from the 32 patients, 16 (50%), 11 (34%), and 31 (97%) showed HI titers less than the protective level (<40) to H1N1, H3N2, and B, respectively. Eight (25%) patients were susceptible to all subunits of virus in vaccine. In the healthy control group, the percentage of children with titer less than 40 for H1N1, H3N2, and B were, respectively, 17 (57%), 10 (34%), and 26 (87%). Five (17%) healthy children were susceptible to all subunits of virus in vaccine.

Vaccine-Mediated Protection

Pre-vaccine protection in patients with ALL and healthy controls in the present study were 16 (50%) versus 13 (43%) against H1N1, 21 (66%) versus 20 (66%) against H3N2, and 1 (3%) versus 4 (13%) against B antigen ($P = 0.141$, $P = 0.132$, and $P = 0.072$, respectively).

The patients and healthy controls were evaluated for the protective titer analysis to determine the proportion of patients/healthy controls with increase in HI titers from <40 to ≥40. The increase of HI titers from <40 to ≥40 in the patients were 11 (43.4%) for H1N1, 7 (63.3%) for H3N2 and 8 (26%) for B, whereas 15 (88%), 8 (80%), and 19 (73%) of the healthy controls showed protective response for H1N1, H3N2, and B, respectively. Further study of the results revealed that the protective response (HI titer ≥40) for virus subunits among the susceptible patients and healthy

TABLE I. Comparison of Patients With ALL and Healthy Control in Terms of Pre-Vaccine Immune Status

	H1N1 (GMT, 95% CI)	H3N2 (GMT, 95% CI)	B (GMT, 95% CI)
Patients	32.5 (24.8–42.6)	54 (40–77)	12.8 (10.6–15.2)
Control	31.5 (23–44)	54 (35.2–83)	17 (12.3–23.4)
P	0.873	0.946	0.136

TABLE III. Pre- and Post-Vaccination GMT to Each Virus Following Vaccination in Patients With ALL

	Pre-vaccine, GMT (95% CI)	Post-vaccine, GMT (95% CI)	*P*
H1N1	32.57 (24.88–42.65)	55.86 (39.33–79.29)	0.001
H3N2	55.08 (39.58–76.65)	101.69 (71.45–144.74)	0.001
B	12.75 (10.66–15.24)	27.32 (19.03–39.23)	0.001

controls for H1N1 and H3N2 antigens are not significantly differed, but response rate for B antigen in patients was significantly lower than healthy controls ($P = 0.033$).

GMT Analysis

The antibody titers to each of the three strains of influenza virus before vaccination are shown in Table I. The pre-vaccine GMT for H1N1, H3N2, and B antigens shows no significant difference between patients and healthy controls ($P = 0.873$, $P = 0.946$, $P = 0.136$, respectively). The results of comparing post-vaccine GMT values for each of the three antigens for both patient and healthy control groups are depicted in Table II. This table reveals that a significant difference was observed ($P = 0.041$) only for H3N2. The significant response to each virus in patients following vaccination was demonstrated in the analysis of pre- and post-vaccination GMT ($P = 0.001$). The results are depicted in Table III, showing the response to each virus in patients with ALL.

Immune Response

The response rate analysis showed that the percentage of patients with fourfold increase in HI titers were 56.2% for H1N1, 40.6% for H3N2, and 59.4% for B, while the percentages of healthy controls with fourfold increase in HI titer were 80%, 53.3%, and 83.3% for H1N1, H3N2, and B, respectively (Table IV). The results given in Table IV showed that the response rate for H1N1 and B was significantly lower in the patients than the healthy controls ($P = 0.04$ and $P = 0.038$, respectively).

Adverse Reaction Assessment

Complete reactogenicity data were available from 35 of overall 62 participants (56%). The vaccine was well tolerated in the two patient and healthy control groups and did not cause any significant local or systemic adverse reactions. Axillary temperature >37.8°C during 5 days after vaccination was reported only in two patients and one healthy control. Rates of mild to moderate pain, swelling and redness at the injection site 24 hr after vaccination were 0%, 1%, and 1%, respectively, in the patient group and 0%, 0%, and 1%, respectively, in the healthy control group. Rates of adverse reaction did not differ by dose or numbers of vaccine. No relapse was observed during study and at least 3 months after completion of vaccination.

TABLE II. Comparison of Patients With ALL and Healthy Control in Terms of Post-Vaccine Immune Response

	H1N1 (GMT, 95% CI)	H3N2 (GMT, 95% CI)	B (GMT, 95% CI)
Patient	52.87 (37.7–73.8)	81.87 (55.8–120)	25.41 (18.5–35)
Control	76.38 (55–106.4)	145.41 (100–212.5)	38.07 (26.6–54.3)
P	0.13	0.041	0.106

DISCUSSION

This study, which was carried out for the first time in the Middle East, demonstrated that the trivalent, inactivated influenza vaccine was tolerated well in children on maintenance chemotherapy for ALL with acceptable but limited immune response compared to the healthy controls. Results of recent studies on inactivated trivalent influenza vaccine have shown immune response in children with any kind of malignancy but weaker as compared to healthy control [8–19]. There were, however, conflicting findings about the effect of different variables such as age, type of malignancy, intensity and type of chemotherapy, leukocyte count at the time of vaccination, time of vaccination, correlation to chemotherapy, vaccine dose and previous history of influenza vaccination [8–19]. For example, the study carried out by Porter et al. [15] indicated that sero-response in children receiving maintenance therapy for ALL were significantly low to the subunits of viruses in the trivalent inactivated influenza vaccine as compared to the healthy control group. This finding was in contrast with that concluded in similar studies both carried out by Brydak et al. [25,26], in which significant sero-response to the influenza vaccine were found. These conflicting results could be related to the history of previous vaccination against influenza in a great percentage of healthy controls in the study by Porter et al. [15] and the completion of chemotherapy by most of the patients in the study by Brydak et al. [25,26]. In the present study, we tried to minimize some of these confounding variables, including the type of malignancy, intensity and type of chemotherapy, leukocyte count at the time of vaccination, time of vaccination, correlation to chemotherapy, and previous history of influenza vaccination. Also, another important feature of the present study was the enrollment of patients' siblings in the study as the healthy controls.

In the present study, sero-responses were detected in 56.2%, 40.6%, and 59.4% of patients compared to 80%, 53.3%, and 83.3% of the healthy controls against H1N1, H3N2, and B, respectively, indicating that immune response in patients is comparable to the healthy controls at least for H3N2. These findings are in agreement with those obtained by Matsuzaki et al. [17] and Hsieh et al. [27] in which sero-response in children with ALL were 40–63.3% and 24–60%, respectively. However, they are in contrast to earlier results of Porter et al. [15] in patients treated for ALL, in which significantly low response to the subunits of viruses in the vaccine was found. This could be related to the possible contribution of the history of

TABLE IV. Comparison of Patients With ALL and Healthy Control in Terms of Post-Vaccination Sero-Response* Against H1N1, H3N2, and B Influenza Viruses (95% CI)

	H1N1 (%)	H3N2 (%)	B (%)
Patients (N = 32)	56.2 (26.9–73.1)	40.6 (11.6–55.1)	59.4 (38.6–83.6)
Control (N = 30)	80 (81.9–105.6)	53.3 (53.8–96.2)	83.3 (81.9–105.6)
P	0.04	0.31	0.038
Odds ratio	3.1 (1.1–9.7)	—	3.4 (1.1–11.3)

*Fourfold rise in antibody titer.

previous vaccination in the vast majority of healthy controls to their respective results.

The most recent studies of trivalent influenza vaccination in children with ALL have demonstrated protective response rates of 60–65% [15] or 24–60% [27] for different viral strains. In the present study, the protective response ($\geq$40) was in 26–65.6% patients and 73–88% healthy controls. These results show that protective response in patients is comparable with that suggested in similar studies on ALL patients [15,27] but is significantly lower than healthy controls.

The values of protective pre-vaccination HI antibody titers are in agreement with those obtained by Chisholm et al. [18] for patients with solid tumor (35%, 58%, and 10% against H1N1, H3N2, and B, respectively). Also, the pre-vaccine ratios of protection in both studies are low against subunit B in both the patient and healthy control groups. In a similar study carried out by Bektas et al. [19], the respective values were 52%, 53%, and 44%. In the present study, there was a significant percentage of patients and healthy controls with protective pre-vaccination antibody titer against at least one of the influenza strains in the vaccine. This could be due to previous natural exposure to wild influenza viruses in the community.

Similar to the study done by Chisholm et al. [18] on patients with solid tumors, the present study showed poorer response rate to influenza B in both patients with ALL and healthy siblings as compared to influenza A. Furthermore, the analysis of pre- and post-vaccination GMT showed significant response to each viral subunit in patients ($P = 0.001$).

The interpretation of the results of various studies is difficult because of small sample size and other uncontrollable variables. The present study detected the immune response in patients with cancer, but the clinical efficacy of the vaccine for prevention of influenza infection is still unknown.

In view of acceptable immune response in patients with cancer and no reported serious adverse effect to the vaccine and regarding mortality and morbidity of influenza infection, clinicians can follow the AAP and ACIP recommendations for annual vaccination of children and immunosuppressed people [5,6]. However, further research with larger sample size could shed more light to approving the pre- and post-vaccination status of immunity to influenza in children with cancer as well as answering the questions on timing and dose of the vaccine.

ACKNOWLEDGMENT

The authors would like to thank Mrs. Jalili-Tahmasbi for preparing the manuscript and Mrs. Saadatmand for preparing laboratory data.

REFERENCES

1. Ljungman P, Anderson J, Aschan J, et al. Influenza A in immunocomprised patients. Clin Infect Dis 1993;17:244–247.
2. Kempe A, Hall CB, Macdonald NE, et al. Influenza in children with cancer. J Pediatr 1989;115:35–39.
3. Ruben FL. Inactivated influenza virus vaccines in children. Clin Infect Dis J 2004;38:678–688.
4. Lange B, Shapiro SA, Waldman MTG, et al. Antibody response to influenza immunization of children with acute lymphoblastic leukemia. J Infect Dis 1979;140:402–406.
5. Harper SA, Fukuda K, Uyeki TM, et al. Prevention and control of influenza. Recommendation of the advisory Committee on immunization practice (ACIP). MMWR Recomm Rep 2004; 53: 1–40.
6. American Academy of Pediatrics. Prevention of influenza: Recommendations for influenza immunization of children 2008–2009. Pediatrics 2008;122:1–18.
7. Royal College of Pediatrics and Child Health. Immunization of the Immunocompromised Child: Best practice statement. United Kingdom: Royal College of Pediatrics and Child Health; 2002.
8. Ring A, Marx G, Steer C, et al. Influenza vaccination and chemotherapy. (A shot in the dark?) Support Care Cancer 2002; 10:462–465.
9. Gross PA, Gould L, Brown AE. Effect of cancer chemotherapy on the immune response to influenza virus vaccine review of published studies. Rev Infect Dis 1985;7:613–618.
10. Sumaya CV, William TE, Brunnel PA. Bivalent influenza vaccine in children with cancer. J Infect Dis 1977;136:656–660.
11. Smithson WA, Seirn RA, Ritts RE, et al. Response to influenza virus vaccine in children receiving chemotherapy for malignancy. J Pediatr 1978;93:632–634.
12. Feldman S, Webster RG, Sugg M. Influenza in children and young adult with cancer. Cancer 1977;39:350–353.
13. Steinherz PG, Brown AE, Gross PA, et al. Influenza immunization of children with neoplastic disease. Cancer 1980;45:750–756.
14. Brown AE, Steinherz PG, Miller DR, et al. Immunizations against influenza in children with cancer. Results of a three dose trial. J Infect Dis 1982;145:126.
15. Porter CC, Edwards KM, Zhy Y, et al. Immune response to influenza immunization in children receiving maintenance chemotherapy for acute lymphoblastic leukemia. Pediatr Blood Cancer 2004;42:36–340.
16. Chisholm JC, Devine T, Charlett A, et al. Response to influenza immunization during treatment of cancer. Arch Dis Child 2001; 84:496–500.
17. Matsuzaki A, Lumino A, Koga Y, et al. Immune response after influenza vaccination in children with cancer. Pediatr Blood Cancer 2005;45:831–837.
18. Chisholm J, Howe K, Taj M, et al. Influenza immunization in children with solid tumors. Eur J Cancer 2005;41:2280–2287.

19. Bektas O, Karadeniz C, Oguz A, et al. Assessment of the immune response to trivalent split influenza vaccine in children with solid tumors. 2007;49:914–917.
20. Porter CC, Piehling KA, Hamilton R, et al. Influenza immunization practice among pediatric oncologists. J Pediatr Haematol Oncol 2005;25:134–138.
21. Bridges CB, Harper SA, Fukuda K, et al. Prevention and control of influenza; recommendation of the Advisory Committee on Immunization Practices (ACIP). MMWR 2003;52:1–34.
22. Lee MS, Mahmood K, Adhikary L, et al. Measuring antibody responses to a live attenuated influenza vaccine in children. Pediatr Infect Dis J 2004;23:852–856.
23. Michell DR, Ruben FL, Gravenstein S. Immuogenicity and safety of inactivated influenza vaccine in young children in 2003–2004. Pediatr Infect Dis J 2005;24:925–927.
24. Bridges CB, Katz JM, Levandowski RA, et al. Inactivated influenza vaccines. In: Plotkin S, Orenstein W, Offit P, editors. Vaccines, 5th edition. Philadelphia: Saunder's; 2008.
25. Brydak LB, Rokika-Milewska R, Machala M, et al. Immunogenicity of sub-unit trivalent influenza vaccine in children with acute lymphoblastic leukemia. Pediatr Infect Dis J 1998;17:125–129.
26. Brydak LB, Rockicka-Milewska R, Jackowska T, et al. Kinetics of humoral response in children with acute lymphoblastic leukemia immunized with influenza vaccine in 1993 in Poland. Leuk Lymphoma 1997;26:163–169.
27. Hsieh YC, Lu MY, Kao CL, et al. Response to influenza vaccine in children with leukemia undergoing chemotherapy. J Formas Med Assoc 2002;101:700–704.