Original Research Article

Immune Responses to Hepatitis B Vaccine in Type 2 Diabetic Patients

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: *This study was conducted to determine the immune responses to hepatitis B vaccine in type 2 diabetic patients.*

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 percentage of seroprotective titer (anti-HBs titer ≥10 mIU/mL) did not differ ($p>0.05$) between the diabetic (90.91%) and the non-diabetic group (91.18%) following hepatitis B vaccination. The percentage of high seroprotective titer (anti-HBs titer >100 mIU/mL) was more significant in the healthy control (80.65%) than in the diabetic group (63.33%), but the difference was not significant ($p>0.05$). The mean value of anti-HBs titer was lower in the diabetic group (357.81 mIU/ml) than in the non-diabetic group (621.24 mIU/ml), but the difference was not significant ($p>0.05$). The mean value of IFN-γ was lower in the diabetic group (0.1480 IU/ml) than in the non-diabetic group (0.2788 IU/ml), and here, the difference was significant ($p<0.05$). The mean value in the serum IL-2 level was also lower in the diabetic group (0.26111U/ml) than in the non-diabetic group (0.3691 IU/ml) but the difference was not significant ($p<0.05$).*

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

INTRODUCTION

Diabetes mellitus and hepatitis B virus infection both are life threatening conditions. They are being considered as great burden for global public health and their frequencies are dramatically increasing day by day all over the world. In 2000, the prevalence of diabetes for all age groups worldwide was estimated as 2.8% and the number of diabetic people was 171 million [1]. At that time the number of diabetic patients in Bangladesh was about to be 3.2 million and Bangladesh was 10 highest diabetic populated country [1]. In 2030, the prevalence of diabetes mellitus may rise from 2.8% to 4.4% if the current flow continues and then the number of diabetic patients will increase from 171 million to 366 million. At that time the number of diabetic patients in Bangladesh will increase from 3.2 million to 11.9 million and Bangladesh will be 7th highest country according to the mass of diabetic patients [1]. About 1.1 million people died from diabetes in 2005 and WHO projects that death from diabetes will be double within 2030 [2]. The global pandemic principally involves type 2 diabetes (85%-95%) to which several factors contribute, including obesity, sedentary life style, greater longevity, unsatisfactory diet and increasing urbanization [3,4]. The prevalence of non-insulin-dependent diabetes mellitus (NIDDM) in sub-urban population of Bangladesh was about 4.1% [5]. In rural Bangladesh, the prevalence of type 2 diabetes increased from 2.3% to 6.8% between the year of 1999 and 2004 [6]. In urban area the prevalence was more threatening; it was about 8.1% [7]. On the other hand, worldwide about two billion people have been infected with hepatitis B virus and about 350 million live with chronic infection. An estimated 6,00,000 person die each year due to the acute or chronic consequence of hepatitis B virus infection. Hepatitis B is endemic in Asia, where 8% to 10% of the adult population is chronically infected. In

the Middle East and Indian sub-continent, an estimated 2% to 5% of the general population is chronically infected [8]. Bangladesh is situated in intermediate endemic area according to the prevalence on hepatitis B infection where 3% of the general population was identified as seropositive for hepatitis B virus surface antigen in 2003 [9]. Seropositive case was also detected in pregnant woman of Bangladesh as 3.5% [10]. Higher seroprevalence was observed in special group of people, for example Bangladeshi truck drivers, intravenous drug users and commercial sex workers where the prevalence was distributed as 5.9%, 6.2% and 9.7% respectively [11-13]. The prevalence of hepatitis B virus infection is higher in diabetic patient than the healthy people [14]. This higher prevalence of hepatitis B infection in diabetes mellitus may be due to decrease immunity or increase frequency of skin puncture because they have to monitor blood sugar frequently, they have to inject insulin specially during pregnancy or surgery or any other severe illness, their rate of hospitalization and duration of staying hospital is higher, at that time they have to face various injecting procedure for diagnostic and therapeutic purpose. Diabetes mellitus worsen the hepatic condition of hepatitis patient and increase the risk of complication [15]. Diabetes mellitus is related to increase the risk of development hepatocellular carcinoma (HCC) and chronic liver disease (CLD). It is also associated with more advanced lesion and poor outcome in patients with hepatocellular carcinoma [16,17]. Diabetes mellitus accelerates liver fibrosis and the incidence of bacterial infection in cirrhotic patients which are associated with increase mortality. In hepatic disease metabolic homeostasis of glucose is impaired as a result blood glucose level may rise (hepatogenous diabetes) that makes the total diabetic condition very severe. So, when an individual becomes affected with diabetes mellitus and hepatitis simultaneously the situation becomes very hard to manage and his life span becomes shorten [15]. Hepatitis B virus infection can be prevented by terminating the root of hepatitis B transmission but it is not always easy and sometimes very difficult or impossible, especially in accidental or uncommon way of transmission. At least 30% of reported hepatitis B among adult cannot be associated with an identifiable risk factor [18]. Another easy and reliable way of prevention the infection is vaccination with hepatitis B vaccine but there remains uncertainty of vaccine response in diabetic patients because diabetic patients face abnormality in different stage of immunity [19-22]. From the administration of antigen to development of immunity, vaccine is to pass through some vital steps to which several factors contribute such as cellular factor (APC, T cell, B cell) and chemical factor (MHC, Transcription factor, Cytokine) etc. IL-2 and IFN-γ are the two important cytokine that play vital role in different stage of immune response. IL-2 activates verity of cell of immune system including helper T cells, cytotoxic T cells, B cells, macrophages, natural killer cell. In different research work an increased serum level of IL-2 and IFN-γ was observed following infection and vaccination, this observation indicates the relationship of IL-2 and IFN-γ with immune response [23,24]. Therefore, if serum anti-HBs, IL-2 and IFN-γ in type 2 diabetic and non-diabetic individuals following hepatitis B vaccination can be analyzed and compared, it may give a comparable account of immune response to hepatitis B vaccine between type 2 diabetic and non-diabetic individuals. If the seromarkers (anti-HBs, IL-2 and IFN-γ) of type 2 diabetic patients can be correlated with duration of diabetes, it may also give an idea about the relation of immune response to the vaccine with duration of diabetes.

METHODOLOGY & MATERIALS

The study was designed to determine the immune responses in type 2 diabetic patient against hepatitis B vaccine. The study was an experimental type of study where the sample was selected by non-probability purposive sampling. 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 & 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. The study was done under the Department of Immunology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic disorder (BIRDEM), Shah bag, Dhaka, Bangladesh. The duration of the study was 1 year (2009-10). 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.

Experimental group:

- Type 2 diabetic patients.

Control group:

- Non diabetic comparatively healthy individuals.

- Sample number: diabetic sample was 33 in number and non-diabetic sample was 34 in number. So, total sample was 67 in number.

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. (WHO 1999, WHO 2009).

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.

Anti-HBs was assayed in 28 day's serum as serum anti-HBs reaches its peak level after 4 weeks of hepatitis B vaccination [25]. Anti-HBs was also assayed in pre-vaccination serum. IFN-γ was assayed in 14th day's serum, IL-2 was assayed in7th day's serum. We faced difficulties to determine which blood sample (7th or 14th day's) should be used to analyze for IFN-γ and IL-2 because in previous studies it was not clear that in which post-vaccinated day the parameters reach their maximum level. In different studies different post-vaccinated day's (1", 2nd week) blood were analyzed but in conclusion these studies only showed that after vaccination the serum level of IFN-γ and IL-2 rises from the base line without mentioning the time frame [23,24,26,27]. To solve this problem, we collected samples both of 1" (7th day) and 2nd (14th day) weeks after last vaccination. Just before starting the main analysis, we assayed five participants' blood (both 7° and 14 day's) for both IFN-γ and IL-2. In these pilot studies, higher value of serum IL-2 was observer in 7th (mean OD- 0.059) day's serum than 14th (mean OD-0.057) day's serum and in case of IFN-γ higher value was observer in 14 (mean OD-0.066) day's serum than 7th (mean OD-0.069) day's serum. Therefore, we fixed the samples assay date for Anti-HBs at 28 day's samples, for IFN-γ at 14 day's samples and for IL-2 at 7 day's samples. 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.

RESULT

The mean age value of diabetic (experimental) group was 51.76% and non-diabetic (control) group was 50.74%. Statistically there was no age difference between the diabetic and the non-diabetic group (p-0.05). The mean values of serum bilirubin were calculated as 0.527mg/dl and 0.489mg/dl in diabetic and non-diabetic group respectively. The difference of serum bilirubin level between diabetic and non-diabetic group was not significant (p>0.05). The mean values of serum ALT were calculated as 29.67 U/L and 28.90 U/L in diabetic and non-diabetic group respectively. The difference of two values was not significant (p>0.05). The calculated mean values of serum creatinine were 0.791 mg/dl in diabetic group and 0.742 mg/dl in non-diabetic 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 diabetic and non-diabetic group respectively (Table 1). Among the 33 diabetic subjects 26 (78.79%) were male and 7 (21.21%) were female, among the 34 non diabetic subjects 26 (76.47%) were male and 8 (23.53%) were female. So, the male female ratio was also nearly same (p>0.05) (Figure 1). Among the responder group low protective titer (10-100mIU/mL) was observed in 11 diabetic and in 6 non-diabetic sample, on the other hand high protective titer (>100mIU/mL) was observed in 19 diabetic and in 25 non-diabetic sample. In general view the percentage of low protective titer was higher in diabetic group and the percentage of high protective titer was higher in non-diabetic group but the differences could not be proved statistically significant (p>0.05) (Table-2). The mean values of post vaccination anti-HBs titer were 357.81 mIU/mL in diabetic group and 621.24 mIU/mL. In non-diabetic group. The mean value of anti-HBs titer looked lower in diabetic group than that of non-diabetic group but the p value was non-significant (>0.05) (Table-4). A significant difference was observed between the values of IFN-γ in the maple of diabetic and non-diabetic group. The mean value of IFN-γ was in diabetic group as (0.1480 IU/ml) which was significantly lower han that of non-diabetic group (0.2768 IU/ml) Here the p value was calculated as <0.05 that indicates significant difference of the mean values (Table-5). The mean values of serum IL-2 were calculated as

0.2611 IU/ml and 0.3691 IU/ml in diabetic and non-diabetic group respectively following hepatitis B Vaccination. But the analyzed p value was found as >0.05 that indicates non-significant difference of the mean values (Table-6).

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

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

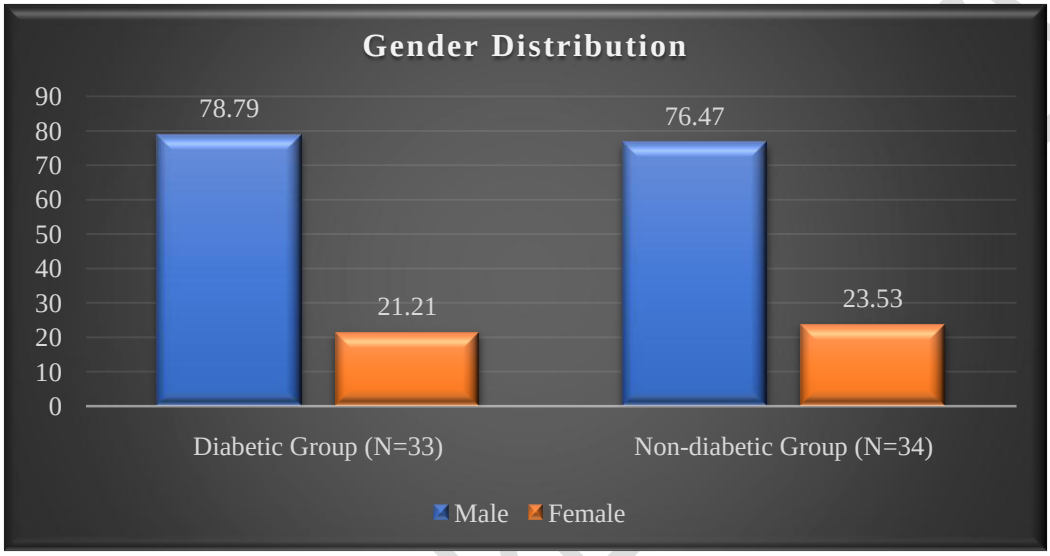

Figure 1: Gender distribution of the study population based on two groups.

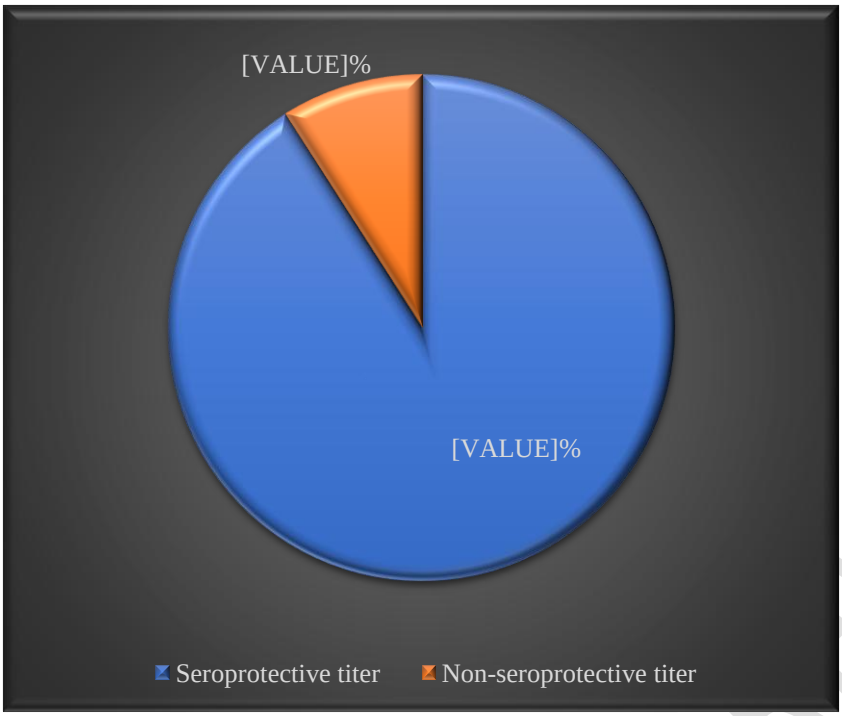

Figure 2: Overall percentage of seroprotective anti-HBs titer development following hepatitis B vaccination.

Table 2: The number and percentage of seroprotective titer in diabetic and non-diabetic group 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 3: The number and percentage of high and low seroprotective titer in diabetic and non-diabetic group following 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 mean value of post-vaccination anti-HBs titer in diabetic and non-diabetic group.

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 mean value of IFN-ɣ in diabetic and non-diabetic group Allowing hepatitis B vaccination.

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 mean value of IL-2 in diabetic and non-diabetic group following 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 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 [28]. 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 [29] so, in our study the hepatitis B vaccination response fulfilled the gold standard vaccine response criteria. 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) [28]. 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 significant statistically ($p>0.05$). The mean value of anti-HBs titer was lower (357.81 mIU/mL) in diabetic group than that of non-diabetic group (621.24 mIU/mL). Here the Standard Deviations were calculated as 375.59 and 832.34 for diabetic and non-diabetic group respectively. The values of Standard Deviations were large in both groups because the raw data were fluctuated. The difference of the two mean values was not small but it also could not touch the significant level ($p>0.05$). The mean serum IFN-γ level (0.1480 IU/ml) was lower in diabetic group than that of non-diabetic (0.2788 IU/ml) group. The values of Standard Deviations were observed in diabetic group and non-diabetic group as 0.202 and 0.288 respectively. Here the difference of serum IFN-γ level between the diabetic group and non-diabetic group was proved as significant ($p<0.05$) In case of serum IL-2 level the mean values were calculated as 0.2611 IU/ml and 0.3691 IU/ml in diabetic and non-diabetic group respectively. The observed Standard Deviations of serum IL-2 were 0.201 for diabetic group and 0.283 for non-diabetic group. The mean value of IL-2 for diabetic group was lower than that of the non-diabetic group and the difference of the two mean values was big but here also the difference could not be proved significant ($p>0.05$). The correlation between duration of type 2 diabetes and serum anti-HBs titer fallowing hepatitis B vaccination was negative but not significant because here 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 vaccination, 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. During immune response cytokine is secreted into the body and they play important role in different stage of immune response [24,26,27,30]. As hepatitis B vaccine acts under active immune response, it should present a positive correlation among serum anti-HBs level, serum IFN-γ and serum IL-2 level. In this study, the correlation between anti-HBs titer and IFN-Γ fallowing hepatitis B vaccination was found positive and significant because here the r value was found as 0.29 and p value of correlation was found as <0.05. In case of correlation between serum anti-HBs level and serum IL-2 level following hepatitis B vaccination the r value was calculated as 0.53 that also indicates a positive relationship between the serum anti-HBs level and serum IL-2 level. The calculated p value of the correlation was found as <0.05 that also indicates significant correlation. As, the correlations between serum IFN-γ and anti-HBs titer and between serum IL-2 and anti-HBs titer were positive and significant, theoretically it should have same type of correlation between serum IFN-γ and IL-2 level. Practically, after statistical analysis the correlation between serum IFN-γ and IL-2 was identified as positive and significant (r 0.35, p=0.05). These significant positive correlations among serum anti-HBs titer, IFN-γ and IL-2 following hepatitis B vaccination ultimately support the data of previous studies [23,24,26,27]. So, this result actually ensures the accuracy of our research procedures. On the other hand, the significant positive correlations suggest that, in general when any one of the seromarkers (anti-HBs titer, IFN-γ & IL-2) will be higher the other will be higher. From this conception, it can be hoped that a good immunological response of vaccine can

be achieved with injecting of IFN- or IL-2 from outside of the body during vaccination especially in the person who is unable to show an effective immune response against vaccination or whose body is unable to produce same amount of IFN-γ or IL-2 compare to the healthy individuals. In some research works good immune response was achieved by using IFN-γ and IL-2 as vaccine adjuvant [31-33]. In the study, in every case (anti-HBs, IFN-γ & 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 & 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. 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.

Limitations of the study: Every hospital-based study has some limitations and the present study undertaken is no exception to this fact. The limitations of the present study are mentioned. Therefore, the results of the present study may not be representative of the whole of the country or the world at large. The number of patients included in the present study was less in comparison to other studies. Because the trial was short, it was difficult to remark on complications and mortality.

CONCLUSION AND RECOMMENDATIONS

The study total 67 (Type 2 diabetic 33, control 34) subjects were vaccinated with hepatitis B vaccine, among which 61 (91%) showed seroprotective titer (anti-HBs 210 mIU/mL). The percentage of seroprotective titer was nearly same (>0.05) for both type 2 diabetic (90.91%) and non-diabetic group (91.18%) but the percentage of high seroprotective titer (anti-HBs >100 mIU/mL) was lower in diabetic group (63.33%) than non-diabetic group (& 80.65%) though the difference was not significant (>0.05). The mean values of serum anti-HBs titer, IFN-γ and IL-2 were lower in diabetic group than non-diabetic group but only in case of seromarker IFN-γ, the difference was proved significant ($p<0.05$) and in case of other two seromarkers (anti-HBs titer & IL-2 level) the differences could not be proved significance (>0.05). The correlations of serum anti- HBS titer, IFN-γ and IL-2 with duration of diabetes were found negative (r= -0.059, -0.105 & -0.139 respectively) but the correlations were not significant ($p>0.05$). The correlations persisted between anti-HBs titer and IFN-γ, between anti-HBs titer and IL-2 and between IL-2 and IFN-γ were positive (r-0.29, 0.53 & 0.35 respectively) and significant ($p<0.05$) that indicate the accuracy of the research work. If extended research works can be conducted in this respect more interesting and reliable result can be achieved.

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

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