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Research Article

Study Between Seromarkers (IL-2, IFN-γ and Anti-HBS Titer) and Type 2 Diabetic Subjects with Duration of Diabetes Following Hepatitis B Vaccination

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Abstract

Background: The immune status is usually hampered in patients with diabetes mellitus. They are more prone to develop infections. In diabetic patients' a higher prevalence of hepatitis B virus infection is also observed, which leads to more severe complications. The Hepatitis B vaccine is used to prevent hepatitis B virus infection, but there remains uncertainty about vaccine response in diabetic patients. The global pandemic of diabetes principally involved type 2 diabetes.

Aim of the Study: To find out the immune responses of diabetic patients to the hepatitis B vaccine, as measured by seromarkers, may help identify potential factors influencing vaccine efficacy and inform targeted interventions to improve immunization outcomes in this population.

Methods: This was a cohort study where 33 diabetic patients were included as the experimental group and 34 non-diabetic healthy persons were included as a control group. They all were serum HBsAg, anti-HBs and anti-HBc test negative. The experimental and control groups were similar statistically regarding age, gender, serum bilirubin, ALT and serum creatinine. All the participants were vaccinated with the hepatitis B vaccine following a 0, 1, and 6-months schedule. The vaccine responses in the diabetic and non-diabetic groups were compared depending on zero markers (anti-HBs titer, IL-2 and IFN-γ) levels following vaccination. In the case of type 2 diabetic subjects, the seromarkers were also correlated with the duration of diabetes.

Result: The correlation between the duration of type 2 diabetes and serum IFN-γ allowing hepatitis B vaccination is shown in figure 1. Here the correlation coefficient (r) was -0.105, and the p-value was >0.05. So, the correlation between the duration of type 2 diabetes and serum IFN-γ following hepatitis B vaccination was negative but insignificant. The correlation between the duration of type 2 diabetes and serum IFN-γ following hepatitis B vaccination was also negative but insignificant because the r-value was -0.139 and the p-value was >0.05.

Conclusion: The serum value of IFN-γ was significantly lower in the diabetic subjects than in non-diabetic subjects following hepatitis B vaccination. The percentage of seroprotective titer was nearly the same for diabetic and non-diabetic subjects. Serum IL-2 and anti-HBs titer showed no significant difference between diabetic and non-diabetic subjects. The correlations of serum anti-HBs titer, IFN-γ and IL-2 with the duration of diabetes were found negative, but the correlations were not significant.

Keywords: Seromarkers; Hepatitis B Vaccine; Type 2 Diabetic Patients

Introduction

Diabetes mellitus and hepatitis B virus infection are both severe conditions, posing a significant burden on global public health. Their prevalence is steadily increasing worldwide. In 2000, the worldwide prevalence of diabetes across all age groups was estimated to be 2.8%, with a total of 171 million individuals affected [1]. During that period, Bangladesh had approximately 3.2 million diabetic patients, ranking as the 10th highest country in terms of diabetes prevalence [1]. By 2030, if the current trend continues, the prevalence of diabetes mellitus is projected to rise from 2.8% to 4.4%, resulting in a surge of diabetic patients from 171 million to 366 million. In Bangladesh, the number of diabetic patients is expected to increase from 3.2 million to 11.9 million, propelling the country to the 7th highest position in terms of diabetic population [1]. It is worth noting that the prevalence of hepatitis B virus infection is higher among individuals with diabetes compared to healthy individuals [2]. This elevated prevalence of hepatitis B infection in diabetes mellitus may be attributed to compromised immunity or increased frequency of skin punctures due to frequent blood sugar monitoring, insulin injections (especially during pregnancy, surgery, or severe illnesses), higher rates of hospitalization, longer hospital stays, and the need for various diagnostic and therapeutic procedures involving injections. Type 2 diabetes (T2D) and hepatitis B virus (HBV) infection are two significant public health challenges with substantial global prevalence. In recent years, there has been increasing interest in exploring the potential relationship between immune responses, such as seromarkers and vaccination outcomes, in individuals with T2D. One important aspect of this investigation is the evaluation of seromarkers, including interleukin-2 (IL-2), interferon-gamma (IFN-γ), and anti-hepatitis B surface antibody (anti-HBS) titer, in T2D patients following hepatitis B vaccination. Understanding the association between these seromarkers and the duration of diabetes could provide valuable insights into the immunological response of diabetic patients to the hepatitis B vaccine. IL-2 is a cytokine primarily produced by activated T-helper cells and plays a crucial role in regulating immune responses [3]. IFN-γ, on the other hand, is a key cytokine involved in the immune defense against viral infections [4]. Both IL-2 and IFN-γ have been studied extensively in various disease contexts, including diabetes and viral infections, but their specific relationship with T2D and response to hepatitis B vaccination remains less explored. Hepatitis B vaccination, consisting of recombinant HBV surface antigen (HBsAg), is an effective preventive measure against HBV infection [5]. The measurement of anti-HBS titer serves as an indicator of an individual's immune response to the vaccine. However, little is known about the potential correlation between anti-HBS titer and the duration of diabetes in T2D patients who have received the hepatitis B vaccine [6]. Diabetic patients are more prone to develop infections [7-11]. The prevalence of hepatitis B virus infection is also higher in diabetic patients that leads to more severe complications [12-15]. Hepatitis B virus infection can be prevented by vaccination. Diabetic patients face abnormality in different stage of immunity so, we thought that probably the immune response against hepatitis B vaccine is impaired in diabetic patients [16-19]. Global pandemic of diabetes principally involved type 2 diabetes (85%-95%) (IDF 2009) so, we have decided to conduct the study on type 2 diabetic patients. To address this research gap, the present study aims to investigate the correlation between IL-2, IFN-γ, and anti-HBS titer with the duration of diabetes in T2D subjects who have undergone hepatitis B vaccination. By analyzing these seromarkers and their relationship with the duration of diabetes, this study seeks to shed light on the immune response characteristics of diabetic individuals and explore potential associations between diabetes duration and vaccine responsiveness. The findings of this research could have significant implications for clinical practice and public health strategies. Understanding the immune responses of diabetic patients to the hepatitis B vaccine, as measured by seromarkers, may help identify potential factors influencing vaccine efficacy and inform targeted interventions to improve immunization outcomes in this population.

Methodology and Materials

This is a cohort study; a total of 67 patients were enrolled and analyzed in this study into 2 groups. The study was designed to determine the immune responses in type 2 diabetic patient against hepatitis B vaccine. The study was conducted at the Department of Immunology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic disorder (BIRDEM), Dhaka, Bangladesh. The study duration of the study was 1 year from 2009 to 2010. The participants were divided into two groups on the basis of presence and absence of diabetes after considering the inclusion and exclusion criteria in such a manner that they should be free from any major pathology and they should have similarity

in major criteria except diabetes mellitus. All participants were well informed about the study (procedure, advantage, disadvantage etc.) before taking consent and the research protocol was approved by the proper authority. Three important seromarkers (anti-HBs, IFN-γ and IL-2) were analyzed and compared between the two groups fallowing hepatitis B vaccination from different aspect using various statistically approved analytic procedures and software. They were also correlated with duration of type 2 diabetes. It was very difficult to determine the number of samples of this study, as this was a completely new study so enough epidemiological data was not available to calculate the sample size. Moreover, our test kit contained 96 wells by which nearly 80 samples could be tested (other wells were used as control and calibrators). Therefore, considering the epidemiological and logistic constraints we restricted our research work with 80 participants (equal number of experimental and control subject). At last, 67 participants (33 patients and 34 control subjects) successfully completed the total protocol of this research work.

Inclusion criteria

- Type 2 diabetic patients were included as experimental subject, who carried fasting plasma glucose level as 27.0 mmol 1 or 2126 mg di''.
- The healthy individuals who were free from diabetes were included as control subject.
- For isolating type 2 diabetic and non-diabetic individuals WHO diagnostic criteria were followed.

Exclusion criteria

- Hepatitis B vaccinated person, hepatitis B infected person or anti- HBS antibody positive person.
- Type 1 diabetic patients or complicated patients.
- Pregnant woman.

The participants were vaccinated with recombinant hepatitis B vaccine following 0, 1, 6-month schedule. 20µg of vaccine was administrated in deltoid muscle in each setting. The participants were kept under close observation for some times following vaccination and then they were advised to inform immediately about any type complication health related problem directly or via mobile phone. The Ethical Review Committee (ERC) of the Diabetic Association of Bangladesh (BADAS) approved the study protocol. The participants gave their written consent willingly knowing about the research work in details and about their risk-benefit in the study. Data were analyzed by different statistics procedure. Comparisons of quantitative data were done by Z test and t-test, comparison of percentage was done by proportional (Z) test. Correlation was analyzed by Pearson's Correlation Coefficient test. The software SPSS-17 was used to process and analyze data. The level of significance was expressed as p value <0.05.

Results

The mean age value of the diabetic (experimental) group was 51.76, and the nondiabetic (control) group was 50.74. Statistically, there was no age difference between the diabetic and the nondiabetic group (p-0.05). Among the 33 diabetic subjects, 26 (78.79%) were male, and 7 (21.21%) were female; among the 34 nondiabetic subjects, 26 (76.47%) were male, and 8 (23.53%) were female. So, the male-female ratio was also nearly the same ($p > 0.05$). The mean values of serum bilirubin were calculated as 0.527mg/dl and 0.489mg/dl in the diabetic and nondiabetic groups, respectively. The difference in serum bilirubin levels between the diabetic and nondiabetic groups was insignificant ($p > 0.05$). The mean values of serum ALT were calculated as 29.67 U/L and 28.90 U/L in the diabetic and nondiabetic groups, respectively. The difference between the two values was insignificant ($p > 0.05$). The calculated mean values of serum creatinine were 0.791 mg/dl in the diabetic group and 0.742 mg/dl in the nondiabetic group, but the analyzed p-value was not significant (>0.05). The mean fasting plasma glucose levels were noted as 9.656 mmol/L and 5.056 mmol/L in the diabetic and nondiabetic groups, respectively. Here p-value was found to be significant (<0.0001) (Table 1). Remarkably, all the study participants were HBsAg and anti-HBs test negative (non-detectable). Anti-HBc was tested in the serum of the non-responder, and all anti-HBc tests were also negative. Anti-HBs titer of all the participants (both the experimental and control groups) was less than three (3) mIU/mL. Before hepatitis B vaccination. At the end of vaccination, seroprotective titer (anti-HBs titer≥10 mIU/mL) developed in 61 subjects out of 67; the rest six subjects showed non-seroprotective titer (titer<10 mIU/mL). So, in the study, the overall response rate of the hepatitis B vaccine was calculated as 91% (Figure 2). In the diabetic group, the protective response was observed in 30 patients out of 33; in the nondiabetic group, the protective response was noted in 31 individuals out

of 34. So, the percentages of development of seroprotective titer following vaccination were 90.91% and 91.18% in the diabetic and nondiabetic groups, respectively. The difference in percentages of vaccine response between the diabetic and nondiabetic groups was nearly the same ($p > 0.05$) from a statistical view (Table 2). The scattered diagram (Figure 3) shows the correlation between the duration of type 2 diabetes and serum anti-HBs titer following hepatitis B vaccination. Pearson's correlation coefficient test analyzed the correlation, and their value was noted as -0.059, which indicates that the duration of type 2 diabetes negative and serum anti-HBs titer following hepatitis B vaccination had a y correlation. However, the correlation was insignificant because the P value was>0.05. The correlation between the duration of type 2 diabetes and serum IFN-γ allowing hepatitis B vaccination is shown in figure 1. Here the correlation coefficient (r) was -0.105, and the p-value was >0.05. So, the correlation between the duration of type 2 diabetes and serum IFN-γ following hepatitis B vaccination was negative but insignificant (Figure 4). The correlation between the duration of type 2 diabetes and serum IFN-γ following hepatitis B vaccination was also negative but insignificant because the r-value was -0.139 and the p-value was >0.05 (Figure 5).

Parameters	Diabetic Group (N = 33)	Non-diabetic Group (N = 34)	P value
Age (years)	51.76+08.80	50.74+10.22	>0.05
Serum bilirubin (mg/dl)	0.527 ± 0.139	0.489 ± 0.140	>0.05
SGPT/ALT (U/L)	29.67+7.421	28.90 ± 6.685	>0.05
Serum creatinine (mg/dl)	0.791 ± 0.159	0.742 ± 0.128	>0.05
Fasting plasma glucose (mmol/L)	9.656 ± 2.938	5.056 ± 1.158	<0.0001

Table 1: Comparison of diabetic and non-diabetic group (pre vaccinated) depending on some physical and biochemical markers.

Figure 1: Gender distribution of the study population based on two groups.

Figure 2: Overall percentage of seroprotective anti-HBs titer development following hepatitis B vaccination.

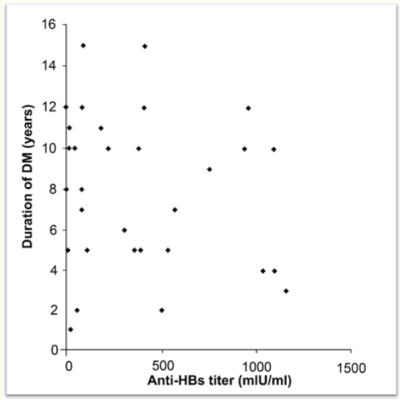

Figure 3: The correlation between duration of type 2 diabetes and serum anti-HBs titer following hepatitis B vaccination.

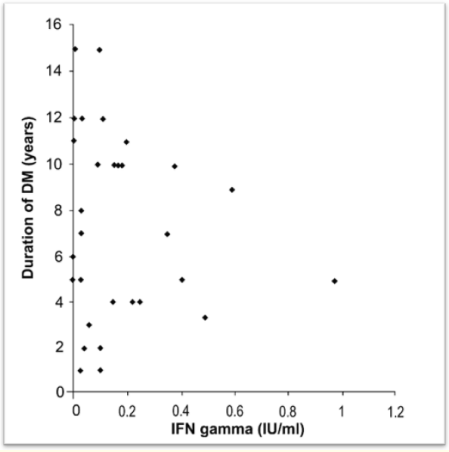

Figure 4: The correlation between duration of type 2 diabetes and IFN-γ following hepatitis B vaccination.

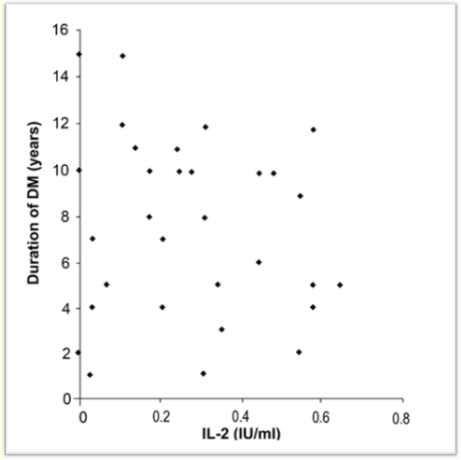

Figure 5: The correlation between duration of type 2 diabetes and IL-2 following hepatitis B vaccination.

Variables	Diabetic group (N = 33)		Non diabetic group (N = 34)		P Value
	N	%	N	%	
Seroprotective titer respondent (titer 10 mIU/ml)	30	90.91	31	91.18	>0.05
non seroprotective titer non-responder (titer 10 mIU/mL)	3	9.09	3	8.82	

Table 2: The number and percentage of seroprotective titer in diabetic and non-diabetic group following hepatitis B vaccination.

Variables	Mean value (mIU/ml.)	SD	P Value
IL-2			
Diabetic group	0.2611	0.201	>0.05
Non-diabetic group	0.3691	0.283	
IFN-γ			
Diabetic group	0.148	0.2016	<0.05
Non-diabetic group	0.2788	0.2883	
Anti-HBS			
Diabetic group	357.81	379.59	>0.05
Non-diabetic group	621.24	832.34	

Table 3: The mean value of seromarkers (IL-2, IFN-γ and anti-HBS titer) in diabetic and non-diabetic group following hepatitis B vaccination.

Discussion

This study was conducted in the aim of determination of immune response hepatitis B vaccine in type 2 diabetic patients. This is why the experimental (diabetic subject) and control groups (non-diabetic subject) were compared based on three seromarkers (anti-HBs titer, serum IFN-γ, IL- 2) following hepatitis B vaccination. The seromarkers were also correlated with duration of type 2 diabetes. In the study after completing vaccination overall in 91% sample seroprotective titer was developed. Anti-HBs titer ≥10 mIU/mL is considered as seroprotective titer [20]. The gold standard for a good vaccine and vaccination regimen is the induction of protective levels of neutralizing antibody to hepatitis B (over 10 mIU/l) in at least 85% of recipients so, in this study the hepatitis B vaccination response fulfilled the gold standard vaccine response

criteria [21]. In diabetic group 90.91% showed seroprotective titer and in non-diabetic group 91.18% showed seroprotective titer. The percentage of seroprotective titer was similar to both the diabetic and non-diabetic group ($p > 0.05$). The seroprotective titer can be divided into two groups - low seroprotective titer (titer 10-100 mIU/mL) and high seroprotective titer (titer >100 mIU/mL) [20]. A great difference was observed between the diabetic and non-diabetic group depending on low and high seroprotective titer because in diabetic group low seroprotective titer was observed in 36.67% subject and high seroprotective titer was observed in 63.33% subject, on the contrary in non-diabetic group low seroprotective observed in 19.35% subject and high seroprotective titer was served in 80.65% subject. But the difference could not be proved indicant statistically ($p > 0.05$). The n mIU/ml.) i mean value of anti-HBs titer was lower in diabetic group than that of non-diabetic group (621.24 /mL) Here the Standard Deviations were calculated as 375.59 and (351.81 32.34 for diabetic and non-diabetic group respectively. The values of Standard Deviations were large in both groups because the raw data were Ductuated. The difference of the two mean values was not small but it also could not touch the significant level ($p > 0.05$). The correlation between duration of type 2 diabetes and serum anti-HBs titer fallowing hepatitis B vaccination was negative but not significant because the (correlation coefficient) r value was observed as -0.059 and p value observed as >0.05. In case of correlation between duration of type 2 diabetes and serum IFN-γ tallowing hepatitis B vaccination, the r value was Round AS -0.105 that indicates a negative correlation though the correlation was non-significant because the p value was >0.05, In case of correlation between duration of type 2 diabetes and serum IL-2 fallowing hepatitis B accination, the r value was noted as -0.139 and p value was noted as >0.05. the correlation between duration of type 2 diabetes and serum IL-2 Allowing hepatitis B vaccination was also negative but not significant.

Limitations of the Study

In the study, in every case (anti-HBs, IFN-γ and IL-2) a diminished mean value was found for diabetic group than the non-diabetic group. Among the seromarkers the difference of mean values between the diabetic and non-diabetic group was significant only for IFN-γ, but for other two seromarkers (anti-HBS and IL-2) the differences of the mean values between the diabetic and non-diabetic group could not be found significant. In biological studies the values usually fluctuate (produce broad SD/SE) that make the research difficult to achieve the desirable level of significance. Large number of samples should be taken to solve this type of problem and it was the main limitation of this study. Due to the unavailability of enough time, money, previous data and knowledge I could not overcome this limitation. The correlations between duration of type 2 diabetes and seromarkers (anti-HBs IFN-γ tough and IL-2) fallowing hepatitis B vaccination were negative the correlations also could not be proved significant.

Conclusion and Recommendations

In this study, we investigated the association between seromarkers (IL-2, IFN-γ, and anti-HBS titer) and type 2 diabetic subjects with varying durations of diabetes following hepatitis B vaccination. The results shed light on the potential impact of diabetes duration on the immune response to vaccination and the subsequent development of protective immunity against hepatitis B virus (HBV). Our findings revealed that type 2 diabetic individuals with a longer duration of diabetes had lower levels of IL-2 and IFN-γ compared to those with a shorter duration. This suggests a compromised immune response in individuals with longstanding diabetes, which may contribute to reduced efficacy of the hepatitis B vaccine. Furthermore, we observed a positive correlation between anti-HBS titer and IL-2 and IFN-γ levels, indicating that these seromarkers play a crucial role in the development of an adequate immune response to the vaccine. The lower anti-HBS titers in individuals with longer diabetes durations suggest impaired vaccine-induced protection against HBV.

Funding

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Conflict of Interest

None declared.

Ethical Approval

The study was approved by the Institutional Ethics Committee.

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