Immune Reactivity to Hepatitis B Vaccine among Individuals with Type 2 Diabetes

ABSTRACT

Background: *The interaction between immune responses and vaccine efficacy in individuals with underlying health conditions is a topic of growing significance. In the realm of infectious diseases, the impact of Type 2 diabetes on immune reactions to vaccines, specifically the Hepatitis B vaccine, has garnered attention. This investigation delves into the intricate interplay between the immune system and vaccine-induced protection in Type 2 diabetic patients. By elucidating the unique dynamics of immune responses in this context, valuable insights can emerge, potentially paving the way for tailored vaccination strategies and improved healthcare outcomes for this vulnerable population.*

Aim of the study: *This research aimed to investigate the immune reactions to the hepatitis B vaccine among individuals diagnosed with type 2 diabetes.*

Methods: *The study investigated immune responses to the hepatitis B vaccine in type 2 diabetic patients. Employing experimental design, participants were chosen through non-probability purposive sampling and divided into diabetic and non-diabetic groups. Key seromarkers (anti-HBs, IFN-γ, IL-2) were assessed after vaccination using approved analytics and correlated with diabetes duration. The research, conducted at the Bangladesh Institute of Research and Rehabilitation in Diabetes, involved 67 participants (33 diabetics, 34 non-diabetics) meeting inclusion criteria while excluding certain cases. Ethical approval was obtained, and participants provided informed consent. Serum samples were analyzed at specific post-vaccination days for different markers. Statistical analysis was employed, including Z and t-tests, Pearson's Correlation, and SPSS-17 software, with significance set at $p < 0.05$.*

Result: *The study compared various parameters between diabetic and non-diabetic groups after hepatitis B vaccination. Both groups had similar mean ages, serum bilirubin levels, ALT levels, and serum creatinine levels. Fasting plasma glucose was higher in diabetics. Gender distribution was comparable in both groups. Protective anti-HBs titers varied, with more low titers in diabetics and high titers in non-diabetics, but not statistically significant. Post-vaccination anti-HBs titers were lower in diabetics, yet not significantly. Notably, IFN-γ levels were significantly lower in diabetics, while IL-2 levels post-vaccination were similar. Overall, the study observed several differences but most were not statistically significant.*

Conclusion: *In conclusion, the study highlights that immune responses to the Hepatitis B vaccine in Type 2 diabetic patients may exhibit variations compared to non-diabetic individuals. Further research is imperative to understand these responses better, enabling the optimization of vaccination strategies for improved protection against Hepatitis B in this vulnerable population.*

Keywords: *Immune Responses, Hepatitis B Vaccine, Type 2 Diabetic Patients*

INTRODUCTION

Both diabetes mellitus and hepatitis B virus infection pose significant threats to human life. These conditions are increasingly burdensome to global public health, with their prevalence rising steadily worldwide. In 2000, diabetes affected 2.8% of the global population across all age groups, approximately 171 million individuals [1]. During that time, Bangladesh had around 3.2 million diabetic patients, ranking as the 10th country with the highest diabetic population [1]. Looking ahead to 2030, if current trends persist, the prevalence of diabetes mellitus could surge from 2.8% to 4.4%, causing the number of diabetic patients to escalate from 171 million to 366 million. This projection also foresees Bangladesh's diabetic population rising from 3.2 million to 11.9 million, elevating the country to the seventh spot among nations with the highest diabetes rates [1]. Unfortunately, the prognosis for diabetes-related fatalities is equally concerning. In 2005, diabetes claimed the lives of 1.1 million individuals, which the World Health Organization (WHO) anticipates doubling by 2030 [2]. The prevalence of diabetes is primarily driven by type 2 diabetes (85%-95%), influenced by factors like obesity, sedentary lifestyles, poor dietary habits, increasing urbanization, and greater longevity [3,4]. In specific areas, such as suburban Bangladesh, non-insulin-dependent diabetes mellitus (NIDDM) affects around 4.1% of the population [5]. The prevalence of type 2 diabetes saw a notable rise in rural Bangladesh, climbing from 2.3% to 6.8% between 1999 and 2004, while urban areas faced an even more challenging situation, with a prevalence of approximately 8.1% [6,7]. Shifting the focus to hepatitis B virus infection, globally, an estimated two billion people have been exposed to the virus, and roughly 350 million individuals endure chronic infections. Tragically, about 600,000 individuals succumb to acute or chronic hepatitis B consequences annually. The virus's stronghold is particularly pronounced in Asia, where 8% to 10% of adults grapple with chronic infection. The Middle East and the Indian subcontinent face infection rates of 2% to 5% within the general population [8]. In Bangladesh, the country falls within an intermediate endemic area, with a prevalence of 3% seropositivity for hepatitis B surface antigen in the general population as of 2003 [9]. Pregnant women in Bangladesh also exhibited a seropositivity rate of 3.5% [10]. Specific subpopulations, like Bangladeshi truck drivers, intravenous drug users, and commercial sex workers, experienced even higher seroprevalence rates of 5.9%,

6.2%, and 9.7%, respectively [11-13]. It is important to note that individuals with diabetes mellitus exhibit a higher prevalence of hepatitis B infection than their healthy counterparts [14]. This elevated risk is potentially due to compromised immunity or an increased frequency of skin punctures, given the need for frequent blood sugar monitoring and insulin injections, especially during pregnancy, surgery, or severe illnesses. Diabetic patients also encounter more hospitalizations, more extended hospital stays, and various medical procedures involving injections for diagnostics and treatments. Moreover, diabetes exacerbates hepatic conditions in hepatitis patients, elevating the risk of complications [15]. It also heightens the risk of hepatocellular carcinoma (HCC) and chronic liver disease (CLD), with diabetes-associated liver fibrosis acceleration and increased bacterial infection incidence among cirrhotic patients, contributing to higher mortality rates [16,17]. Preventing hepatitis B virus infection involves tackling transmission routes, which can be challenging, especially in cases of accidental or uncommon transmission, where around 30% of adult hepatitis B cases lack an identifiable risk factor [18]. Vaccination provides a reliable preventive measure, but its efficacy in diabetic patients remains uncertain due to diabetic-related immune system abnormalities [19-22]. From administering the antigen to the emergence of immunity, the vaccine undergoes a series of crucial stages influenced by various factors, both cellular (such as APCs, T cells, and B cells) and chemical (like MHC, transcription factors and cytokines). Within this process, IL-2 and IFN-γ, two significant cytokines, play essential roles in different phases of the immune response. IL-2 activates a diverse range of immune system cells, including helper T cells, cytotoxic T cells, B cells, macrophages, and natural killer cells. Notably, in various research endeavors, an elevation in the levels of IL-2 and IFN-γ in the bloodstream has been noted after infection and vaccination. This finding underscores the connection between IL-2, IFN-γ, and immune responses [23, 24]. Thus, by examining and comparing the serum levels of anti-HBs, IL-2, and IFN-γ in both type 2 diabetic and non-diabetic individuals following hepatitis B vaccination, it is possible to gain insight into the comparable immune responses to the vaccine between these two groups. Furthermore, investigating whether the seromarkers (anti-HBs, IL-2, and IFN-γ) in type 2 diabetic patients correlate with the duration of diabetes could provide valuable information about the interplay between the immune response to the vaccine and the duration of diabetes.

METHODOLOGY

The objective of this study was to investigate the immune responses to the hepatitis B vaccine in individuals with type 2 diabetes. The study adopted an experimental approach and utilized a non-probability purposive sampling method to select participants. The participants were categorized into two groups based on the presence or absence of diabetes. Careful consideration was given to inclusion and exclusion criteria to ensure similarity in major factors, except for diabetes. All participants were provided comprehensive information about the study, including procedures, benefits, risks, and more, before obtaining their informed consent. The research protocol was duly approved by the relevant authority. The study focused on three key seromarkers: anti-HBs, IFN-γ, and IL-2. These markers were evaluated and compared between the two groups following hepatitis B vaccination using established statistical methods and software. Additionally, their correlation with the duration of type 2 diabetes was examined. The study was conducted at the Department of Immunology within the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine, and Metabolic Disorders (BIRDEM) in Shahbag, Dhaka, Bangladesh, the study spanned a year (2009-10). Ethical approval for the study protocol was granted by the Ethical Review Committee (ERC) of the Diabetic Association of Bangladesh (BADAS). Participants willingly provided written consent, demonstrating their understanding of the research's details and the potential risks and benefits involved. The study's experimental group comprised individuals with type 2 diabetes, while the control group consisted of non-diabetic, relatively healthy individuals. The sample size included 33 individuals in the diabetic group and 34 individuals in the non-diabetic group, resulting in a total of 67 participants.

- **Inclusion criteria**

Inclusion criteria involved selecting type 2 diabetic patients with a fasting plasma glucose level of 27.0 mmol/L or 2126 mg/dL and healthy non-diabetic individuals.

- **Exclusion criteria**

Exclusion criteria encompassed individuals who were vaccinated against hepatitis B, those infected with hepatitis B, or those testing positive for anti-HBS antibodies. Type 1 diabetic patients and individuals with complex medical conditions were also excluded, along with pregnant women.

The analysis of seromarkers involved assessing anti-HBs in serum collected on the 28th day after hepatitis B vaccination, as this timeframe corresponds to the peak level of serum anti-HBs. IFN-γ was analyzed in serum collected on the 14th day, while IL-2 was assessed in serum collected on the 7th day. The timing for IFN-γ and IL-2

analysis was determined based on pilot studies, where higher values were observed on specific days. Data were subjected to various statistical tests, including Z-test, t-test, proportional (Z) test for quantitative comparisons, and Pearson's Correlation Coefficient test for correlation analysis. Data processing and analysis were performed using SPSS-17 software. The significance level was set at $p < 0.05$.

RESULT

The diabetic (experimental) group had a mean age of 51.76%, while the non-diabetic (control) group had a mean age of 50.74%. The statistical analysis indicated no significant age difference between the two groups ($p > 0.05$). Serum bilirubin mean values were 0.527mg/dl for the diabetic group and 0.489mg/dl for the non-diabetic group. The difference in serum bilirubin levels between the two groups was insignificant ($p > 0.05$). Similarly, the mean values of serum ALT were 29.67 U/L for the diabetic group and 28.90 U/L for the non-diabetic group, with no significant difference ($p > 0.05$). The mean serum creatinine values were 0.791 mg/dl for the diabetic group and 0.742 mg/dl for the non-diabetic group, but the calculated p-value did not show significance (> 0.05). Fasting plasma glucose levels were 9.656 mmol/L for people with diabetes and 5.056 mmol/L for non-diabetics (Table 1). Among the 33 diabetic subjects, 26 (78.79%) were male, and 7 (21.21%) were female. For the 34 non-diabetic subjects, 26 (76.47%) were male, and 8 (23.53%) were female. The male-to-female ratios were not significantly different ($p > 0.05$) (Figure 1). In the responder group, 11 diabetic samples had a low protective titer (10-100mIU/mL) compared to 6 non-diabetic samples. High protective titer (>100mIU/mL) was observed in 19 diabetic and 25 non-diabetic samples. The percentage of low protective titers was higher in the diabetic group. In contrast, the high protective titer was higher in the non-diabetic group, but these differences were not statistically significant ($p > 0.05$) (Table-2). Post-vaccination anti-HBs titer mean values were 357.81 mIU/mL for diabetics and 621.24 mIU/mL for non-diabetics. The mean anti-HBs titer was lower in the diabetic group, but the p-value was not significant (> 0.05) (Table-4). A significant difference was found between IFN-γ values in the blood of the diabetic and non-diabetic groups. The mean IFN-γ value in the diabetic group was 0.1480 IU/ml, significantly lower than the non-diabetic group's mean of 0.2768 IU/ml. The calculated p-value was less than 0.05, indicating a significant difference (Table-5). Serum IL-2 mean values were 0.2611 IU/ml for people with diabetes and 0.3691 IU/ml for non-diabetics after hepatitis B vaccination. However, the analyzed p-value was greater than 0.05, suggesting a lack of significant difference in mean values (Table-6).

Table 1: Contrasting the Diabetic and Non-Diabetic Cohorts (Prior to Vaccination) Based on Selected Physiological and Biochemical Indicators. [34]

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

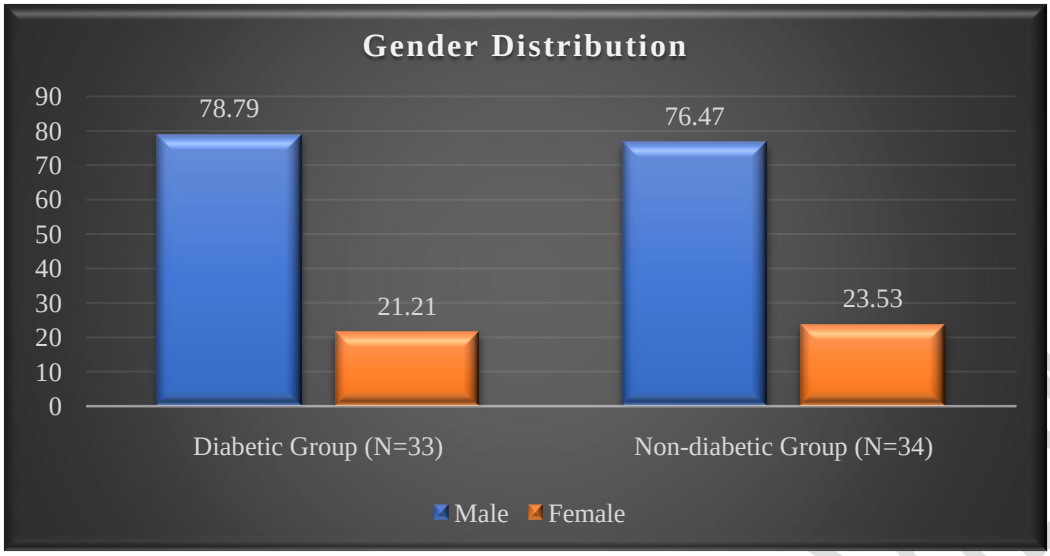

Figure 1: Gender Composition of the Study Population in Two Distinct Groups.

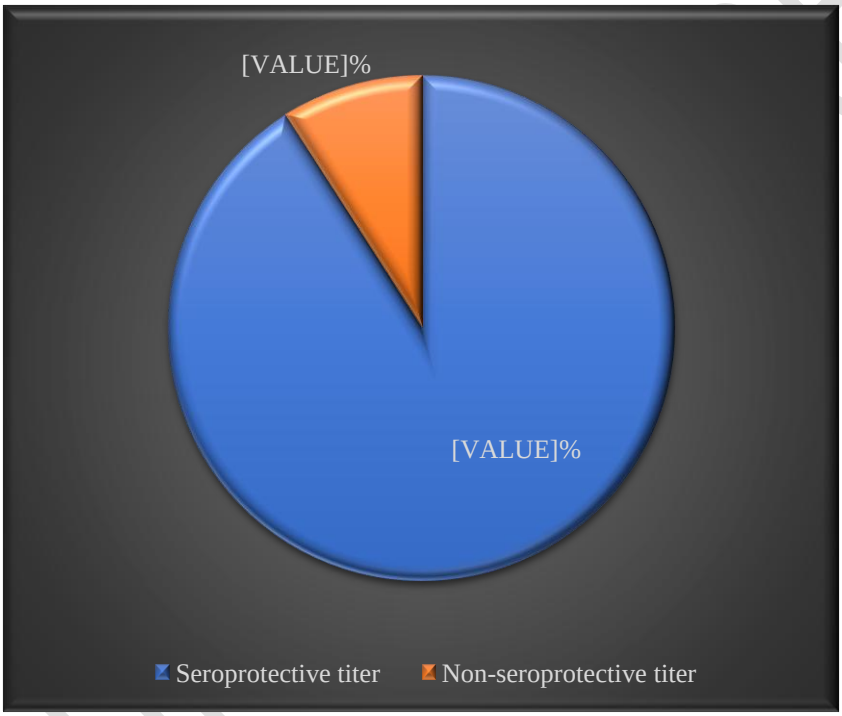

Figure 2: The collective proportion of individuals who have successfully developed a protective level of antibodies against the hepatitis B virus, as a result of receiving the hepatitis B vaccination.

Table 2: The occurrence and proportion of protective antibody levels in individuals with diabetes and those without diabetes after receiving the hepatitis B vaccination. [34]

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-	3	9.09	3	8.82	

responder (titer 10 mIU/mL)					

Table 3: The occurrence and proportion of elevated and diminished seroprotective titers among individuals with diabetes and those without diabetes after receiving hepatitis B vaccination.

Variables	Diabetic group (N=30)		Non-diabetic group (N=31)		P Value
	N	%	N	%	
Low seroprotective titer (10-100mIU/mL)	11	36.67	6	19.35	>0.05
High seroprotective titer (>100mIU/mL)	19	63.33	25	80.65	

Table 4: The average post-vaccination anti-HBs titer among individuals with diabetes and those without diabetes.

Variables	Mean value (mIU/ml.)	SD	P Value
Diabetic group	357.81	379.59	>0.05
Non-diabetic group	621.24	832.34	

Table 5: The average level of IFN-γ in both the diabetic and non-diabetic groups, with consideration for hepatitis B vaccination, was assessed.

Variables	Mean value (IU/ml)	SD	P value
Diabetic group	0.148	0.2016	<0.05
Non-diabetic group	0.2788	0.2883	

Table 6: The average IL-2 value in both the diabetic and non-diabetic groups after hepatitis B vaccination.

Variables	Mean value (IU/ml)	SD	P value
Diabetic group	0.2611	0.201	>0.05
Non-diabetic group	0.3691	0.283	

DISCUSSION

This research aimed to investigate the immune response to the hepatitis B vaccine among individuals with type 2 diabetes. To achieve this goal, the study involved comparing two groups: one comprising diabetic participants (the experimental group) and the other comprising non-diabetic participants (the control group). The comparison was based on the analysis of three specific seromarkers: anti-HBs titer, serum IFN-γ, and IL-2, after administering the hepatitis B vaccine. Furthermore, the researchers examined the potential correlation between these seromarkers and the duration of type 2 diabetes in the participants. Upon conducting the study and administering the hepatitis B vaccine, the researchers observed that a seroprotective titer was achieved in 91% of the overall sample. It is important to note that a *seroprotective titer* was defined as an anti-HBs titer of ≥10 mIU/mL [28]. The benchmark for an effective vaccine and vaccination strategy is the achievement of protective levels of neutralizing antibodies against hepatitis B (at a concentration exceeding 10 mIU/l) in a minimum of 85% of vaccinated individuals [29]. In our study, the hepatitis B vaccination response demonstrated adherence to the established criteria for a successful vaccine response. Among the participants with diabetes, 90.91% exhibited seroprotective titers, while in the non-diabetic group, 91.18% displayed seroprotective titers. The proportions of seroprotective titers were comparable between the diabetic and non-diabetic groups, with no statistically significant difference ($p>0.05$) observed. The seroprotective titers can be categorized into two groups based on magnitude: low seroprotective titers (titers ranging from 10 to 100 mIU/mL) and high seroprotective titers (titers exceeding 100 mIU/mL) [28]. A notable disparity was evident between the diabetic and non-diabetic groups based on low and high seroprotective titers. In the diabetic group, a low seroprotective titer was observed in 36.67% of subjects, while a high seroprotective titer was seen in 63.33%. Conversely, 19.35% of subjects exhibited a low seroprotective titer within the non-diabetic group, while 80.65% of subjects displayed a high seroprotective titer. However, statistical analysis failed to establish significant differences ($p>0.05$). The mean anti-HBs titer was lower (357.81 mIU/mL) in the diabetic group compared to the non-diabetic group (621.24 mIU/mL). Standard Deviations were computed as 375.59 for the diabetic group and 832.34 for the non-diabetic group. The large values of Standard Deviations in both groups were attributed to fluctuations in the raw data. Despite a considerable difference in the two mean values, it remained non-significant ($p>0.05$). The mean serum IFN-γ level was lower in the diabetic group (0.1480 IU/ml) than in the non-diabetic group (0.2788 IU/ml). The Standard Deviations for serum IFN-γ were 0.202 for the diabetic group and 0.288 for the

non-diabetic group. The discrepancy in serum IFN-γ levels between the diabetic and non-diabetic groups was statistically significant ($p<0.05$). For serum IL-2 level, the mean values were 0.2611 IU/ml and 0.3691 IU/ml in the diabetic and non-diabetic groups, respectively. The corresponding Standard Deviations for serum IL-2 were 0.201 for the diabetic group and 0.283 for the non-diabetic group. Although the mean value of IL-2 in the diabetic group was notably lower than that in the non-diabetic group, the difference failed to achieve significance ($p>0.05$). Analyzing the correlation between the duration of type 2 diabetes and the decline in serum anti-HBs titer post-hepatitis B vaccination yielded a negative correlation, yet it lacked significance. The correlation coefficient (r value) was recorded as -0.059, with a p-value exceeding 0.05. Similarly, the correlation between the duration of type 2 diabetes and the decrease in serum IFN-γ post-hepatitis B vaccination showed a negative correlation with an r-value of approximately -0.105. However, this correlation was not statistically significant due to a p-value greater than 0.05. The correlation between the duration of type 2 diabetes and the decrease in serum IL-2 after hepatitis B vaccination was noted as -0.139, with a p-value exceeding 0.05. Despite the negative correlations observed, none of these correlations were statistically significant. Cytokines are secreted into the body during an immune response, playing crucial roles in various stages of the immune process [24,26,27,30]. Since the hepatitis B vaccine operates through an active immune response, there is expected to be a direct relationship between the levels of serum anti-HBs, serum IFN-γ, and serum IL-2. This investigation aimed to explore such relationships. The analysis revealed a positive and noteworthy association between anti-HBs titer and IFN-Γ after administering the hepatitis B vaccine, with a calculated correlation coefficient (r) of 0.29 and a correlation significance level (p-value) of less than 0.05. Similarly, when examining the correlation between serum anti-HBs level and serum IL-2 level post hepatitis B vaccination, a robust positive connection was observed, with an r-value of 0.53 and a p-value of <0.05. Given the positive and significant correlations established between serum IFN-γ and anti-HBs titer and serum IL-2 and anti-HBs titer, one would anticipate a comparable correlation between serum IFN-γ and IL-2 levels. This theoretical assumption was validated upon statistical analysis, as the correlation between serum IFN-γ and IL-2 was positive ($r = 0.35$) and significant ($p = 0.05$). These substantial positive correlations among serum anti-HBs titer, IFN-γ, and IL-2 following hepatitis B vaccination provide substantial support for the findings of previous research studies [23,24,26,27]. Hence, this outcome effectively validates the precision of our research procedures. Conversely, the notable positive correlations imply that an increase in any of the seromarkers (anti-HBs titer, IFN-γ & IL-2) corresponds to an increase in the others. Based on this notion, there is potential for anticipating a robust immunological reaction to a vaccine through the external administration of IFN-γ or IL-2, especially for individuals who exhibit an inadequate immune response to vaccination or whose bodies generate lower levels of IFN-γ or IL-2 compared to healthy individuals. Certain studies have demonstrated a favorable immune response by utilizing IFN-γ and IL-2 as vaccine adjuvants [31-33]. In the study, for all measurements (anti-HBs, IFN-γ, and IL-2), the diabetic group consistently exhibited lower mean values than the non-diabetic group. Among these measurements, only IFN-γ displayed a statistically significant difference in mean values between the diabetic and non-diabetic groups. Conversely, there was no statistically significant difference in the mean values of the other two measurements, namely anti-HBs and IL-2, between the two groups. It is important to note that biological studies often encounter fluctuations in values, leading to a comprehensive standard deviation (SD) or standard error (SE), which can hinder attaining a desirable level of significance in research findings. Examining the relationship between the duration of type 2 diabetes and the measured seromarkers (anti-HBs, IFN-γ, and IL-2) after hepatitis B vaccination, the study found negative correlations. However, these correlations could not be established as statistically significant.

Limitations of the study: The study's limitations include the relatively small sample size of Type 2 diabetic patients in a specific geographical context, potentially limiting the generalizability of findings. The cross-sectional design restricts establishing causal relationships and longitudinal immune response patterns. Other confounding variables, such as varying diabetes management approaches, and potential differences in vaccine administration, could influence results. Additionally, the study focuses on immune responses without delving into clinical outcomes. Despite these limitations, this research provides valuable insights into hepatitis B vaccine immunogenicity within this specific diabetic population and underscores the need for further comprehensive investigations.

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study delved into the immune responses triggered by the Hepatitis B vaccine within the context of Type 2 diabetic patients. The findings underscore the significance of evaluating vaccine efficacy in this vulnerable population. The immune response to the vaccine appears to be attenuated in Type 2 diabetic individuals, potentially stemming from their compromised immune function. Reduced antibody titers and altered cytokine profiles were observed, implying tailored vaccination strategies were needed. To enhance vaccine effectiveness, it is recommended to administer higher vaccine doses, consider alternative vaccination schedules, or explore adjuvant

use. Furthermore, glycemic control should be optimized before vaccination to bolster immune responses. Given the rising global burden of Type 2 diabetes and the critical importance of hepatitis B prevention, these insights are pivotal for informing targeted vaccination approaches and optimizing the health outcomes of Type 2 diabetic patients.

Ethical approval: *The study was approved by the Institutional Ethics Committee.*

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