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Immune Responses toBNT162b2 SARS-Cov-2 mRNA Vaccine in T2DM Individuals in Bangladesh

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Abstract:
Background*: Given the high incidence of diabetes as a comorbidity in COVID-19, vaccination against SARS-CoV-2 is deemed a priority for this population. In this prospective longitudinal study, 19 individuals with T2DM and 16 apparently healthy individuals were assessed prior to receiving the first dose, 4 weeks after the first dose, and 2-4 weeks after the second dose of the BNT162b2 mRNA vaccine to evaluate the SARS-CoV-2 spike protein-specific IgG for humoral immune responses andto evaluate the T-cell mediated immune responses, IFN-γ and IL-2 secreting PBMCs against the SARS-CoV-2 BNT162b2 mRNA vaccine were assessed and compared.*
Materials and Methods*: 19 known cases of type 2 diabetes and 16 healthy controls were included through purposive sampling and were regularly sampled before 1st dose, after 1st and 2nd dose of vaccination. The serum samples were evaluated for HbA1c status. SARS-CoV-2 spike protein-specific IgG was measured by the chemiluminescent immunoassay method using an automated analyzer. Ex-vivo ELISpot assays for IFN-γ and IL-2 were done using isolated PBMCs. A p-value < 0.05 was considered significant.*
Results*: All participants exhibited seroconversion and a robust rise in SARS-CoV-2 spike protein-specific IgG titers (p<.001) at 4 weeks after the first dose, however, geometric mean titers and SARS-CoV-2-specific trimeric IgG titers (BAU/mL) among individuals with T2DM (median = 264.1 BAU/mL; GMT = 304.90 BAU/mL) were inferior to those observed in healthy individuals (528.07 BAU/mL) at 4 weeks after the first dose of the vaccine. A robust rise of IFN-γ and IL-2 secreting PBMCs counts in T2DM participants irrespective of hypertension, gender and age after 2 doses of vaccination (p <0.001) was also observed. However, IFN-γ secreting PBMCs counts were significantly lower in type 2 diabetic group than healthy control group after 1st dose (p =0.021).Spearman's correlation test showed significant negative correlation of HbA1c to IFN-γ secreting PBMCs counts (Spearman's rho=-0.47, p =0.043) after 2 doses in T2DM group.*
Conclusion:*Although type 2 diabetic participants had a lower response initially,particularly in those with poor glycemic control, BNT162b2 mRNA vaccine overall induces a robust T-cell mediated immune response*
Key Word*:BNT 162b2 Vaccine; diabetes; immune response; SARS-CoV-2.*

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I. Introduction

Diabetes causes intensification of the severity of COVID-19 disease by compromising innate immunity, triggering an enhanced pro-inflammatory response, and lowering angiotensin-converting enzyme 2 (ACE2) expressions.[1] Diabetes patients have been reported to have a lower clearance of SARS-CoV-2 from their bloodstream, which along with poor T cell activity, and associated cardiovascular illness lead to the vulnerability of diabetic individuals to COVID-19 infections.[2]The intricate interaction between COVID-19 infection and diabetes get worse significantly with increasing age of patients and longer period of uncontrolled diabetes.[3]Furthermore, dysregulation in immune response is also aided by an adverse hormonal environment in Type 2 diabetes mellitus (T2DM) patients.[4]Hence, T2DM patients are among the priority group to receive vaccination against SARS-CoV-2 worldwide as per recommendation from WHO.[5]

Immunocompromised patients have demonstrated decreased immune response compared to the healthy group. While in prior studies the humoral response (IgG) in healthy control was found to be > 99% against BNT162b2 (Pfizer-BioNTech), Bergman et al.(2021) showed that only 72.2% of the patients (solid organ

recipients and patients living with the human immunodeficiency virus) had seroconverted 2 weeks after administrating the 2nd dose of the BNT162b2 (Pfizer-BioNTech) vaccine.[6–8]In the case of diabetes, the evidences regarding immune responses are mixed worldwide.[9–11] However, the paucity of data on cell-mediated response to the SARS-CoV-2 vaccine in T2DM patients and data on the immunological vaccine responses related to glycemic control in participants with T2DM in the hitherto available clinical trials of COVID-19 vaccines, calls for more research in this subgroup.[12]Moreover, no study on a comprehensive and integrated analysis of immune parameters with response kinetics to SARS-CoV-2 vaccination in correlation with glycemic control in type 2 diabetic patients has ever been conducted in Bangladesh. In Bangladesh, with more than 13.1 million people living with diabetes, the question regarding the effect of glycemic control over immune response following vaccination against SARS-CoV-2 remains.[13]

In these circumstances, this study aimed to evaluate SARS-CoV-2 specific IgG for humoral immune responses and to assess and compare IFN-γ and IL-2 secreting peripheral blood cells (PBMCs) for T-cell mediated immune responses against the SARS-CoV-2 BNT162b2 (Pfizer-BioNTech) mRNA vaccine in type 2 diabetic individuals attending a tertiary care hospital in Bangladesh.

II. Material And Methods

Study design

This prospective longitudinal observational study was carried out to evaluate the humoral and T-cell mediated immune response against SARS-CoV-2 mRNA vaccine in individuals at or above 30 years of age with type 2 diabetes mellitus attending a tertiary care hospital of Bangladesh. This study included 19 knownadultscases of type 2 diabetes mellitus and 16 apparently healthy adults as type 2 diabetic group and healthy control group respectively. Participants were studied before 1st dose (T1), 4 weeks after 1stdose (T2) and 2-4 weeks after 2nd dose (T3) of BNT162b2 mRNA (Pfizer-BioNTech) vaccination. Study participants were selected from November 2021 to April 2022 by purposive sampling method from the Endocrinology outpatient department and COVID-19 Vaccine Centre of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. All laboratory procedures were performed in the Department of Virology, BSMMU. For the purpose of this study, the cut-off value of HbA1c was set at 7% and participants were categorized as follows: type 2 diabetes mellitus patients with HbA1c >7% as good glycemic control and type 2 diabetes mellitus patients with HbA1c ≤7% as inadequate or poor glycemic control.[14]Body Mass Index (BMI) (kg/m^2) was measured and categorized as BMI <25 kg/m^2 and BMI ≥25 kg/m^2. Hypertension was defined by either a documented diagnosis of hypertension, the patient taking antihypertensive medications, or the latest (within three months) blood pressure readings (either systolic ≥140 or diastolic ≥90).

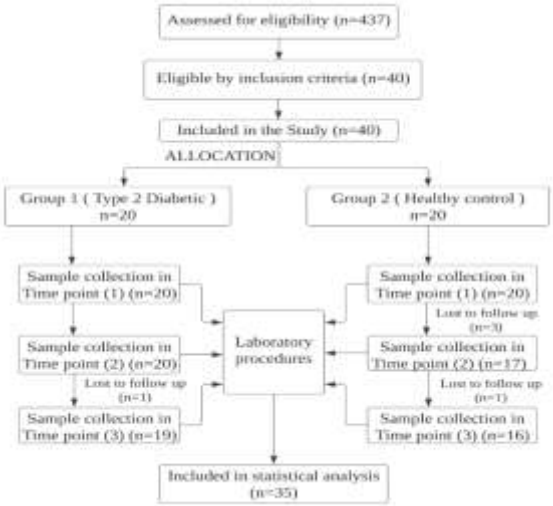

Figure no 1: Study flow chart showing the participation of the study subjects in different time points.

Laboratory procedures

Blood sample collection and processing:

After obtaining the informed written consent, with aseptic precautions 4 ml venous blood samples were collected at each time in plain vacutainer tubes (1ml) and sodium heparin vacutainer tubes(3ml) and processed accordingly. An extra 1ml Blood was collected in EDTA vacutainer tubes from the participants before 1st dose of vaccination and HbA1c status determination by an automated analyzer (Sebia capillary).

Detection of IgG antibodies specific to SARS-CoV-2:

Before 1st dose and 4 weeks after 1st dose of BNT162b2 mRNA (Pfizer-BioNTech) vaccination, using processed blood serum, IgG antibody specific to SARS-CoV-2 infection were detected by Automated LIAISON® XL Analyzer using FDA approved SARS-CoV-2 IgG chemiluminescence enzyme immunoassays kit (LIAISON® SARS-CoV-2 TrimericS IgG assay, REF 311510, Diasorin, Italy) following manufacturer instructions to assess the seroconversion rate. Detection range of assay was 4.80 to 2080 BAU/mL (Binding Antibody Unit/mL) and test results were reported as positive (≥ 33.8 BAU/mL) or negative (<33.8 BAU/mL).

Ex-vivo IFN-γ and IL-2 ELISpot assay:

On each time point for each participant, sample collected in the sodium heparin vacutainer tubes was processed for the collection of PBMCs by density gradient centrifugation using Histopaque®-1077 (SigmaAldrich, Germany) by previous method[15]and ex-vivo ELISpot assays for IFN-γ and IL-2 were done using ELISpot assay-kit (AutoImmuneDiagnostika GmbH, Germany) according to manufacturer instructions. AID ELISpot Reader 7.0 System was used for evaluation.

Statistical analysis:

All collected data were processed using Microsoft Excel® 2019. The statistical analysis was conducted and the 95% confidence intervals for each category were calculated at 10% acceptable error level using IBM® SPSS Statistics 25.0, jamovi 2.2.5 (based on R software), and DATAtab online statistical calculator. Dataset was tested for normality (Shapiro-Wilk and Kolmogorov-Smirnov) and for non-normal data non-parametric test as Mann-Whitney U-Test (two-tailed), Friedman Test etc. was used. A p value of <0.05 were considered significant.

III. Result

Out of 35 participants, 19 (54.3%) were diagnosed cases of type 2 diabetes mellitus (Group 1) and 16 (45.7%) were non-diabetic healthy control (Group 2). The mean age of type 2 diabetic group and healthy control group was 53.2±9.12 (SD) and 50.4±11.4 (SD) years respectively. Among type 2 diabetic participants (n=19), male and female ratio was 1.11:1, while it was 1:1.29 in case of healthy control group (n=16). Majority of the participants, were from urban areas (n=20, 57.1%) and belonged to middle-income families (n=23, 65.7%). Mean duration of sample collection after 2nd dose was 24.2±3.39 days. Among all participants, 16 (45.7%) were hypertensive and 19 (54.3%) were normotensive. Subdividing based on body mass index (BMI), 12 (34.29%) had a BMI ≥25 kg/m^2 and 23 (65.71%) had a BMI <25 kg/m^2 (Table 1).

Table no 1: Characteristics of the study participants.

Features	Type 2 Diabetic		Healthy control	
	Male (n=10) (52.63%)	**Female** (n=9) (47.37%)	**Male** (n=7) (43.75%)	**Female** (n=9) (56.25%)
Age (years) (mean±SD)	54.9 ± 6.9	51.33 ±11.22	53.14 ±12.56	48.33 ±10.69
Residence				
Urban	6 (31.58%)	4 (21.05%)	3 (18.75%)	7 (43.75%)
Rural	4 (21.05%)	5 (26.32%)	4 (25%)	2 (12.5%)
Socio-economic status				
High	1 (5.26%)	2 (10.53%)	-	-
Middle	6 (31.58%)	5 (26.32%)	5 (31.25%)	7 (43.75%)
Low	3 (15.79%)	2 (10.53%)	2 (12.5%)	2 (12.5%)
Hypertension				
Hypertensive	5 (26.32%)	6 (31.58%)	1 (6.25%)	4 (25%)
Normotensive	5 (26.32%)	3 (15.79%)	6 (37.5%)	5 (31.25%)
BMI level				
Normal	9 (47.37%)	6 (31.58%)	5 (31.25%)	3 (18.75%)
Overweight	1 (5.26%)	1 (5.26%)	2 (12.5%)	6 (37.5%)
Obese	-	2 (10.53%)	-	-

All participants received BNT162b2 (Pfizer-BioNTech) mRNA vaccine, which was well tolerated with no serious AEFI (Adverse events following immunization) was observed. According to the measured HbA1c status, 5 participants (26.32%) with type 2 diabetic had good (<7.0%) glycemic control and 14 (73.68%) had

inadequate or poor (≥7.0%) glycemic control. The mean of HbA1c was 6.3±0.45 (SD) in type 2 diabetic participants with good glycemic control and 9.27±1.77 (SD) in type 2 diabetic participants with inadequate or poor glycemic control. Participants of the healthy control group had a mean HbA1c of 5.74±0.294 (SD). All participants of both groups were found seroconverted at 4weeks after 1st dose of vaccination.

Humoral immune response:

In the comparison of SARS-CoV-2 specific trimeric IgG titers (BAU/mL) in type 2 diabetic participants and healthy control by Mann-Whitney U-Test (two-tailed) (Table no 2), there was significant difference (p = 0.029) in SARS-CoV-2 specific trimeric IgG titers (BAU/mL) between the type 2 diabetic group (n = 19; median = 264.1 BAU/mL) and healthy control group (n = 16; median = 449.6 BAU/mL) at 4 weeks after 1st dose (T2). Analysis also showed significant rise (p <0.001) in the titers value in the repeated measures for both type 2 diabetic group and healthy control group from before 1st dose (T1) to 4 weeks after 1st dose (T2). At 2-4 weeks after 2nd dose (T3), a number of test results (19 out of 35) were clustered upon the upper end point of the detectable range (2080 BAU/mL), hence, statistical analysis was not done for 2-4 weeks after 2nd dose (T3).

Table no 2: Shows the comparison of SARS-CoV-2 specific trimeric IgG titers (BAU/mL) in type 2 diabetic participants and healthy control.

Time points	Type 2 diabetic (n=19)	Healthycontrol(n=16)	*p*-Value
Before 1st dose (T1)	18.1 (7, 23)	15.0 (11.8, 22.2)	0.791
4 weeks after 1st dose (T2)	264.1 (148, 571)	449.6 (402, 706)	0.029*
p-value#	<.001*	<.001*	

Data expressed as median (IQR). * p < .05. *p*-Value= determined by Mann-Whitney U-Test (two-tailed), *p*-Value#=determined by Wilcoxon rank test.

Participants of healthy control group had a higher geometric mean titer (GMT) at before 1st dose (T1) (15.21 BAU/mL) and at 4 weeks after 1st dose (T2) (528.07 BAU/mL) than type 2 diabetic group at before 1st dose (T1) (13.41 BAU/mL) and at 4 weeks after 1st dose (T2) (304.90 BAU/mL) respectively (Table no 3). The overall regression was statistically non-significant.

Table no 3: Shows geometric mean of SARS-CoV-2 specific trimeric IgG titers in type 2 diabetic and healthy control group.

Time points	SARS-CoV-2 specific trimeric IgG titers (BAU/mL)		Ratio	*p*-Value
	Type 2 diabetic (n=19)	Healthy control (n=16)		
Before 1st dose (T1)	13.41	15.21	0.88:1	0.497
4 weeks after 1st dose (T2)	304.90	528.07	0.58:1	0.815

p-Value= determined by Linear regression of geometric mean. * p < .05.

The comparison of SARS-CoV-2 specific trimeric IgG titers (BAU/mL) in type 2 diabetic group in context of hypertension, BMI level, gender and age, where analysis determined by Mann-Whitney U-Test (two-tailed) presented non-significant differences in the SARS-CoV-2 specific trimeric IgG titers (BAU/mL) values at before 1st dose (T1) and at 4 weeks after 1st dose (T2) (Table no 4) showing that normotensive, male and aged at or under 50 years had more titers value than hypertensive, female and aged over 50 years. A robust rise of SARS-CoV-2 specific trimeric IgG titers (BAU/mL) was shown from before 1st dose (T1) to 4 weeks after 1st dose (T2) in type 2 diabetic group irrespective of hypertension status, gender and age. Type 2 diabetic participants with BMI <25 kg/m^2 also had robust rise of SARS-CoV-2 specific trimeric IgG titers (BAU/mL) across time, contrary to the type 2 diabetic participants with BMI level ≥25 kg/m^2 who had a statistically non-significant rise. A significant negative correlation was observed between HbA1c (%) and SARS-CoV-2 specific trimeric IgG titers (BAU/mL) at 4 weeks after 1st dose (T2) in type 2 diabetic group. Spearman correlation co-efficient matrix for inter-relationship of biomarkers within Type 2 diabetic (n = 19) and healthy control (n = 16) showed significant negative correlation of HbA1c (%) to SARS-CoV-2 specific trimeric IgG titers (Spearman's rho, r= -0.62, p=0.006) at 4 weeks after 1st dose (T2) in type 2 diabetic group.

Table no 4: Shows the comparison of SARS-CoV-2 specific trimeric IgG titers in type 2 diabetic group (n=19) in terms of hypertension, BMI level, gender and age at different time points.

Feature(s)	Sub-group(s)	Before 1st dose (T1)	4 weeks after 1st dose (T2)	*p*-Value#
Hypertension	Hypertensive (n=11)	18.1	199.7	0.02*
	Normotensive (n=8)	16.75	465.25	0.025*
	***p*-Value**	0.967	0.129	
BMI level	<25 kg/m^2(n=15)	18.7	264.1	0.006*
	≥25 kg/m^2(n=4)	10.9	433.45	0.176
	***p*-Value**	0.515	0.411	
Gender	Male (n=10)	18.9	360.75	0.017*
	Female (n=9)	14.5	264.1	0.002*
	***p*-Value**	0.437	0.447	
Age	≤50 years (n=5)	13.8	410.5	0.0.47*
	> 50 years (n=14)	18.8	200.6	0.016*
	***p*-Value**	0.286	0.156	

Data expressed as Median. *p*-Value= determined by Mann-Whitney U-Test (two-tailed). *p*-Value#=determined by paired samples t test. * $p < .05$.

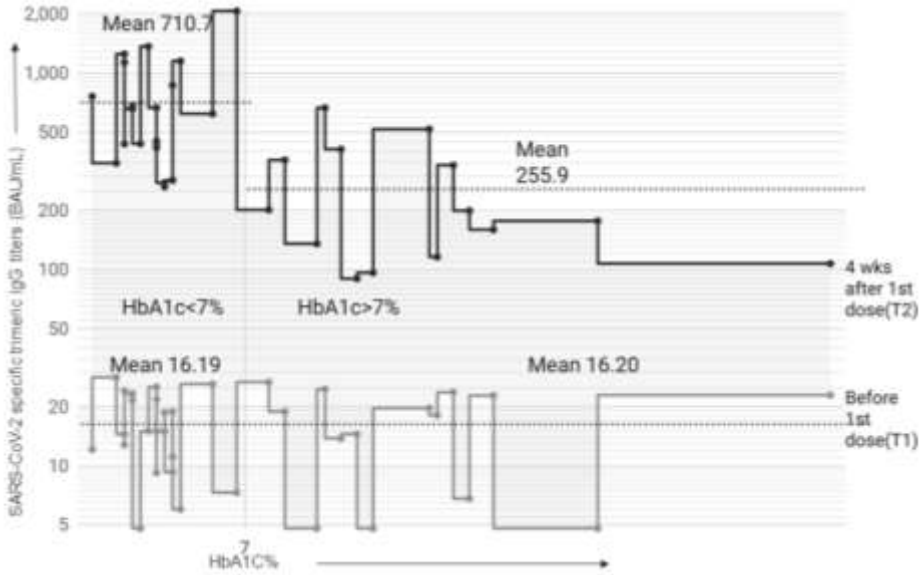

Figure no 2: Shows lower IgG responses at 4 weeks after 1st dose (T2) as glycemic control deteriorates.

T-cell mediated immune response:

Comparisons of IFN-γ and IL-2 secreting PBMCs counts from ex-vivo ELISpot assay in type 2 diabetic participants and healthy control showed significant differences ($p < 0.001$) in the repeated measures of both IFN-γ and IL-2 secreting PBMCs counts in both type 2 diabetic and healthy control groups with time. A significant difference ($p = 0.021$) was also observed in the IFN-γ spot counts between T2DM group and control group at 4 weeks after 1st dose (T2) showing a lower response in T2DM group.

Analysis was performed to compare IFN-γ and IL-2 secreting PBMCs against SARS-CoV-2 BNT162b2 (Pfizer-BioNTech) mRNA vaccine in type 2 diabetic participants in context of hypertension, BMI level, gender and age, which showed robust rise of IFN-γ and IL-2 secreting PBMCs counts with time in type 2 diabetic group irrespective of hypertension, BMI level, gender and age. The results of Spearman's correlation test showed significant negative correlation of HbA1c to IFN-γ secreting PBMCs counts (Spearman's rho=-0.47, $p = 0.043$) at T3 in T2DM group. Non-significant correlations of HbA1c to other parameters were shown in control group.

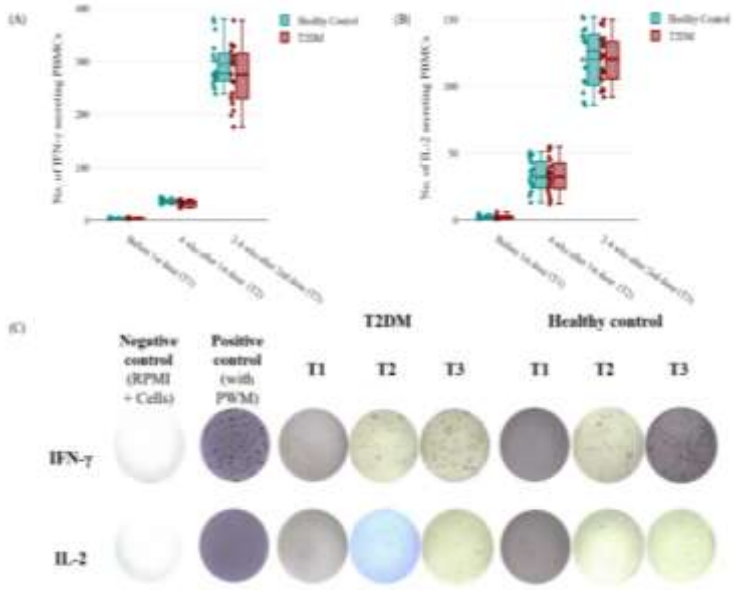

Figure no 3:A robust rise of both IFN-γ (A) and IL-2 (B) secreting PBMCs was shown in both the healthy control group and the T2DM group after ex-vivo ELISpot assays. (C) Representative ELISpot wells of the IFN-γ and IL-2 ELISpot assay with a positive control (stimulated with PWM) and a negative control (RPMI medium and cell suspension).

IV. Discussion

Since the emergency approval, vaccination against SARS-CoV-2 remained the mainstay for preventing COVID-19 notably severe disease, along with social distancing, application of personal protective equipment (e.g., face mask) and maintenance of proper hygiene. The immune response after vaccination, developed by both innate and adaptive immune systems, were found to be slowed and delayed in immunocompromised groups.[12] However, the immunological reactions to COVID-19 vaccinations in type 2 diabetic individuals were little understood up to this point.[16]

In the present study, SARS-CoV-2-specific IgG antibodies along with IFN-γ and IL-2 secreting PBMCs were evaluated in known cases of type 2 diabetes and healthy individuals aged 53.2±9.12 years and 50.4±11.4 years respectively. Through purposive sampling an approximately equivalent distribution of both male and female gender was also taken with a minute female predominance. Most of the participants in this study had a BMI <25 kg/m^2. A major percentage of participants in the type 2 diabetic group had inadequate or poor (HbA1c≥7.0%) glycemic control. Poor glycemic control was an effective indicator of disease severity and mortality in patients with COVID-19.[17]Participants were studied at 3 time points, baseline (T1) being at the day of 1st dose of vaccination, at the day of 2nd dose of vaccination (T2) which was 4 weeks after 1st dose and lastly between the time period of 2-4 weeks after the 2nd dose (T3) with a mean duration of 24.2±3.39 days.

All participants had negative IgG titer at the baseline time point before 1st dose (T1). SARS-CoV-2 specific IgG antibody was assessed in this study to perceive any differences in seroconversion rate between T2DM participants and the healthy control group, and both groups showed a 100% seroconversion rate at 4 weeks after 1st dose of BNT162b2 (Pfizer-BioNTech) mRNA vaccine and showed a significant increase in SARS-CoV-2-specific trimeric IgG titers (BAU/mL) from before 1st dose (T1) to 4 weeks after 1st dose (T2). IgG is the major antibody isotype of blood and extracellular fluid, and plays a crucial role in blocking viral attachment to ACE2 on host cells, thereby preventing viral entry.[18] At the final time point (T3), however, a number of test results (19 out of 35) were clustered at the upper end point of the detectable range (2080 BAU/mL), and statistical analysis could not be performed due to reagent constraints. Significant differences (p=0.029) in SARS-CoV-2-specific trimeric IgG titers (BAU/mL) were observed between the type 2 diabetes mellitus and healthy control groups at 4 weeks after 1st dose (T2). The geometric mean titers (GMT) in the type 2 diabetes mellitus group were lower than that in the healthy control group, indicating a lower IgG response in the former (ratio: 0.58:1) after the 1st dose (T2). This finding is consistent with those of other studies by Lustig *et al*. (2021), Islam *et al*. (2022), and Papadokostaki*et al*. (2022), which have demonstrated lower concentrations of IgG in patients with diabetes, particularly after the 1st dose (T2).[19–21] These studies suggest that individuals with T2DM may have a weaker immune response to vaccination compared to healthy individuals, which could have implications for their protection against certain infections. This could mean that they are more susceptible to contracting infections or experiencing more severe symptoms if they do become infected. In the context of

infectious diseases such as COVID-19, this could have significant implications for the health outcomes of individuals with T2DM. It highlights the need for the development of targeted vaccination strategies to improve their protection against infectious diseases.

The participants in both type 2 diabetic group and healthy control group had a significant rise in both IFN-γ and IL-2 secreting PBMCs counts from T1 to T3. However, the type 2 diabetic groups showed a lower IFN-γ secreting PBMCs counts after 1st dose (T2) than healthy control group indicating a lower IFN-γ response which was also demonstrated in a study where Van Praet et al. showed the association of diabetes with a lower cellular response against BNT162b2 (Pfizer-BioNTech) mRNA vaccine.[22]Prior studies showed that diabetic subjects display a delayed adaptive immune response to pathogen.[23]Dysfunction of both innate immune response (including dysfunction of neutrophils and macrophages) and adaptive immune response (including T cells) are supposed to be accountable for the feeble immune system against invading pathogens in diabetic subjects.[24]

In the current investigation, a significant negative correlation was observed between HbA1c (%) and SARS-CoV-2 specific trimeric IgG titers (BAU/mL) at 4 weeks after 1st dose (T2) in type 2 diabetic group (Figure no 2). This finding is consistent with the results of the CAVEAT study conducted by Marfella*et al*. (2022), in which the authors reported that type 2 diabetic patients with HbA1c >7% had a significantly reduced virus-neutralizing antibody capacity compared to normoglycemic individuals and type 2 diabetic patients with good glycemic control.[16] However, in contrast to the present study, Sourij*et al*. (2022) demonstrated that HbA1c (%) levels did not have a significant impact on antibody levels following COVID-19 vaccination.[25] Previous research has shown that patients with hyperglycemia and insulin resistance have reduced lymphocyte proliferative responses CD4+ to CD8+ lymphocyte ratio, decreased macrophage or monocyte function, and abnormalities with antigen presentation, which could result in this delayed immune response.[26] As T2DM is a complex chronic disease, it requires a comprehensive and multidisciplinary approach to management. Despite the availability of several treatment options, the management of T2DM remains challenging. Therefore, there is a need for targeted interventions at different levels of care, including individual, community, and healthcare system levels. The primary goal of individual-level intervention is to improve glycemic control through lifestyle modifications and pharmacological therapy. Lifestyle modifications include diet and exercise, which have been shown to improve insulin sensitivity, reduce insulin resistance, and improve glycemic control in T2DM patients. Pharmacological therapy includes oral hypoglycemic agents, injectable glucagon-like peptide-1 receptor agonists, and insulin. The choice of pharmacological therapy depends on several factors, including the patient's age, comorbidities, and disease duration. Community-level interventions include diabetes education and support programs, peer support groups, and community-based physical activity programs. Healthcare system-level interventions include electronic health records, quality improvement programs, and care coordination programs. The successful implementation of targeted interventions requires the involvement of patients, healthcare providers, community members, and policymakers. Targeted interventions can improve the outcomes of T2DM patients by addressing the individual, community, and healthcare system-level factors that contribute to the disease. Also,in the future, vaccination authorities should consider placing emphasis on monitoring the diabetic status of known diabetic patients before vaccination to prevent any suboptimal immune responses.

In the present study, robust rise of IFN-γ and IL-2 secreting PBMCs counts were observed from T1 to T3, irrespective of hypertension status, BMI level and age of the type 2 diabetic participants. Also, similar to the findings by Van Praet et al., there was no discernible difference in IFN-γ and IL-2 secreting PBMCs counts from ELISpot assays between the genders in present study, however, Schwarz et al. detected delayed and reduced antibody and T-cell responses in elderly people, to which the present study differs.[22,27]

Further analysis was performed to compare SARS-CoV-2 spike protein-specific IgG levels between type 2 diabetic participants who received the BNT162b2 mRNA vaccine, with respect to hypertension, BMI level, gender, and age as they are amongst the important co-factors of T2DM. Results revealed a robust rise in SARS-CoV-2 specific trimeric IgG titers (BAU/mL) from before 1st dose (T1) to 4 weeks after 1st dose (T2) in type 2 diabetic participants, regardless of gender, and age. However, this rise was statistically non-significant in those with a BMI level $\geq$25 kg/m^2. One possibility is that obesity may be associated with a weaker immune response to vaccines due to chronic inflammation and metabolic dysregulation. Additionally, it is possible that other factors, such as age or comorbidities, may have influenced the immune response to the BNT162b2 vaccine in individuals with a BMI of 25 kg/m^2 or higher. There was no discernible difference in SARS-CoV-2 spike protein-specific trimeric IgG titers (BAU/mL) between genders. One possibility is that the immune response to SARS-CoV-2 infection or vaccination may be similar between males and females due to the similar expression of immune receptors and genes involved in immune responses in both sexes. However, it is important to note that other factors, such as hormonal differences between males and females, may potentially play a role in the immune response to SARS-CoV-2 infection or vaccination. Hence, the decline of sex hormones in the older group for both males and females may also have resulted in a similar immune response to the BNT162b2 mRNA vaccine. Furthermore, no significant differences in SARS-CoV-2 spike protein-specific trimeric IgG

titers (BAU/mL) were observed in sub-groups divided by hypertension status. These findings are consistent with the study by Ali *et al*. (2021), which reported that hypertension did not have a significant impact on IgG levels.[28]Hypertension is a chronic condition that is associated with immune dysregulation and inflammation, which could potentially affect the immune response to vaccination. However, this particular study did not find any significant differences in IgG levels between individuals with hypertension and healthy controls. However, it is possible that the limitations of the present study may have influenced the results. Firstly, due to recruitment restrictions, financial limitations, and time constraints, the study was relatively small and was complicated by low statistical power. Additionally, the study was performed at a single center and only included individuals who received the BNT162b2 (Pfizer-BioNTech) mRNA vaccine. Another challenge was the high individual variability inherent to human studies. To account for this, robust statistical methods, such as median value and two-sided Mann-Whitney-U test, were used. Finally, an additional titration of SARS-CoV-2 specific trimeric IgG titers (BAU/mL) at 2-4 weeks after the 2nd dose (T3) could reveal values beyond the upper endpoint of the detectable range. Further research is warranted to better understand the relationship between glycemic control and the humoral immune response to COVID-19 vaccines.

V. Conclusion

In conclusion, this study found that both the type 2 diabetes mellitus and healthy control groups were seroconverted after the first vaccine dose. In type 2 diabetes mellitus, significant increases in SARS-CoV-2-specific trimeric IgG titers were observed after the first dose irrespective of hypertension, age and gender differences. However, the outcomes of this study also indicated that patients with type 2 diabetes mellitus had a lower IgG response compared to healthy controls after the first vaccine dose. Furthermore, there was a significant negative correlation between HbA1c (%) and SARS-CoV-2 specific trimeric IgG titers (BAU/mL) at 4 weeks after 1st dose in the type 2 diabetes mellitus group. This study also demonstrated that BNT162b2 (Pfizer-BioNTech) mRNA vaccine induces robust T-cell mediated immune response in both type 2 diabetic participants and healthy individuals. However, type 2 diabetic group showed lower immunological responses specially after 1st dose when compared with healthy control. This finding suggests that good glycemic control is essential for an optimal humoral immune response to the BNT162b2 mRNA vaccine. Additionally, it's important for patients with diabetes to work with their healthcare providers to achieve good glycemic control, which can help to improve overall health outcomes and reduce the risk of complications from COVID-19 and other infections.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Statement

This study was reviewed and approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (reference: BSMMU/2021/10168, registration number: 3692, date: 06-11-2021). Participants were assured about their confidentiality and were free to withdraw them from the study at any time.

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Author contributions

All authors contributed to the article and approved the submitted version.

Data availability and pre-print publication statement

The raw data supporting the conclusions of this article will be made available by the authors upon request. Part of this study is available as pre-print (DOI: 10.22541/au.166871560.02594959/v3).

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