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Long-Term T-Cell-Mediated Immunologic Memory to Hepatitis B Vaccine in Young Adults Following Neonatal Vaccination.

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Background: The long-term duration of cell-mediated immunity induced by neonatal hepatitis B virus (HBV) vaccination is unknown.
Objectives: Study was designed to determine the cellular immunity memory status among young adults twenty years after infantile HB immunization.
Patients and Methods: Study subjects were party selected from a recent seroepidemiologic study in young adults, who had been vaccinated against HBV twenty years earlier. Just before and ten to 14 days after one dose of HBV vaccine booster injection, blood samples were obtained and sera concentration of cytokines (interleukin 2 and interferon) was measured. More than twofold increase after boosting was considered positive immune response. With regard to the serum level of antibody against HBV surface antigen (HBsAb) before boosting, the subjects were divided into four groups as follow: GI, HBsAb titer < 2; GII, titer 2 to 9.9; GIII, titer 10 to 99; and GIV, titers ≥ 100 IU/L. Mean concentration level (MCL) of each cytokines for each group at preboosting and postboosting and the proportion of responders in each groups were determined. Paired descriptive statistical analysis method (t test) was used to compare the MCL of each cytokines in each and between groups and the frequency of responders in each group.
Results: Before boosting, among 176 boosted individuals, 75 (42.6%) had HBsAb 10 IU/L and were considered seroprotected. Among 101 serosusceptible persons, more than 80% of boosted individuals showed more than twofold increase in cytokines concentration, which meant positive HBsAg-specific cell-mediated immunity. MCL of both cytokines after boosting in GIV were decreased more than twofold, possibly because of recent natural boosting.
Conclusions: Findings showed that neonatal HBV immunization was efficacious in inducing long-term immunity and cell-mediated immune memory for up to two decades, and booster vaccination are not required. Further monitoring of vaccinated subjects for HBV infections are recommended.

Keywords: Cell-Mediated Immunity; Hepatitis B Vaccine; Booster Vaccination

1. Background

Hepatitis B (HB) vaccine is highly immunogenic and efficacious in preventing hepatitis B virus (HBV) infection (1-6). Long-term protection by HB vaccination is dependent on the persistence of strong immunologic memory (7-11). Immune memory is a key characteristic of specific immune response and resides in memory B and T lymphocytes that are sensitized through an initial exposure to a specific antigen (12-14). The presence of prolonged HBV-specific immune memory after HB vaccination is suggested by a number of epidemiologic studies showing the absence of disease in vaccinated population and demonstration of an anamnestic response after revaccination (15-20). However, the most important question is that how-long the protection lasts. Some recent studies indicated disappearance of immune memory in a significant number of vaccinees, most of whom showed a good initial response to primary course of vaccination (21, 22). Several studies aimed to detect and measure the HBV surface antigen (HBsAg)-specific T-cells and B-cells reactivity in vaccinees to show the presence of specific immune memory; however, the results were contradictory (10, 21-26). this study aimed to determine whether the HBs Ag-specific T-cell memory could persist for a long period of time after neonatal HB vaccination, particularly in vaccine recipients whose serum antibodies levels against HBsAg (HBsAb) was less than protective (<10 IU/L) to make an optimal policy of booster vaccination.

2. Objectives

This study was designed to evaluate the long-term cell-mediated immune memory to booster vaccination in

vaccine recipients twenty years after neonatal HB immunization.

3. Patients and Methods

3.1. Population

The study subjects were partly selected from an epidemiologic study, which was planned to determine the effect of neonatal HB immunization program on prevalence rate of HBV infection seromarkers among vaccinees, twenty years after program had been launched in Iran. For that study, 510 young adults with the age ranging from 18.6 to 20.5 years (female, 52%) were enrolled. Participants had received a complete series of recombinant HB vaccine since birth and had not received any additional dose of HB vaccine thereafter, had not received immunoglobulin, blood, or blood products during the preceding three months, and had no history for chronic illnesses. The study was approved by Ethic Committee on Human Research of Mazandaran and Tehran Universities of Medical Sciences. Written informed Consent was obtained from all participants and their parents. Serum HBsAg, HBsAb, and antibody against HBV core antigen (anti-HBc) were measured and history of symptomatic clinical hepatitis in the subjects or their household members was investigated. The results of this study were reported previously (27). All collected sera were stored at -20 °C for further evaluation. For laboratory studies, 176 young adults (female to male ratio, 93:83) were randomly selected and were boosted by one adult dose of HB vaccine (EuvaxB 20 μg Life Sciences. Korea). Ten to 14 days after boosting, the blood samples were obtained. Cell-mediated immune response to boosting was evaluated by measuring the serum concentrations of two Th1-related cytokines, namely, interleukin 2 (IL-2) and interferon (IFN), by ELISA method using Human IL-2 ELISA kit (Bioassay Technology Laboratory, Shanghi, Crystal Day Biotech Co Ltd. Cat No: E 0105 Hu) and Human interferon ELISA kit, (Bioassay technology Technology laboratory Laboratory, Shanghi,. Crystal Day Biotech Co Ltd. Cat No: E 0105 Hu), according to manufacturer instructions. Based on the subjects' HBsAb titer before booster injection, they were divided into four groups as follows: GI, titer 0.1 to 1.9; GII, 2 to 9.9; GIII, 10 to 99; and GIV, ≥ 100 all IU/L. serum concentrations of both cytokines for each subject were measured. The proportion of individuals who showed positive immune response to booster injection in each group was calculated. Positive immune response (the presence of cell-mediated immune memory) in each person was defined as more than twofold increase in each cytokine in comparison with the level before boosting. Mean concentration level (MCL) of both cytokines for each group before and after boosting were determined.

3.2. Data Analysis

The collected data were analyzed using descriptive statistical methods. Paired-samples t test was used to compare the proportion of responders for each cytokines between different groups. MCL of both cytokines after and before boosting were compared in each group. The MCL between different groups was compared using paired-samples t test. A P value < 0.05 was considered statistically significant. Finally, to estimate the provided long-term immunity by neonatal HB vaccination, the cumulative numbers of seroprotected individuals before booster injection and the numbers of seronegative subjects who responded to boosting were determined.

4. Results

The booster dose of vaccine was tolerated well and there were no serious adverse events. The relative proportions of allocated subjects in each group were as follow: GI, 61; GII, 40; GIII, 29; and GIV, 46 persons.

4.1. Injection

After booster injection, the MCL titer of both cytokines, ie, IL-2 and IFN, in all groups except for GIV were increased significantly. The MCL of both cytokines were decreased significantly after boosting in group GIV (Table 1). The proportion of subjects who did responded to boosting by more than twofold increase in cytokines levels varied between different groups. In this regard, 47 out of 61 (77%) and 44 out of 61 (72%) subjects in GI and 37 out of 40 (92.5%) and 37 out of 40 (92.5%) persons in GII showed more than two fold increase in the level of IL-2 and IFN after booster injection, respectively. These response rates in two groups were statistically significant (P = 0.05). After booster administration, the serum levels of both cytokines decreased significantly in 27 out of 46 (58.7%) subjects with high levels of seroprotection (GIV) (Table 2). In this study, two subjects in GII, 4 in GIII and one in GIV were positive for anti-HBc antibodies. All of these but one in GIV responded to booster injection with more than twofold increase in both cytokines. However, more than twofold decrease in the cytokines levels in one subject of GIV was seen after boosting. Based on collected data, 75 out of 176 vaccinees with prebooster seroprotection, and also 84 of 101 serosusceptible studied subjects with preservation of cell-mediated immune memory[(overall 159 of 176 (90.35%)] maintained their vaccine- induced immunity , up to 2 decades.

Table 1. Mean Concentration Levels of Interleukin 2 and Interferon γ Hepatitis B Vaccine Booster Injection in Different Groups of Studied Youths [a,b]

Groups (No.), HBsAb level, IU/L	IL-2			INF-γ	
	Before	After	P Value	Before	After
GI (61), 0.1-1.99	272.36 ± 111.15	1200.91 ± 552.6	0.000	100.40 ± 43.64	270.21 ± 133.
GII (40), 2-9.9	301.82 ± 152.11	1515.45 ± 417.7 9	000	92.52 ± 49.36	318.27 ± 121.2
GIII (29), 10-99	449.65 ± 271.68	1348.72 ± 614.6	000	119.62 ± 76.30	256.55 ± 151.0
GIV (46), ≥ 100	1368.65 ± 443.28	537.47 ± 577.69	000	327.39 ± 233.05	121.32 ± 133.4
GV [c] (101), 0.1-9.9	284.0 ± 129.02	1325.47 ± 524.6 3	000	92.28 ± 45.91	289.24 ± 130.2

[a] Abbreviations: IL-2, interleukin 2; and INF γ, interferon γ.
[b] Data are presented as mean ± SD.
[c] GV, including the sum of all with anti-HBs.

Table 2. Frequencies of cell–Mediated Immune Response to Hepatitis B Vaccine Booster Injection in Different Groups of Studied Subjects According to Their ant-HBS Antibodies Concentrations, 20 Years After Neonatal HB Vaccination [a,b]

Groups (No.), HBsAb level, IU/L	IL-2	INF-γ	P value, IL-2: G1 vs. G2	P value, G1 vs. G3	P value, G2 vs. G3
	Positive Response [c]	Positive Response [c]			
GI (61),0.1-1.99	77	72	0.05	NS	0.01
GII (40), 2-9.9	92.5	92.5	0.05	NS	0.01
GIII (29), 10-99	65.5	62			
GIV (46) [d], ≥ 100	58.7 [d]	58.7 [d]			
GV (101), < 10	83	80			

[a] Abbreviations: IL-2, interleukin 2; and INF-γ, interferon γ.
[b] Data are presented as percentage.
[c] Positive response, more than twofold increase in cytokine level after boosting.
[d] More than twofold decrease in cytokine level after booster injection.

5. Discussion

In this study, long-term persistence of the HBsAg-specific cell-mediated immune memory in response to HB vaccine booster administration among vaccinated young adults was studied. Twenty years after neonatal immunization, 75 out of 176 of vaccinees (42.6%) were still seroprotected (HBsAb titer ≥ 10 IU/L). In addition, more than 83% (84 out of 101) and 80% (81 out of 101) of seronegative subjects (HBsAb levels < 10 IU/L) with marked increasing in the MCL of IL-2 and IFN, showed positive response after booster injection, indicating the presence of cell-mediated immune memory. The presence of HBsAg-specific immune memory after HB vaccination was suggested in a number of studies by epidemiologic data (16-18, 27, 28), showing the absence of disease in vaccinated population (5,19,20) and proven by demonstration of an anamnestic response after revaccination (10, 20, 26, 28-30). Thus, the presence of specific immunologic memory after HB vaccination is now undisputed in principle as well as its minimum duration. However, demonstrating of this memory in the vaccinated individual, especially when serum HBsAb is disappeared, apart from the possibility to revaccinate and look for an anamnestic response, is still remained a problem. During recent years, several studies have attempted to detect and measure HBsAg-specific T-cells and B-cells immunologic responses among vaccinated individuals with different results (10, 21-27, 31). In a study by Bauer and Jilg (24) 4-8 years after successful HB vaccination, the reactivity of vaccine-induced HBsAg-specific T cell and B cells were studied. Both effector and memory phenotype T cells were isolated, stimulated with HBsAg, and IFN (product of Th1 activity) and IL-5 (indicator of humoral response) secretion were tested using ELISPOT. In addition, to detect even small numbers of specific T cells, appropriate subpopulation of peripheral blood monocyte were enriched, memory B cells were analyzed by co-cultivation of B cells with CD4 T cells, and HBsAb secreting cells using ELISPOT was identified. The study showed that significant numbers of HBsAg-specific memory T and B cells were present in all vaccinees even in the absence of specific HBsAb. However, these cells activity were short-lived after revaccination. Researchers concluded that fully vaccinated subjects who had lost their seroprotection overtime showed T cell immunologic memory that were capable to activate HBsAg-specific T and B cells and trigger production of HBsAb in revaccina-

tion or re exposure. In other study by Wang et al. (25), T-cell immune memory to HBsAg challenge in 29 fully vaccinated healthcare workers (HCWs) and 9 unvaccinated HCWs using ELISPOT were studied. To determine their HBsAb status just before experiment, blood samples were obtained. In vitro proliferative responses to HBsAg challenge in both groups were measured using ELISPOT. Ten years after primary successful vaccination, 19 out of 29 vaccinated HCWs (61.3%) retained their seroprotection. After one dose HB vaccine booster injection, the highest T cell proliferative response was observed in those with HBsAb > 100 IU/L. In addition, T cell reactivity was found positive in 7 out 12 vaccinees (58%) who had lost their seroprotection (HBsAb < 10 IU/L). Researchers concluded that T cell memory to HBsAg can be demonstrated by lymphocyte proliferation many years after HB vaccination, even in the majority of persons with HBsAb <10 IU/L, and further booster injection is not necessary in healthy responders to HB vaccine. Also, in a study by Zhu et al. (31), specific-T cell immunity in 44 fully vaccinated Chinese adults negative for HBV markers were studied. Ten to 12 days after HB vaccine boosting HBsAg and HBeAg-specific T-cell immune responses were measured. Result indicated that specific T cell immune memory were detected in 41 out of 44 subjects including seven out of ten persons whose HBsAb titer were < 10 IU/L. They concluded that neonatal HB vaccination-induced long-term immune memory, and prolong follow-up and surveillance of vaccinees who had been vaccinated at early age should be continued. Our study findings and mentioned studies results were different from those reported recently from Taiwan (21) and Thailand (22), two hyperendemic countries with more than two decades universal infants HB vaccination. In a Taiwanese study (21) on 872 HBsAb seronegative students, the immune response to HB vaccine booster dose administration was evaluated. HBsAg-specific T cell immune response (IFN production) measured by ELISPOT was negative in 27.2% of boosted individuals 15 to 18 years after early infancy HB vaccination. Based on study findings, they concluded that a notable proportion of vaccinated adolescents did lost their immune memory induced by a plasma-derived HB vaccine. This decay of immune memory raised concerns about the need for a booster vaccine for high-risk groups in the long run. In addition, in another study in Thailand (22) on 87 high-risk adolescents who had received a complete primary course of recombinant HB vaccine 15 to 18 years earlier, the presence of long-term humoral and cellular immune memory were investigated. Overall, 58.6% of subjects were still positive for humoral immunity (HBsAb titer, ≥ 10 IU/L), and 50.6% were positive for cellular immunity as measured by ELISPOT. There were no correlation between the levels of HBsAb and positive ELISPOT. However, the majority of participants who were positive for IFN-producing cells were seropositive (81.8%). Researchers concluded that 15 to 18 years after early infancy HB immunization, a second booster dose should be considered, especially in high-risk groups. In a recent study (26), T-cell immune memory responses to HBsAg challenge in persons vaccinated 13 to 18 years earlier were also evaluated. Results indicated the presence of immunologic memory even in those with undetectable HBsAb titer. However, further follow-up studies to define the susceptibility to HBV infection among vaccinees that did not responded to HBsAg challenge and requirement for possible additional booster dose administration was recommended. In our study, after booster administration of HB vaccine to those with HBsAb > 100 IU/L, the MCL of cytokines were decreased markedly in comparison with the levels before boosting. Revaccination or re-exposure to HBsAg in previously immunized population will results in a typical anamnestic antibody response that may persist for more than several months and produce a significant changes in the specific cellular immune system with the release of short-lived cytokines, which would diminish to rather low or undetectable concentrations within few weeks (9, 12, 13, 15). This phenomenon could explain the possible causes for decreasing the cytokines MCL after boosting among seroprotected individuals, especially those with HBsAb titer > 100 IU/L. It is speculated that individuals in GIV were naturally boosted recently, which resulted in an anamnestic response and production of long-lived antibodies (HBsAb) accompanied by release of short-lived cytokines weeks to months before booster injection, suggestive for the presence both types of immune memory. Currently, HB vaccine booster administrations are not recommended (14). Our study finding provide additional support for current recommendation by demonstrating long-lasting immunity and immune memory despite loss of seroprotection in more than 90% of vaccinated young adult. However, nearly 20% of subjects with HBsAb < 10 IU/L failed to respond to booster injection. Fallow-up studies are required to determine the susceptibility to HBV infection among vaccinees that failed to demonstrate response to HBsAg challenge and to define the requirement for booster vaccination to maintain long-term protection. In most studies, the presence of T-cells and B-cells reactivity in response to HBsAg challenge have been evaluated by ELISPOT. Furthermore, in a different recent published study, specific T-cell immunity in two groups of children vaccinated with two different types of hexavalent HB containing vaccines were investigated. T-cell immunologic reactivity to HBs Ag challenge was assessed by in vitro measuring of peripheral blood mononuclear cells responses. After six days of incubation, T-cells proliferation were enumerated using flow cytometry, supernatants were collected to measured secreted INF-γ concentrations by specific ELISA, and intracellular cytokines production (INF-γ and IL-2) were quantified using appropriate methods. Results indicated that there was a good correlation between different methods including ELISA in measuring T-cell immune responses. The authors concluded that, although two vaccines were different in generating serum antibody titers, both vaccines were effica-

cious in generating strong T-cell recall responses (28).

For our study, in vitro ELISPOT was not feasible; therefore, we used ELISA to measure the related cytokines concentration in serum of boosted individuals, which was a limitation to our study. However, to increase the accuracy of study method, we examined much larger subjects and compared the postbooster cytokines levels with those before challenge. Based on recent study, in some instances, ELISA could be a possible substitute for ELISPOT.

Our study results showed that twenty years after primary HB vaccination, from 176 studied subjects, 75 vaccinees (29 with HBsAb titer of 10 to 99 and 46 with titer of 100 IU/L) retained their seroprotection against HBV infection and 84 out of 101 seronegative individuals showed a T-cell-specific immune mediated response to HBsAg challenge, indicating the presence of immune memory. These finding indicated that 90.35% of vaccinated youths retained their specific immunity against HBV. Moreover, 9.65% of studied vaccinees remained possibly susceptible to HBV. It is not clear whether all of these youths had lost their vaccine-induced immunity overtime or partly they were non-responders to primary course of vaccination, and this is another limitation to this study.

HB vaccine was highly effective in providing long-term immunity in vaccinated individuals. The presence of immunity and T-cell-specific immune memory was demonstrated in response to booster injection twenty year after neonatal HB vaccination even in those who had lost their seroprotection. In accordance with recently published reports (14, 32), our findings did not recommend booster dose because specific cell-mediated immune memory of HB vaccine probably still existed, which means that protective antibodies will reappear rapidly in re-exposure to specific antigen (24). Hence infection can be prevented or eliminated rapidly after re-exposure to HBV. Further follow-up studies and seroepidemiologic surveillances to monitor acute and chronic HBV infections in vaccinated individuals and in the community are recommended.

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Authors' Contributions

Saffar Hiva, Saffar Mohammed Jafar, and Ajami Abolghasem: conceptualization, design, data collection, analysis, and writing the draft, Shams-Esfandabad, Kian, and Mirabi Araz-Mohammad: data collection and laboratory testing. Khalilian Ali-Reza: statistical analysis.

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