Original Article *Iran J Ped Hematol Oncol. 2022, Vol 12, No 1, 1-9*

Dwindled serum IgG levels of Rubella, Diphtheria toxin, Hepatitis B virus and Tetanus Toxoid after chemotherapy; a report from Iranian children with malignancy

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Received: 25 July 2021 **Accepted:** 03 October 2021

Abstract

Backgrounds: Epigenetic regulation such as DNA methylation plays a major role in chromatin organization Background: Chemotherapy suppresses immunoglobulin production as a result of cell toxicity. Decreased immunoglobulin levels can result in the onset of opportunistic infections. The aim of the current study is to compare the immunoglobulin G (IgG) levels of the selected vaccine-preventable disease (VPD) before and six months after chemotherapy in a group of Iranian children with malignancies.

Materials and Methods: In this interventional study, serum levels of Rubella, Diphtheria toxin, Hepatitis B virus (HBV), Tetanus Toxoid, Mumps, and Measles IgG were measured in 30 children with malignancy and previously vaccinated for these diseases. Six months after chemotherapy, serum IgG levels were reassessed and compared with their corresponding pre-chemotherapy levels.

Results: In this study, 17 (56.7%) male and 13 (43.3%) female were included. The mean age was 7.69±3.09 years. After chemotherapy, Rubella IgG levels dropped from 73.88±85.11 to 56.59±72.84 IU/mL ($P<0.05$; $r= 0.956$; 33.4% become serum negative (SN)). Diphtheria toxin IgG was diminished from 0.683±0.454 to 0.174±0.248 IU/mL ($P<0.05$; $r=0.601$; 26.7% SN). Anti-HBV IgG showed a reduction from 46.26±101.56 to 25.56±80.49 IU/mL ($p<0.05$; $r= 0.524$; 60% SN) and Anti-Tetanus Toxoid IgG fell down from 1.031±0.582 to 0.321±0.408 IU/mL ($p<0.05$; $r= 0.365$; 33.4% SN). Anti-Measles and Anti-Mumps IgGs showed no significant change ($p>0.05$).

Conclusion: Pediatric chemotherapy was associated with dropped serum IgG levels of most VPDs. A good correlation was also observed between serum levels of IgG before and six months after chemotherapy. Revaccination of children with malignancies may be necessary upon declined serum IgG titers.

Keywords: Chemotherapy, Immunoglobulin G, Neoplasm, Vaccine-preventable diseases

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[DOI: 10.18502/ijpho.v12i1.8355]

Introduction

Infectious diseases, both viral and bacterial types, are prevalent in Iran due to the tropical weather of Iran, posing high risks of communicable diseases for special populations. For instance, hepatitis B virus prevalence is reported to be 20% between patients with thalassemia in Iran. Accordingly, patients with thalassemia receiving blood products or patients suffering from selected cancers and those who receive immunosuppressive therapies such as chemotherapy are among the higher-risk population (1, 2). Vaccination is an efficient approach for prevention and decrement of the mortality and morbidity rate of several infective diseases (3). Immunodeficiency or impaired immunity could be due to medical interventions, immunomodulatory drug usage, surgery, trauma, environmental factors, severe

infections, malnutrition, vitamin D3 deficiency, and metabolic disorders (4). Various types of cancer have been associated with immunodeficiency status. There is an increased risk of infectious diseases in patients suffering from malignancies. In some special types of cancers, the infective agent was the major cause of death (5-7).

Roughly, 1/600 children with cancer die before adulthood (8-10). Chemotherapy in the early stages of cancer can cure the disease; while in the advanced stages, it can control the malignancy. However, chemotherapy can be used in patients who suffer from severe toxicity to normal cells (11). It has been widely used for children with different cancers due to its valuable contribution to the patients' survival (12). Cancer chemotherapy has been associated with hematological toxicity and transient immunosuppression usually lasting three to six months post-chemotherapy (13-15). Younger patients are more sensitive to intensive chemotherapy since the remission to the normal status lasts for a longer time (16).

Investigations have shown an increased risk of lymphatic malignancies in patients suffering from severe immunodeficiency (17). Moreover, malignant patients are prone to bacterial infections due to their disability to produce immunoglobulin. In this regard, antibodies measurement is essential in selecting the proper prophylactic option and the intervention strategy (18).

After chemotherapy, both normal and abnormal bone marrow cell types are suppressed, and the patient needs blood transfusion, which is associated with a set of complications (19). Bacterial infection and viral particles are the most threatening transmittable agents during transfusion (20). Impotent humoral immunity after intensive chemotherapy increases the chance of invasion, reactivation, or remission of vaccine-preventable infectious agents due to the dropped immunoglobulin concentration to the suboptimal levels. Thus, revaccination is recommended for diseases associated with significantly reduced immunoglobulin blood levels (15, 21).

Unfortunately, the knowledge about the effect of chemotherapy on the protective antibodies against vaccine-preventable diseases in cancerous pediatric patients is very limited in Iran. No revaccination program was carried out on children suffering from malignancies and treated with chemotherapy approaches. In this context, the present study aimed to evaluate serum levels of immunoglobulin G (IgG) against Rubella, Diphtheria toxin, hepatitis B virus (HBV), Tetanus toxoid, Mumps, and Measles in a group of pediatric patients before and after chemotherapy.

Materials and Methods

The current interventional survey included the cancer-suffering children referring to the hematology-oncology ward of Amirkabir hospital, a university hospital in Arak city, Markazi province, Iran. Children with cancer, older than two years and younger than 15 years old who completed the vaccination program according to the Iranian health system were included in this study. In addition, written consent forms were completed and signed by the patient's parents. The exclusion criteria included any hematologic disorder other than leukemia, immunodeficiency disorder, parent's dissatisfaction, and patient's circumstances that necessitated exclusion from the study, based on the oncology sub-specialist decision.

Thirty pediatric candidates of chemotherapy with different malignancies were included (Table I). Electronic repository information of the deputy of the health was used to confirm the complete vaccination program for each child based on the national Iranian vaccination schedule. The serum samples were collected before and six months after the chemotherapy.

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Chemotherapy sessions were carried out for each patient based on the subspecialist decision and the newest guidelines for each malignancy and patient's status (tumor grade and the disease stage). HBV vaccination was conducted one month before and six months after chemotherapy. Six months after chemotherapy, the blood samples were again obtained, and serum samples were reassessed in terms of the intended antibodies. The IgG serum levels were explored against Rubella, Diphtheria toxin, HBV (Anti-HBV), Tetanus Toxoid, and Mumps using enzyme-linked immunosorbent assay (ELISA) methods according to the manufacturer's instruction. ELISA Kits Company's name, decision ranges (negative and positive), and the unit of the report are listed in Table I. Figure 1 depicts the study workflow including vaccination, cancer development during life, and IgG assessments before and after chemotherapy.

Statistical analysis

As the design of the study was interventional and the distributions of the data were not normal, the Wilcoxon Signed Ranks test was employed. Serum IgG levels of each infectious agent were compared before and after chemotherapy with at least 95% confidence interval. Besides, Spearman's correlation was used to evaluate the serum IgG level association before and after chemotherapy.

Ethical Consideration

The current interventional survey was approved by the ethical review board of Arak university of Medical Sciences. This study also follows the ethical guidelines of the Declaration of Helsinki (IR.ARAKMU.REC.1397.147).

Results

Among the 30 participants, 17 (56.7%) individuals were male and 13 (43.3%) were female. Their mean age was 7.69±3.09 years (Range: 3-15 years). The most frequent malignancy was acute lymphoblastic leukemia (ALL) (56.7%) (Table II).

Rubella, Diphtheria toxin, HBV, and Anti-Tetanus Toxoid serum IgG levels were significantly decreased after chemotherapy ($p< 0.001$). Rubella IgG was decreased from 73.88±85.11 to 56.59±72.84 IU/mL; Diphtheria toxin IgG showed a reduction from 0.683±0.454 to 0.174±0.248 IU/mL; Anti-HBV IgG was decremented from 46.26±101.56 to 25.56±80.49 IU/mL, and Anti-Tetanus Toxoid IgG dropped from 1.031±0.582 to 0.321±0.408 IU/mL. Meanwhile, Anti-Mumps and Anti-Measles serum IgG levels were decreased and increased, respectively although not significant (Table III).

Spearman's correlation values clarified a powerful, positive, and significant correlation for Rubella IgG levels before and after chemotherapy. The good and significant positive correlations were observed for Diphtheria toxin and Anti-HBV IgGs. An acceptable positive and significant correlation was detected for Anti-Tetanus Toxoid IgG levels. Nonetheless, an insignificant positive association was seen for Anti-Mumps serum IgG level; and an insignificant negative association was resulted from association analysis before and after chemotherapy (Table III).

Figure 2 depicts the frequency of negative, positive, and borderline cases in terms of serum IgG levels, before and after chemotherapy. Based on the reference ranges defined for different kits, after chemotherapy, the percentages of the positive cases were decreased for Rubella, Diphtheria toxin, Anti-HBV and Anti-Tetanus toxoid IgGs from 86.7%, 30%, 86.7%, and 96.7% to 53.3%, 3.3%, 26.7%, and 63.3%, respectively. However, the percentages of Anti-Measles IgG positive cases were increased from 66.7% to 83.3%; this increment was from 33.3% to 56.7% for Anti-Mumps IgG.

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Table I: Laboratory test kits characteristics used in current study; reference ranges are presented

Immunoglobulins-G (IgG) kit	Negative	Positive	Company/country
Rubella (IU/mL)	< 9	> 11	Pishtaz Teb Co; Iran
Diphtheria toxin (IU/mL)	< 0.1	> 1	IBL; Germany
Anti-HBV (mIU/mL)	< 10 mIU/mL	≥ 10 mIU/mL	Pishtaz Teb Co; Iran
Anti-Tetanus Toxoid (IU/mL)	< 0.1	≥ 0.1	EUROIMMUN; United Kingdom
Anti-Mumps (U)	< 9	> 11	IBL; Germany
Anti-Measles (NTU; GenWay Units)	< 9	> 11	GenWay Biotech; USA

Table II: Demographic and basic data of investigated individuals

	Status	Frequency (%)
Gender	Male (%)	17 (56.7)
	Female (%)	13 (43.3)
Types of malignancy	ALL (%)	17 (56.7%)
	AML (%)	6 (20%)
	Brain tumor (%)	1(3.3%)
	Hodgkin lymphoma (%)	2 (6.7%)
	Ewing tumor (%)	1 (3.3%)
	Rhabdomyosarcoma (%)	1 (3.3%)
	Neuroblastoma (%)	2 (6.7%)

Table III: Comparison of serum IgG levels of infectious agents before and 6-months after chemotherapy in cancer patients

Laboratory test	Step	Minimum	Maximum	Mean±SD	Wilcoxon test p-value	R (p-value)
Rubella IgG (IU/mL)	**Before intervention**	4.00	391.00	73.88±85.11	**< 0.001**	**0.956 (<0.0001)**
	After intervention	1.00	328.00	56.59±72.84		
Diphtheria toxin IgG (IU/mL)	**Before intervention**	0.05	1.70	0.683±0.454	**< 0.001**	**0.601 (<0.0001)**
	After intervention	0.01	1.00	0.174±0.248		
Anti-HBV (IgG) (mIU/mL)	**Before intervention**	7.00	571.00	46.26±101.56	**< 0.001**	**0.524 (0.003)**
	After intervention	1.50	445.00	25.56±80.49		
Anti-Tetanus Toxoid (IgG) (IU/mL)	**Before intervention**	0.05	2.50	1.031±0.582	**< 0.001**	**0.365 (0.047)**
	After intervention	0.01	1.60	0.321±0.408		
Anti-Mumps IgG (U)	**Before intervention**	2.00	39.00	11.35±7.97	0.561	0.331 (0.079)
	After intervention	1.17	21.00	9.98±5.44		
Anti-Measles IgG (NTU)	**Before intervention**	1.35	65.00	18.91±14.42	0.121	-0.08 (0.678)
	After intervention	0.001	37.80	22.78±11.04		

* Significant results are written in bold font.

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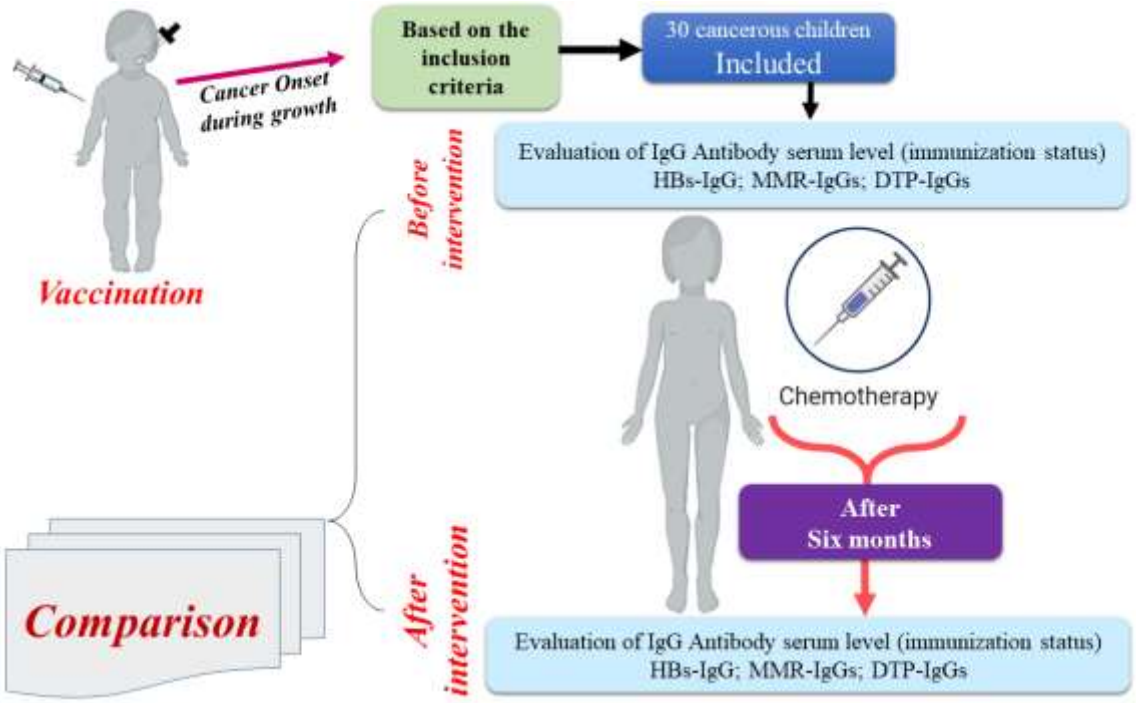


Figure 1. Study design and steps for evaluation of the effect of chemotherapy on the serum IgG levels of a group of vaccine-preventable diseases. Note: this image is created using PowerPoint software and biorender online tools that is available at: https://biorender.com/

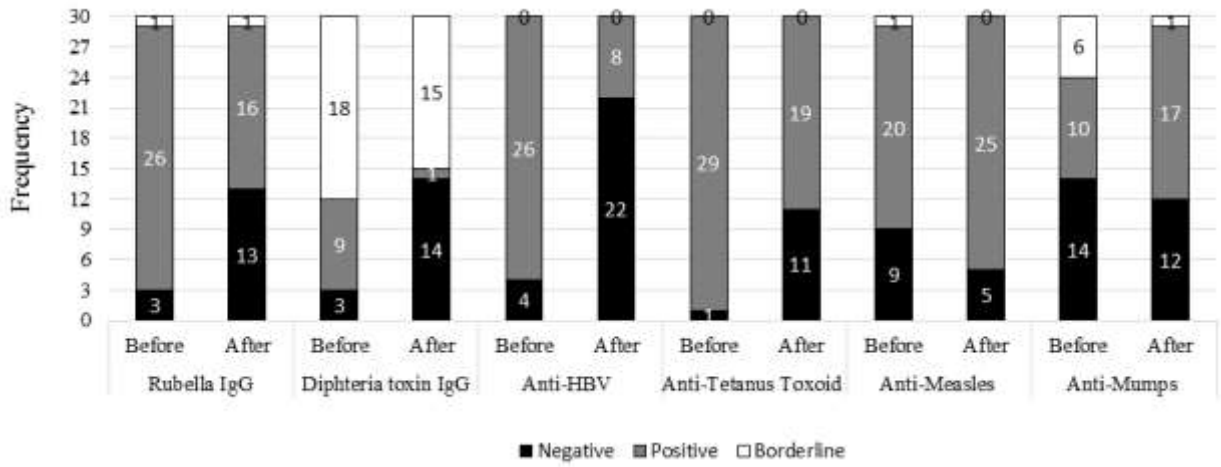


Figure 2: Frequency of each studied disease among children with malignancies, before and after the intervention. Black, gray and white colors represent the frequency of negative, positive and borderline cases. After chemotherapy, positive cases percentages were obviously diminished for Rubella IgG (86.7% to 53.3%), Diphtheria toxin IgG (30% to 3.3%), Anti-HBV IgG (86.7% to 26.7%), and Anti-Tetanus toxoid IgG (96.7% to 63.3%). Anyhow, Anti-Measles and Anti-Mumps IgG positive cases were increased from 66.7 and 33.3% to 83.3and 56.7%, respectively.

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Discussion

This study was done to clarify the immunity status against vaccine-preventable diseases after chemotherapy among pediatric cancer patients referring to this oncology department. The serum IgG levels of Rubella, Diphtheria toxin, HBV, and Tetanus toxoid were decreased after chemotherapy, in a well-correlated manner. On the other hand, serum IgG levels against Mumps and Measles showed an insignificant increase without an acceptable correlation. In other words, after chemotherapy, serum IgG positive cases were respectively decreased by 33.4, 26.7, 60, and 33.4% for Rubella, Diphtheria, HBV, and Tetanus toxoid. Anyhow, Measles- and Mumps-IgG positive cases showed 16.6% and 23.4% enhancement, respectively. The results of Measles and Mumps serum IgG levels were not significant in this study. Bochennek et al. reported the loss of humoral immunity against Measles (27%), Mumps (47%), and Rubella (19%) in children treated for cancer (15). The differences in cancer type and therapeutic regimen could explain the inconsistencies between the presented results and Bochennek's reports.

Garonzi et al. have explored the impact of chemotherapy after pediatric malignancy on humoral immunity to vaccine-preventable diseases. They observed that after chemotherapy, HBV (53%), Rubella (45%), Measles (46%), Mumps (43%), Clostridium Tetani (88%) antibodies changed to negative levels. They confirmed that the seroprotection for studied diseases was affected by malignancy treatment. They used a single booster dose for revaccination and revealed that such an approach can restore vaccine immunity (22). Top and colleagues showed that post-chemotherapy revaccination is immunogenic and well-tolerated by their studied children suffering from ALL (21). In total, the anti-Measles and anti-Mumps IgGs were not significantly changed in this work whereas similar studies showed discrepant findings. Different cancer types and malignancy stages and grades could explain the inconsistency. Furthermore, the sample size is also important; anyway, it is a serious limitation when studying children with malignant disease.

Shahemabadi et al. reported that Anti-HBs antibody levels were positive in 19.12% or borderline in 11.76% of children with malignancies whereas this was only 2.94% positive and 5.88% borderline in the healthy children. They stated that non-immune cases were significantly more frequent between children with cancer (23). Their study was a case-control investigation, but this study is an interventional investigation. Anyway, both studies confirmed low or decreased Anti-HBV IgG among children with cancer. Therefore, HBV revaccination may be necessary as prophylaxis for Iranian children under cancer chemotherapy.

Intensive treatments in childhood ALL were the cause of six-month permanent abnormality in T-, B- and natural killer (NK)-cells (24). After chemotherapy, a decline occurred in the memory B lymphocyte population which is the source of persistent IgG production (24, 25). Therefore, cell toxicity could be the major cause of IgG decline in this investigation, and revaccination probably restores the desired immunity after chemotherapy. Nonetheless, such discussion should be confirmed by the flow cytometric studies.

More precise statistical methods were presented in this study compared to the other published studies (15, 21, 22) since this study offered quantitative reports of IgG antibodies, in addition to the correlation estimations. Quantitative reports help to monitor the values of antibodies with better patient follow-ups. Furthermore, the size of changes in IgG serum levels, after chemotherapy, could be helpful in the prediction of antibody functionality against infectious agents. In

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particular, this is important when a proper correlation exists between pre- and post-chemotherapy results in terms of Rubella, Diphtheria toxin, HBV, and Tetanus toxoid IgG serum levels. This finding suggested that prophylaxis may be necessary for preventable infections among Iranian pediatrics with malignancy. In this regard, one prevention strategy could be revaccination, to keep the patients in a potent immunity status. The therapeutic team will schedule a better management program for a patient if warned about the immunity status against the disease. Hence, the countries without a program of revaccination after pediatric chemotherapy may need a revision on the in-house therapeutic and management guidelines for children chemotherapy.

Conclusion

Pediatric chemotherapy has been associated with reduced serum IgG levels of selected vaccine-preventable diseases. Serum IgG levels were changed in a balanced way meaning that wherever a person had high IgG serum levels before chemotherapy, the IgG values were not severely diminished after chemotherapy. Therefore, keeping IgG levels in high levels values could be protectant against opportunistic agents. As opportunistic infectious agents could threaten children under chemotherapy, a revaccination program was recommended six months after completing the treatment in the cases lacking protective antibody titers. The application of this program is believed to be important to improve the quality of life and survival rate of children with malignancy. Countries without a definite guideline and program of post-chemotherapy immunity status monitoring are recommended to revise their health promotion programs.

Study limitations and strengths

The patient's malignancy and special considerations for their family collaboration in clinical evaluations were the limitations of the current study. Besides, performing such a study with a bigger sample size can help in obtaining the confirmatory results. A report on the Iranian children suffering from cancer, quantitative data presentation, and clarification of revaccination need in the mentioned population are among the strengths of this study.

Acknowledgment

Thanks to the Research Council of Arak University of Medical Sciences, which has provided funding for this research (Grant 3040). We would like to thank all the staff of the Blood and Oncology, Department of Amirkabir Hospital, Arak, Iran.

Conflict of interest

The authors declare no conflict of interest.

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