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Correlation of Robust Immune Response against SARS-CoV-2 Vaccine among Diabetic and Non-Diabetic Participants

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Individuals with Type-2 diabetic mellitus (T2DM) along with several other disease-causing factors are impacted adversely by the SARS-CoV-2 pandemic. In India, BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) vaccines are now being used to limit the spread of SARS-CoV-2 Infection. Adaptive immunity like humoral and T-cell-mediated immunity has a vital role in eliminating SARS-CoV-2. In order to control the disease's course, the infected cells are being destroyed predominantly by cytotoxic CD8+ T cells as well as specific antibodies against SARS-CoV-2 which have the potential to neutralize the virus. This cross-sectional study was done to identify the specific antibodies for SARS-CoV-2 in serum samples from those individuals with and without T2DM by using WANTAI SARS-CoV-2 Total Ab ELISA Kit. The present study comprises 354 study participants, among them T2DM was present in 141 (39.8%) cases and 213 (60.2%) were non-diabetic patients. Hypertension was observed in 95 (26.1%) participants and 259 (73.1%) participants were normotensive. The study participants with T2DM demonstrated lower levels of SARS-CoV-2 total antibodies having an average of 5 AU/ml over those individuals without diabetes showing an average of 12 AU/ml. Among the hypertensive patients, the total antibody levels of SARS-CoV2 are were substantially lowered to showing an average of 8 AU/ml as compared to normotensive subjects showing an average of 14 AU/ml. The results of the current study suggest that regular monitoring of the total SARS-CoV-2 antibody profile may be a useful strategy for assisting people with T2DM and hypertension in determining whether they require SARS-CoV-2 precautionary doses to maintain immunity and protect against infections.

Keywords: Comorbidities; Diabetic; Non-diabetic; SARS-CoV-2 Vaccines; SARS-CoV-2 Total Antibody Levels.

Globally, 651 million people have been affected by SARS-CoV-2 infection as of December 23, 2022, and nearly a population of 6.6 million were deceased as per World Health Organization (WHO)[1]. Individuals with Type-2 diabetic mellitus (T2DM) along with several other disease-causing factors are impacted adversely by the SARS-CoV-2 pandemic that develops SARS-CoV-2 complications[2]. Following the preceding complexities involving T2DM, metabolic syndrome, hypertension, and cardiovascular diseases the fatality rates could upsurge to 10%. The vulnerability of patients with T2DM might be improved by compromised adaptive/ innate/

immune responses to acquire infection due to the occurrence of metabolic inflammation[3].

The first mRNA vaccine is BNT162b2 mRNA (Pfizer–BioNTech vaccine) and it was approved in the United Kingdom through European Medicine Agency on November 02, 2020. BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) are two vaccines in India that have been approved for the Emergency Use Authorization, which was commenced on January 16, 2021[4]. AstraZeneca and Oxford University developed ChAdOx1-nCOV by chimpanzee adenovirus-vector that is recombinant replication-deficient from genetically engineered human embryonic kidney 293 cells that codes SARS-CoV-2 spike antigen. BBV152 is a ß-propiolactone whole virion vaccine that is inactivated and has an adjuvant formed by Toll-like receptor 7/8 and SARS-CoV-2 proteins. This was developed by Bharat Biotech through the Indian Council of Medical Research, Hyderabad[5,6].

Immunological alterations have occurred because of humoral responses to vaccination or by infections acquired. Moreover, when antibody-secreting cells (ASC) are revealed to the same antigen and they are capable of raising recall responses because of the generation of the long-lived memory B cells[7]. Once there is a failure in immune response production by means of circulating antibodies, recall responses conduct the memory B cells for generating new antibodies from the germinal centres [8].

Chronic inflammation provoked by obesity might mitigate the innate cytokine production, macrophage activation, and also reduces pro-inflammatory mechanisms as a result of antigenic exposure[9]. The presence of vaccine escape mechanisms and antiviral resistance among T2DM and/or obese individuals are modified by several obesogenic conditions. Similarly, B and T cell responses among T2DM patients who are obese were impaired by obesity[10]. Another consequence of an adverse hormonal environment occurs due to the dysregulation of immune responses. Typically, obese T2DM patients have weakened innate/adaptive immune responses because of the enhanced several pro-inflammatory cytokines/ chemokines production including IL-1b, TNF-á, IL-18, IFN-g, RANTES, IL-12, MCP-1, and IL-6. Actually, these bioactive inflammatory proteins are associated with the development of SARS-CoV-2 at higher risk[11,12].

Therefore, SARS-COV-2-specific antibody levels between T2DM and without T2DM as well as various metabolic causative factors such as obesity and hypertension and obesity were compared in this study.

MATERIAL AND METHODS

Study Cohort and Participants Recruitment

The Ethical Review Committee (2923/ IEC/2021) has been reviewed and approved by SRM Medical College Hospital and Research Centre, Kattankulathur, Chengalpattu. The participants should have at least completed three weeks following the SARS-CoV-2 vaccine's second dose. Diagnosing of T2DM was done based on self-reporting by the study participants. Inclusion criteria include the participants who had completed the SARS-CoV-2 vaccination course and participants between 18 to 65 years of age. Exclusion criteria include participants who are taking immunosuppressants, those having arthritis, those with autoimmune diseases, and those who had received the second dosage of SARS-CoV-2 vaccination recently. A patient information sheet and an informed consent sheet have been subsequently acquired. All the study participants have been given an authorized questionnaire and completely filled it out. A patient proforma was given to participants which contain gender, age, BMI, previous history of SARS-CoV-2 infection, and other illnesses (hypertension and diabetes).

Blood Sample - Collection and Processing

The venous blood sample was collected by an experienced phlebotomist under aseptic conditions. After the clot formation, the serum was separated after from the blood sample following centrifugation. Then, the serum sample was aliquoted and stored at -80°C.

Measuring serum SARS-CoV 2 total antibodies levels specific to SARS-CoV-2

Specific antibodies for SARS-CoV-2 in serum sample was identified by an Enzyme-linked immunosorbent assay (ELISA) kit (WANTAI SARS-CoV-2 Total Ab ELISA Kit, WANTAI SARS-CoV-2 Diagnostics, India) as per kit instructions. Estimation of total antibodies (IgG and IgM of SARS-CoV-2) from serum samples was

done by using an ELISA. The recombinant antigen of SARS-CoV-2 was already coated in polystyrene micro-well strips. The receptor-binding domain of the SARS-CoV-2 spike protein is an antigen that was employed here.

Addition of calibrators and samples

The appropriate wells were added with 50 µl of Positive calibrator, 50 µl of Negative calibrator, and 100 µl of specimen except for the blank.

Incubation

The plate was incubated at 37°C with the plate covered for 30 minutes.

Washing

Discard the plate cover after incubation. Each well was washed five times using diluted wash buffer and soaked for 30–60 seconds after every wash. After the last washing and tapping were done, the plates were kept on blotting paper to remove the remaining buffer.

Addition of HRP-Conjugate

The remaining wells were added with HRP-Conjugate (100 µl) except Blank.

Incubation

Incubated at 37°C for 30 minutes with a plate covered.

Washing

The plate cover was discarded when the incubation period was over. Each well was washed with diluted wash buffer five times. Soak the microwells for 30 to 60 seconds each time. Following the last wash cycle, the plate was placed on a blotting paper, and tapping was done to take away the leftover buffer**.**

Coloring

Chromogen Solution A (50 µl), and Chromogen Solution B (50 µl) were added to all well along with Blank and delicately stirred it was incubated at 37°C for 15 minutes without a light source. The results for SARS-CoV-2 antibody-positive specimens appeared in blue color between the chromogen solutions and HRP-conjugate in the Positive calibrator.

Stopping Reaction

Stop Solution (50 µl) was added into every well using a multichannel pipette. The positive calibrator displays an intense yellow hue that was exhibited by specimens showing positive for the SARS-CoV-2 antibody.

Calculating Absorbance

The Blank well was calibrated and the absorbance was read at 450.

Table 1. Anti-SARS-CoV-2 serological findings and Clinical Characteristics among individuals stratified by diabetic and non-diabetic status

Variables	Non-Diabetic (n=213)	Diabetic (n=141)	Overall (n=354)
Age (in years)			
18 - 60 years	162 (76%)	134 (95%)	296 (83.6%)
61-65	51 (24%)	7 (5%)	58 (16.4%)
Gender			
Female	108 (50.8%)	61 (43.2%)	169 (47.7%)
Male	105 (49.2%)	80 (56.8%)	185 (52.3%)
Hypertension			
Yes	36 (16.6%)	59 (41.8%)	95 (26.9%)
No	177 (83.4%)	82 (58.2%)	259 (73.1%)
BMI			
Less than 25	62 (29.1%)	25 (17.7%)	87 (24.5%)
Between 25 and 30	99 (46.4%)	65 (46.0%)	164 (46.3%)
Greater than 30	52 (24.5%)	51 (36.3%)	103 (29.2%)
Previous infection of SARS-CoV-2			
Yes	30 (14%)	43 (30.4%)	73 (20.6%)
No	183 (86%)	98 (69.6%)	281 (79.4%)
Total Antibody Titre (AU/ml)			
Mean (SD)	14.22 (4.06)	9 (3.66)	12.1 (4.66)
Median (min, max)	15.7 (0.7,22.4)	10 (0.3,19.8)	11.3 (0.3, 22.4)

RESULTS AND DISCUSSION

Population Factors

This recent study comprises 354 study participants, among them T2DM was present in 141 (39.8%) cases and 213 (60.2%) were non-diabetic patients. Of these, 185 (52.3%) were male and 169 (47.7%) were female. Out of 354 individuals, Hypertension was observed in 95 (26.1%) participants and 259 (73.1%) participants were normotensive. Among them, 296 (83.6%) were d" 60 years and 58 (16.4%) were between 61 to 65 years. Based on the BMI, less than 25 kg/m² (normal weight) was present in 87 (24.5%) individuals, 164 (46.3%) had a 25 and 30 kg/m2 (overweight) and higher BMI than 30 kg/m2 (obese) was observed in 103 (29.2%) individuals. SARS-CoV-2 infection and concomitant conditions were experienced among 73 (20.6%) of the 354 individuals with prior immunizations. Wherein, T2DM was seen in 141 (39.8%) individuals, hypertension was observed in 95 (26.9%), 25 (7.1%) cases had cardiac problems, and 25 (7.1%) study participants had lung illness (Table 1).

Total SARS-CoV-2 antibodies – individuals with or without type 2 diabetes

Based on the T2DM conditions, the study participants with T2DM demonstrated lower levels of SARS-CoV-2 total antibodies having an average of 5 AU/ml over those individuals without diabetes showing an average of 12 AU/ml. Thus, the study participants did not produce antibodies at higher rates in T2DM conditions compared to participants without T2DM (Figure 1).

Total SARS-CoV-2 antibodies - individuals with and without hypertension

Among the hypertensive patients, the total antibody levels of SARS-CoV2 substantially declined to show an average of 8 AU/ml as compared to those who were not having hypertension has an average of 14 AU/ml.

Total SARS-CoV-2 antibodies based on gender, age, and BMI

The present study demonstrates considerably varied circulating total SARS-CoV2 antibodies levels among males and females. Generally, females possess a higher SARS-CoV2 total antibody levels of 15AU/ml over males (13AU/ml). Results of BMI based on regression analysis of obesity status have not demonstrated any important statistical difference.

This research was performed on 354 adults who are diabetic and non-diabetics in order to evaluate the total antibody levels of SARS-CoV-2 succeeding two doses of BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) vaccination. Subsequently, diabetes status, gender, age, time after the second dosage, and hypertension and BMI

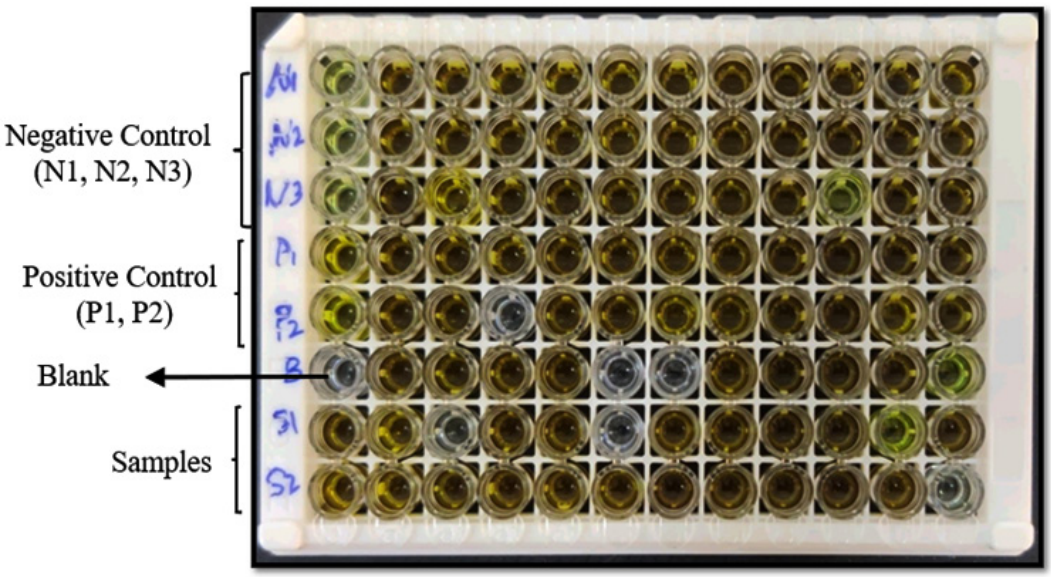

Fig. 1. Total SARS-CoV-2 antibodies test done by ELISA Plate method

were analyzed. The study findings suggest that SARS-CoV-2- total antibodies levels are at higher levels and they are nearly nine times greater than the seropositivity threshold which resulted from the vaccination. Also, this present work has essentially decreased total antibody levels of SARS-CoV-2 within T2DM participants as a result of various immune system defects such as macrophage/ monocyte impairment, functions of neutrophils, dysfunction of the complement system, decreased proliferation of lymphocyte and defects in antigen presentation as they stimulate the resistance for insulin and hyperglycemia.

The robust SARS-CoV-2- total antibodies levels among all the study participants showed efficient processing and presentation of antigens after the administration of two doses of BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) which are proposed in various cohorts on BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) vaccination studies[13,14,15]. The interaction of the ACE2 receptor with the spike (S1) protein of SARS-CoV-2 has been inhibited and they are noticed as positively associated with the experiments on the neutralization of virus strongly[14].

Furthermore, humoral immune response effectiveness to BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) vaccination against T2DM individuals has been studied. So, based on the intention, upraised SARS-CoV-2-levels total antibodies amidst T2DM individuals are certain. The present study also demonstrates that the humoral immune responses of diabetic and those who are non-diabetic are equivalent to SARS-CoV-2 infections depending on the time and antibody titers [15].

Hamad Ali conducted a study and resulted that however there is a lowered vaccine efficacy among those individuals with serious comorbidities such as T2DM, an equal potency has been observed beyond age even though humoral immune responses among the T2DM population are persistent in the study results. Also, humoral immunity in opposition to infections is insufficient among T2DM participants, they have been placed at great risk of abbreviating the infections again [16]. As a result of insufficient prognostic techniques and enhanced infection rate among the T2DM population, the administration of vaccination is needed at first. With respect to BMI, gender, and age among the participants, overall SARS-CoV-2 antibody levels did not show any noticeable differences[17]. As per the study conducted for 3 weeks after the second dose of vaccination on titers of antibody, we were able to recognize that age, BMI, and gender were considerably influenced by humoral immunity. Moreover, the discrepancies among BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) reported in the current study have influenced BMI, diabetic status, age hypertension, gender, and its comorbidities on immune responses might be associated with variations in various study[18].

Study results based on the discrepancies between BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) report the influence of hypertension, gender, age, BMI, diabetic status and its comorbidities on immune responses after the second dose may be related to variations in cohorts[19,20].

CONCLUSION

The present study results demonstrate that the participants with T2DM as well as without T2DM possess a strong total antibody response for SARS-CoV-2 infections, succeeding the two doses of vaccination involving BBV-152 (CovaxinTM) and ChAdOx1-nCOV (CovishieldTM) vaccines. However, titers of antibodies do not greatly influence gender, age, BMI, and hypertension. Yet, T2DM contains significantly lowered titers of antibodies when compared to non-diabetic patients. Therefore, in order to determine the necessity for precautionary doses in maintaining the immune responses of SARS-CoV-2 vaccines, it is always very substantial to notice that total antibody profile monitoring would be a practicable approach to benefit T2DM individuals.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Authors' Contribution

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Ethics Approval

This study was approved by the Institutional Ethics Committee, SRM Medical College Hospital and Research Centre, India with reference number 2923/IEC/2021.

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