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Original Article

Comparative study of diphtheria, tetanus, pertussis and hepatitis B antibodies in children under chemotherapy with healthy children

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ABSTRACT

Immunodeficiency and infectious disease antibody reduction due to chemotherapy can increase the acquisition of infectious diseases and death. The aim of this study was to compare the level of immunity (diphtheria, tetanus, pertussis and hepatitis B antibodies) in children under chemotherapy and healthy children. In this descriptive-analytical study, 35 children with cancer who underwent chemotherapy (case group), and 90 healthy children (control group) were enrolled. Evaluation of specific antibody level against *Corynebacterium diphtheriae*, *Bordotella pertussis*, clostridium, tetanus, and hepatitis B was performed in two groups using enzyme-linked immunosorbent assay (ELISA). Data were analyzed by SPSS version 22 using descriptive statistics, chi-square test, and logistic regression model. The mean antibody titers of hepatitis B, diphtheria, and pertussis were significantly lower in case group ($P < 0.05$). There was a significant difference between the two groups in immunity level only for hepatitis B, with a lower level in the case group ($P < 0.023$). Significant, inverse correlation was found between age and antibody titers of hepatitis B ($r = -0.48$, $P = 0.001$) and pertussis ($r = -0.22$ and $P = 0.01$), and after adjusting for the effect of age by using logistic regression, the immunity levels of hepatitis B and pertussis vaccines were not significantly different between case and control groups ($P > 0.05$). The results of this study showed that the antibody titers were lower in the children under chemotherapy, and only in hepatitis B children, the immunity level was significantly lower in the case group than in the control group.

KEYWORDS: antibody titer, diphtheria, tetanus, pertussis, hepatitis B.

INTRODUCTION

Chemotherapy leads to a wide range of side effects [1-3], including reduction in white blood cell count, impairment and toxicity in the tissues, damage to soft tissue and inflammation, leading to weakening of the immune system [4]. In this regard, if the treatment involves chemotherapy and radiotherapy (combination therapy), it may even have worse complications, such as autoimmune toxicity, which require proper management of the treatment to reduce complications [5]. Children in such conditions are more susceptible to develop certain diseases due to chemotherapy such as

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leukopenia, neutropenia, lymphopenia, and hypogammaglobulinemia [6, 7]. On the other hand, children should be monitored for susceptibility to vaccine-preventable diseases, because immune system impairment also reduces immune response and does not produce acceptable levels of immunity, leading to morbidity and mortality [8]. Immunization is one of the most cost-effective methods currently used to reduce the mortality and morbidity of children under the age of five from vaccine-preventable diseases such as diphtheria, tetanus, pertussis, polio, measles, rubella and hepatitis [9]. Hence, antibody titer measurement is necessary in the case of these diseases, and therefore if immunity to them decreases, the patients under chemotherapy should be revaccinated [10]. Therefore, the present study was conducted to compare the level of immunity (diphtheria, tetanus, tetanus and hepatitis B antibodies) in children under chemotherapy with healthy children.

MATERIALS AND METHODS

In this descriptive-analytical study, 125 cancer patients who underwent chemotherapy and were in the remission stage were enrolled in the study. Because of the study limitations (the lack of availability of many patients), the sample size was determined as 35 for the case group and 90 for the control group. Assuming that the prevalence of lack of response to vaccination (reduction of antibody titers in hepatitis B, tetanus, diphtheria, and pertussis) is 20%, the sample size was calculated to detect an at least 25% difference in the prevalence of lack of response to vaccination among children under chemotherapy and in healthy children, based on the 95% confidence interval and 80% power, as well as the formulas and similar studies [11-13]. Assuming that the prevalence of non-vaccination response (reduction in antibody titers in hepatitis B, tetanus, diphtheria, pertussis disease) is equal to 1%, this sample size represents a difference of at least 1% in the prevalence of non-vaccination response among children under chemotherapy. Therapy in healthy children was chosen based on the following. (1% confidence interval and 2% power).

$$\frac{2(Z1 - \alpha 2 + Z1 - B)\ 2P(1 - P)}{d2}$$

The control group was selected from among children referred to Hajar Hospital who were referred to hospitals affiliated to Shahrekord University of Medical Sciences for a reason other than cancer in 2015-2016.

Inclusion criteria included children aged 1 to 15 years, suffering from cancer, being under chemotherapy and experiencing remission, having received routine national vaccinations, and the patients' and, if necessary, their legal guardians' consent to participate in the study. The history of incomplete vaccination, severe combined immune deficiency (SCID) and common variable immuno-deficiency (CVID) and human immunodeficiency virus (HIV) were considered as exclusion criteria.

Evaluation of the specific antibody level against *Corynebacterium iphtheria*, *Bordotella pertussis*, clostridium, tetanus, and hepatitis B was performed using ELISA.

Serum samples of children under chemotherapy as case group as well as those of healthy children as control group were prepared in special tubes. After a maximum of 2 hours, the serum separation process was performed by centrifugation and the collected sera were kept at -20 °C until the test was performed.

After performing the test according to the manufacturer's protocol and by the ELISA method, the results were evaluated in a form that conformed to the standards used in the kit; the results of the case and control groups were calculated and collected using a checklist to be used in statistical analysis. The kit used to measure the antibody titers of diphtheria, tetanus, and pertussis was prepared by IBL International, with a 96% sensitivity and 98% specificity for diphtheria, a 95% sensitivity and a 98% specificity for tetanus, and a 95% sensitivity and 95% specificity for pertussis. The kit used to measure the Hepatitis B antibody titres was AccuDiag™ HbsAB (Quantitative) ELISA Kit, with a sensitivity of 100% and 99% specificity [14].

It should be noted that before the beginning of the study, for the patients fulfilling the inclusion criteria, necessary explanations about the research project were given to their parents and their informed consent was obtained; and the patients were assured that their personal information would be kept completely confidential.

Data were collected by the SPSS version 22. Data were analyzed by descriptive statistics (frequency and percentage), mean (SD), median (interquartile range), chi-square, Fisher's exact test, and logistic regression.

RESULTS

A total of 35 children with cancer and under chemotherapy (case group) and 90 healthy children (control group) were enrolled. 67 children (53.6%) were living in Shahrekord and rest in other regions. Fifty seven (45.6%) of the studied children were girls and the rest boys. In the case group, 20 (57.1%) and in the control group 37 (41.1%) were girls and the rest boys ($P = 0.106$).

The age of the children was 1-15 years old. 29 patients (82.9%) had non-mass cancer and 6 cases (17.1%) had mass cancer. The personal characteristics of the studied children are presented in Table 1. The mean antibody titers of hepatitis B, diphtheria, tetanus, and pertussis were significantly lower in the case group than in the control group ($P < 0.05$) (Table 2). Regarding immunity level in hepatitis B, 16 (45.7%) in the case group and 61 (68%) in the control group were immune and the difference was statistically significant between the two groups only for hepatitis B vaccine ($P < 0.023$) (Table 3).

Table 4 shows the inter-correlations of vaccine antibody titers and the correlation between vaccine antibody titer and age. Hepatitis B antibody titer ($r = -0.48$, $P = 0.001$) and pertussis ($r = -0.22$, $P = 0.01$) were inversely correlated with age. In other words, with increase in the age, the antibody titer decreased. Due to the fact that the two groups were not equal in age, in order to compare the same groups of case and control in terms of immunity level, the effect of age was modified by applying logistic regression model.

The results showed that age had a significant effect on the hepatitis B immunogenicity level ($P < 0.001$); with a 1 year increase in age,

Table 1. The characteristics of the studied children (healthy children and the children with cancer).

Variable	Case	Control	Significance level (*P*)
	Mean ± standard deviation	Mean ± standard deviation	
Age (yr)	7.00 ± 1.30	5.10 ± 3.20	0.003*
Weight (kg)	23.80 ± 9.40	18.50 ± 8.00	0.004*
Height (cm)	111.2 ± 18.40	99.20 ± 17.70	0.001*
Body Mass Index (kg/m^2)	18.7 ± 3.00	18.00 ± 1.80	0.250

*Significant difference ($P < 0.05$).

Table 2. Hepatitis B, diphtheria, tetanus and pertussis antibody titers in case and control groups.

Groups / Antibody titer	Case		Control		Significance level (*P*)
	Mean ± standard deviation	Median (interquartile range)	Mean ± standard deviation	Median (interquartile range)	
Hepatitis B	124.4 ± 59.8	10 (3.3-45)	157.00 ± 206.00	35.5 (6-302)	0.011*
Diphtheria	5.6 ± 4.9	3.8 (2-9.6)	9.6 ± 12.5	8 (4.3-13.3)	0.002*
Tetanus	7.3 ± 3.35	4.1 (2.3-11.6)	12.00 ± 10.00	10 (4.2-17.1)	0.007*
Pertussis	13.45 ± 12.5	10.3 (3-20)	23.3 ± 27.2	13.6 (8.6-28.6)	0.015*

*Significant difference ($P < 0.05$).

Table 3. Immunity levels of hepatitis B, diphtheria, tetanus, and pertussis in case and control groups.

Groups / Immunity	Case				Control				Significance level (*P*)
	Positive		Negative		Positive		Negative		
	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	
Hepatitis B	16	45.7	19	54.3	61	68.8	29	32.2	0.023*
Diphtheria	34	97.1	1	2.9	90	100	0	0	0.028*
Tetanus	11	31.4	24	68.6	40	44.4	50	55.6	0.184
Pertussis	35	100	0	0	90	100	0	0	-

*Significant difference ($P < 0.05$).

Table 4. Spearman correlation coefficient and significance level between age and antibody titers.

Variable	1		2		3		4	
	Coefficient (*r*)	Significance level (*P*)	Coefficient (*r*)	Significance level (*P*)	Coefficient (*r*)	Significance level (*P*)	Coefficient (*r*)	Significance level (*P*)
Age (yr.)	-0.48	0.001*	-0.14	0.11	-0.15	0.09	-0.22	0.010*
Hepatitis B antibody titer	1	1	0.22	0.01*	0.36	0.001*	0.41	0.001*
Diphtheria antibody titer			1	1	0.62	0.001*	0.67	0.001*
Tetanus antibody titer					1	1	0.75	0.001*
Pertussis antibody titer							1	1

*Significant difference ($P < 0.05$).

Table 5. Logistic regression model in order to compare the immunity level of hepatitis B and subcutis vaccines in case and control groups.

Immunity level	Variable	Coefficient	Standard error	Significance level	Odds ratio	OR: 95% confidence interval
Hepatitis B	Age	0.19	0.62	0.002*	1.211	1.07-1.37
	Group	-0.61	0.433	0.158	0.54	0.23-1.26
Pertussis	Age	0.101	0.062	0.102	1.106	0.98-1.25
	Group	-0.372	0.44	0.398	0.69	0.29-1.63

*Significant difference ($P < 0.05$).

the odds ratio increased to 1.211. In other words, with an increase of one year, the likelihood of negativity to positivity and immune levels increased by 21.1%. Based on the results of Table 5, it can be stated that by adjusting the effect of age, the level of immunity of hepatitis B vaccine and cervical cancer was not significantly different in both case and control groups.

DISCUSSION

This study was conducted to comparatively evaluate the antibody titers of diphtheria, tetanus, pertussis and hepatitis B in children under chemotherapy and healthy children. The results showed that the antibody titers of hepatitis B, diphtheria, tetanus and pertussis were lower in children under chemotherapy than in healthy children, but only the level of hepatitis B immunity in the case group was significantly lower than that of the control group. In the present study, the levels of immunity of hepatitis B, diphtheria, tetanus and pertussis were protective in 54%, 97.1%, 100% and 31.4% of children with cancer, respectively, which are within the range reported by van Tilburg *et al*. [15]. In another study, it was found that tetanus and pertussis antibody levels were protective in 100% and 11% of children with cancer, respectively, who underwent chemotherapy in the last 6 months [16]. In the present study, 100% and 31.4% of cancer patients (aged 1-15 years) under chemotherapy had protective antibodies against tetanus and pertussis, which is consistent with the results of that study. But a study by Cheng *et al*. [17] showed that levels of antibodies of diphtheria, tetanus, and pertussis were reduced in the children under chemotherapy, wherein the baseline antibody levels were reported to be 83.6%, 96.5%, and 96.1%, respectively, and even the antibody titer of hepatitis B was lower than the protective level, but increased after the injection of humoral immune vaccine and therefore increase in vaccination can be a way to protect children under chemotherapy against vaccine-preventable diseases.

Kwon *et al*. conducted a study on the immune responses of children undergoing treatment with anti-neoplastic drugs. In that study, the non-protective antibody titers in diphtheria, tetanus and pertussis vaccines were 6.2%, 11.6% and 62.3%, with a partially protective percentage of 37.2%, 28.1% and 8.9%, respectively. Repeated vaccination raised the antibody against the antigens of each vaccine [18]. In addition, in the study of Viana *et al*. on antibody levels against hepatitis B, rubella, measles and mumps antigens, vaccination was performed in children after chemotherapy. The findings showed that antibody levels decreased after chemotherapy in the case group, and after increasing the frequency of vaccination, immunity to rubella alone did not reach protective level [19]. Similar results were also found in other studies on various vaccinations in children with cancer where after chemotherapy, the antibody titer increased, and repeated vaccination after chemotherapy increased antibody levels in these children [20-24].

In one study on the rate of immunity against hepatitis B after receiving chemotherapy drugs, the anti-hepatitis B antibody titer was lower than the protective level in 33 patients (33%) but did not change in 66 (67%) of them. The highest

decrease (63.6%) was observed in patients with acute lymphoblastic leukemia [25]. In the present study, protective levels of anti-hepatitis B antibodies were observed in 45.7% of children under chemotherapy and lack of protective levels in 54.3% of children. In the study of Viana *et al*., after chemotherapy, 75.9% of children with acute lymphoblastic leukemia had antibody levels lower than protective levels against hepatitis B [19]. In this regard, a similar study reported that the antibody level in children with cancer and under chemotherapy was lower than in healthy children [26]. As observed, various studies have reported varying percentages for immunity levels against tetanus, pertussis, diphtheria, and hepatitis B infection, which may be due to the difference in age at the time of titration, the time of antibody testing after the end of the chemotherapy, the duration and type of treatment, the type of cancer and the time of last vaccination for the subjects, which may affect the antibody titer and the resulting immunity level [24, 27]. However, repeated vaccination after several months of chemotherapy (depending on the type of vaccine) is clearly needed, but continuous monitoring of the antibody titer does not seem to be necessary [28]. The reason for the loss of antibody titers after vaccination in children with malignancy has not been fully determined, but this decrease has been attributed to the high sensitivity of lymphocyte B cells to chemotherapy and their long-term recovery time. Sadjadi *et al*. reported lymphopenia or a severe reduction in B-cell lymphocyte count in 16 patients under maintenance therapy for acute lymphocytic leukemia, which was unpredictable based on total white blood cell count and lymphocyte count [29].

According to the results of our study, the antibody titers against pertussis and hepatitis had a significant correlation with the age of children, but antibody titers against tetanus and diphtheria did not significantly correlate with the age of children. In other studies, the effect of age on immune response varied [7, 21, 26] However, immune system damage appears to be influenced by age, type of cancer, and severity of chemotherapy [15], and some studies have suggested that younger children are at higher risk of immunodeficiency after vaccination [16, 30].

CONCLUSION

Tetanus, diphtheria, pertussis, and hepatitis B antibody levels in children with cancer after chemotherapy are lower than those in healthy subjects. In addition, the level of immunity against hepatitis B in children under chemotherapy was lower than that in healthy children, and it is recommended that these children be re-vaccinated to maintain antibody titers at protective levels. It is suggested that in future studies, the relationship between factors affecting antibody levels be studied in detail and studies be conducted with larger sample size.

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ETHICAL STATEMENT

This research project was approved by the Ethics Committee of Research and Technology Department, affiliated with Shahrekord University of Medical Sciences with ethical number: IR.SKUMS.REC.1395.134.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

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