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Humoral response after breakthrough SARS-CoV-2 infection in type 2 diabetes mellitus patients

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ABSTRACT

Type 2 diabetes mellitus (T2DM) remains an important public health problem in both developing and developed countries. In addition, the recent COVID-19 pandemic has revealed furthel risks for diabetes patients in terms of symptoms and disease progression. Higher mortality and morbidity are related to the complexity of the pathology of this chronic underlying disease, which negatively affects the immune response to the SARS-CoV-2 virus. The humoral response plays an important role in the eradication of the virus; thus, it was analyzed in vaccinated diabetics who underwent COVID-19, as well as in the control group. The aim of this study was to assess the prevalence and level of IgG antibodies raised against the nucleocapsid protein (NCP), S1 subunit receptor binding domain (RBD) and subunit Spike 2 (S2) subunit of the virus's S protein using the Microblot Array test. The results demonstrated significantly lower prevalence and titers of anti-SARS antibodies in diabetic patients compared to the control group. In addition, antibody titers were negatively related to the duration of this chronic disease, body mass index (BMI), comorbidities and HbA1c concentration. Further research is needed to develop the best strategy for specific prevention of SARS-CoV-2 infection in diabetic patients.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a very important global public health problem. According to the International Diabetes Federation (IDF), there are nearly 537 million people with diagnosed T2DM, and by the year 2045, this number will increase to 700 million [1]. In Poland, the prevalence of diabetes is estimated at 8%. By 2040, this number is projected to increase to 11%. Of note, 25-30% of all adults do not know they have diabetes [2].

DM is a complex and chronic disease that occurs either when the beta-cells of the pancreas do not produce enough insulin or when the body cells cannot effectively respond to the insulin hormone (insulin resistance) [3]. High blood glucose (hyperglycemia) is a systemic adverse condition that negatively affects the overall health of the patient. These patients require constant medical care and glycemic control. Many researchers observed a higher prevalence of infections among diabetic patients [4-6].

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The emergence of a new virus – SARS-CoV-2 causing serious illness COVID-19 has become very dangerous for diabetic patients. From the beginning of the pandemic to September 2023, 770,437,327 confirmed cases of COVID-19 and 6,956,900 deaths were reported to the World Health Organization (WHO) [7]. To date, 13,500,135,157 doses of vaccine, the most effective and available prophylactic measure, have been administered worldwide – so far.

T2DM patients are at higher risk for SARS infection than people without diabetes. Moreover, diabetic patients with COVID-19 who have also severe symptoms of the disease have a lower survival rate and a greater mortality rate compared to non-diabetic patients [8,9]. Indeed, diabetes was revealed as the fourth underlying chronic disease among hospitalized COVID-19 patients and the risk of death was 26% higher with diabetes, compared to other populations [10]. Diabetes is often associated with other risk factors, such as increased or decreased synthesis of receptors (ACE2) or discrete metabolites [11], hyperglycemia, hypertension, cardiovascular disease and obesity. In addition, the observed

diabetes caused by COVID-19 through direct damage to beta cells or increasing insulin resistance is a new threat and a serious consequence of this viral disease [12,13]. For these reasons and the risk of fatal outcome, vaccination against COVID-19 is particularly recommended [9,14].

Both innate (the first line of defense) and adaptive immunity (includes the T cells and B cells) play an important role in the progression of type 2 diabetes [15]. In diabetic and obese patients, changes in the proliferation of T lymphocytes and macrophages, as well as impaired function of natural killer cells and B lymphocytes have been described. Macrophages, besides phagocytosis, play a critical role in nonspecific defense and also help initiate specific defense mechanisms. Macrophages are activated by T lymphocytes, which play an essential role in promoting inflammation and insulin resistance by inducing proinflammatory cytokines [16]. Hyperglycemia decreases in immune cells Th1 and Th2 cytokines which are related to high mortality of COVID-19 in diabetic patients [17]. B lymphocytes play an essential role in the humoral response. The antibodies bind to the antigen, neutralizing the pathogen. Memory B cells provide a faster and stronger response to the same pathogen during reinfection, e.g. SARS-CoV-2 breakthrough infection [17].

The aim of this study was to analyze the humoral response in patients with T2DM vaccinated with two doses of the Pfizer vaccine, and, despite vaccination, underwent SARS-CoV-2 infection. The seroprevalence and the level of anti-SARS-CoV-2 IgG antibodies against the nucleocapsid protein (NCP), S1 subunit receptor binding domain (RBD) and subunit Spike 2 (S2) subunit of the virus's S protein were evaluated. The prevalence and the level of antibodies were then compared with the control group (non-diabetic, vaccinated individuals, infected and recovered from COVID-19). The level of antibodies was analyzed depending on sex, age, duration of diabetes, body mass index (BMI), HbA1c level and comorbidities.

MATERIALS AND METHODS

Study Design

Patients with T2DM treated in the outpatient clinic of the Masovian Specialist Hospital in Radom, Poland were enrolled in the study. The qualifying criterion for the study was a history of a mild course of COVID-19 that did not require hospitalization, as well as vaccination with two doses of the Pfizer vaccine. The COVID-19 infection was confirmed by a documented positive RT-PCR test using a nasopharyngeal swab.

Subjects who became infected within 3 months of the second dose of the vaccine were selected for the study. All information on comorbidities, duration of diabetes, HbA1c level and BMI were obtained from the patients' medical history. The results were compared with a control group – 94 individuals from the outpatient clinic in whom diabetes was excluded (HbA1C < 6.0%). The control group was matched by age and sex.

The same criteria applied to people in the control group. All participants answered a questionnaire with demographic, epidemiological, and clinical information regarding previous exposure to COVID-19. The blood for testing the level of specific antibodies was collected from all participants 1 month following the infection.

Sample Collection

Venous blood samples (3-5 mL) from all subjects were centrifuged at 1500 rpm/15 min at room temperature, serum samples were packed in plastic bags and transported in ice to the laboratory. These were then stored at -20°C until analysis.

Detection of SARS-CoV-2 antibody

The serum samples from all individuals were tested using the commercially available Microblot Array COVID-19 IgG assay (TestLine Clinical Diagnostics, Brno, Czech Republic; CE IVD) according to the manufacturer's instructions. The results are given in units of U/mL. The interpretation takes into account the presence or absence of a reaction against at least 1 antigen – NCP, RBD, or S2. Reading and interpretation were performed using Microblot Array reader and software: <185 U/mL = negative, 185-210 U/mL = borderline, >210 U/mL = positive.

Statistical Analysis

The data were analyzed via Graph Pad Prism software version 10.0.2. (San Diego, USA). Pearson's chi-square test was applied to investigate categorical variables. The Shapiro–Wilk test was used to test the normality of continuous variables. Continuous variables were expressed as median (minimum and maximum). The Mann–Whitney U-test was employed to compare the level of antibodies in diabetic patients and the control group and the Kruskal-Wallis Test to analyze the level of antibodies by BMI. Statistical significance was defined as $p < 0.05$.

Ethics

The research was approved by the Medical University of Lublin Ethics Committee and is in accordance with the GCP regulations (no. KE-0254/121/2021, 27 May 2021). Written informed consent was obtained from each participant.

RESULTS

Characteristics of the studied population

A total of 96 patients with diabetes were enrolled in the study. The control group consisted of 94 individuals without a history of diabetes (Table 1). Two age groups were selected among both tested groups: 40-59 ($\overline{x}$ = 51.7) and ≥60 ($\overline{x}$ = 69.1).

Prevalence of NCP, RBD and S2 antibodies in studied groups

The prevalence of particular types of antibodies was similar in the study and the control group. However, there was a trend towards a slightly lower prevalence of antibodies in diabetic patients. This difference is particularly noticeable in the case of S2 antibodies, which were more frequently detected in non-diabetic females (Table 2).

Table 1. Characteristics of diabetic patients

		Diabetic patients N=96	
		N	%
Sex	Male	49	51.0
	Female	47	49.0
Age	40-59	48	50.0
	60-75	48	50.0
Duration of diabetes (years)	5-9	45	46.9
	≥10	51	53.1
Comorbidities	Yes	51	53.1
	No	45	46.9
HbA1c	7.0±0.5	52	54.2
	8.5±0.5	44	45.8
BMI	18.5-24.9	34	35.4
	25.0-29.9	32	33.3
	30.0-39.9	30	31.3

Table 2. Prevalence of NCP, RBD and S2 antibodies in patients with and without of diabetes

Participants' group		NCP		RBD		S2	
		Female	Male	Female	Male	Female	Male
Diabetic Patients	N	40	46	46	47	38	43
	%	85.1	93.9	97.9	95.9	80.9	87.8
Control Group	N	48	42	48	42	46	44
	%	98.0	93.3	98.0	93.3	93.9	97.8
p-Value		0.6597	0.9999	0.9999	0.9999	0.6552	0.3718

Pearson Chi² Test

The serum level of NCP, RBD and S2 antibody in diabetic patients compared to the control group

Statistically significant lower levels of all types of antibodies were observed in diabetic patients compared to the control group (both in women and men) (Table 3). This applied to both age groups and sex.

The relationship between the serum level of NCP, RBD and S2 antibody and duration of diabetes, BMI, comorbidities and HbA1c concentration in diabetic patients

The analysis showed that the duration of diabetes has a significant impact on the level of tested antibodies (Figure 1a). The level of NCP, RBD and S2 antibodies was statistically significantly lower in people suffering from diabetes for ≥10 years ($p < 0.0001$). A similar relationship was observed between antibody levels and BMI (Figure 1b). Patients with normal BMI developed high antibody titers. In obese patients, the titer of all types of antibodies was almost two times lower ($p < 0.0001$).

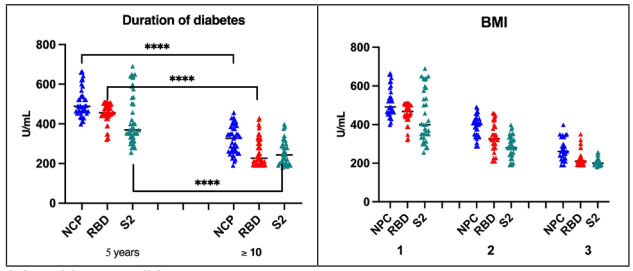
In the study group, comorbidities occurred in 53.1% of the total studied population. Cardiovascular disease and hypertension were the most common. Because these subgroups were not very numerous, they were analyzed together as patients with or without comorbidities (Figure 2a). The level of antibodies in the serum of patients without comorbidities was high. In contrast, patients with comorbidities developed antibodies at a much lower titer.

Table 3. The level of NCP, RBD and S2 antibodies (U/mL) in diabetic patients and controls by sex and age

	NCP	RBD	S2
Female			
Diabetic patients	416.9 (200.5-642.6)	343.1 (190.5-497.4)	308.5 (190.6-590.5)
Controls	604.1 (350.7-989.3)	651.7 (240.9-972.4)	605.0 (200.1-995.5)
p-Value	0.0001*	0.0001*	0.0001*
Male			
Diabetic patients	385.9 (190.1-658.9)	330.8 (190.1-512.1)	328.7 (180.5-650.6)
Controls	564.8 (300.9-865.8)	637.0 (210.1-962.8)	551.1 (190.7-959.3)
p-Value	0.0001*	0.0001*	0.0001*
Age			
40-59			
Diabetic patients	436.5 (254.3-658.9)	422.6 (192.9-512.1)	348.3 (195.7-650.6)
Controls	550.3 (300.9-989.3)	762.5 (210.1-972.4)	525.1 (190.7-995.5)
p-Value	0.0004*	0.0001*	0.0049*
≥60			
Diabetic patients	355.9 (190.1-542.3)	285.4 (190.1-480.1)	249.5 (180.5-360.8)
Controls	541.1 (350.7-734.8)	500.5 (210.9-962.8)	589.9 (200.1-854.2)
p-Value	0.0001*	0.0001*	0.0001*

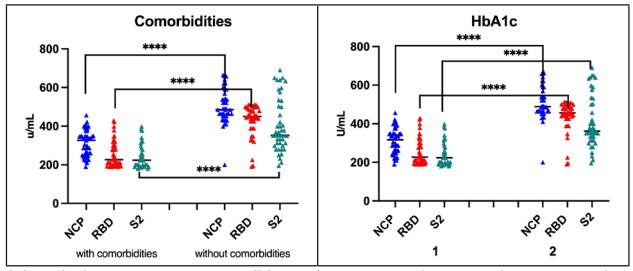
Mann-Whitney U Test (median, min-max)

The antibody level depended also on the HbA1c concentration (Figure 2b). In patients with well-controlled glycemia, the level of antibodies was high, similar to the control group. In contrast, patients with higher HbA1c levels had lower levels of all types of antibodies. This difference was statistically significant ($p < 0.0001$).



(a) and by BMI; (b) BMI: 1 – 18.5-24.9; 2 - 25.0-29.9; 3 - 30.0-39.9; Duration of diabetes – Mann-Whitney U Test; BMI – Kruskal-Wallis Test – p = 0.0001; ****p = 0.0001

Figure 1. The level of NCP, RBD and S2 antibodies among diabetic patients by duration of diabetes



(a) and HbA1c concentration; (b); 1-HbA1c – 8.5+/-0.5; 2-HbA1c – 7.0+/-0.5. Mann-Whitney U Test, ****p=0.0001

Figure 2. The level of NCP, RBD and S2 antibodies in diabetic patients by comorbidities

DISCUSSION

T2DM patients have an increased risk of significant morbidity and mortality from infections and sepsis [18]. Due to

the fact that they are a high-risk group for severe COVID and even death, vaccination is especially recommended for this group of patients.

Many researchers have assessed the effectiveness of preventive vaccinations in various groups of patients, including those with diabetes. Other researchers evaluated the dynamics of antibody levels after vaccination [19]. Systematic reviews of available studies including various types of vaccines, from various countries, underline the risk of limited effectiveness among diabetic patients, including the risk of breakthrough infection [20]. The observations of Rego *et al.* [21] suggest that the presence of IgG antibodies against SARS-CoV-2 spike RBD, the main target of vaccines, is an important survival indicator in both diabetic and non-diabetic individuals.

We analyzed both the prevalence and titers of antibodies against three antigens: NCP, RBD and S2. The results revealed in this study are consistent with the observations of other authors. Our study confirms a lower prevalence and titer of anti-SARS antibodies in diabetic patients compared to the control group. Similar results were also obtained by Ali *et al.* [22], who examined the level of SARS-CoV-2 IgG antibodies and neutralizing antibodies in a group of people with diabetes. They showed that diabetic patients had lower levels of antibodies compared to non-diabetic people.

Other researchers, as in our study, analyzed the level of neutralizing antibodies in patients depending on age [23]. It has already been well documented that older individuals, men and smoking people had significantly lower antibody titers [24].

Our research is distinguished by the fact that both diabetic patients and people from the control group we studied suffered from mild COVID-19 disease despite vaccination, thus acquiring hybrid immunity. We therefore compared these results to a similar group of people without diabetes to determine whether the vaccine was indeed less effective in people with diabetes. It would seem that hybrid immunity should be stronger. However, this assumption has not been confirmed. Despite the breakthrough of the infection, the level of all types of antibodies was significantly lower in diabetics.

Current literature states that Pfizer's vaccine (BNT162b2) elicits a strong and rapid humoral response and is especially important for immunocompromised people who may be at higher risk of breakthrough infections [25].

Our analysis, however, pointed to lower titer of NCP, RBD and S2 antibodies in diabetic patients depending on age. Indeed, patients in the 60 and over group had lower levels of each type of antibody compared to those in the 40-59 age group. This may be related to the better immune status of younger diabetic patients. The weaker antibody response among elderly diabetic residents of long-term care facilities has also been noted by others [26].

All three types of antibodies are elicited during SARS-CoV-2 infection, while vaccinated persons are assumed to have only antibodies against the immunogenic component of the vaccine [27]. Most COVID-19 vaccines are based on the Spike glycoprotein (antigens: RBD, S2). Antibodies against NCP seem to be very helpful in distinguishing natural immunity from that obtained by vaccination [27]. Heinz [27] described people who were vaccinated with BNT162b2 (Pfizer) and then went through and recovered from COVID-19, producing a full spectrum of antibodies.

Analyzing the level of antibodies by age, it was shown that the titers of all antibody types were comparable but lower in diabetic patients. There were no significant differences in the titer of all tested types of antibodies depending on the sex of diabetic patients.

In the next step, we examined whether the duration of diabetes and BMI affected the level of antibodies. We observed that NCP, RBD and S2 antibody titers were associated with the duration of this chronic disease. In people with diabetes lasting more than 10 years, the antibody titer was significantly lower compared to people with diabetes that lasted for 5-9 years. In epidemiological studies in Scotland, a relationship was noted between the duration of diabetes and the cumulative risk of death and COVID-19 treated in an intensive care unit [8]. The results of this study provide data supporting the relationship between antibody titers and duration of diabetes. Interestingly, other studies did not reveal such a relationship [23].

Obesity is a major risk factor for type 2 diabetes. It is estimated that 53% of all adults with diabetes are obese [28]. Many researchers have shown that patients with BMI>30 respond poorly to vaccination [29].

The results obtained in this study indicate a worse immune response in patients with BMI>30. Obese diabetic patients have reduced titers of specific anti-SARS IgG antibodies. Frasca *et al*. (2021) [30] studying the effect of obesity on the level of SARS-CoV-2 specific antibodies in the serum of COVID-19 patients, showed a similar relationship.

Patients with diabetes very often have comorbidities. In the scientific medical literature, data on the incidence and titer of antibodies against SARS-CoV-2 in vaccinated patients with comorbidities are limited. According to released data, arterial hypertension and cardiovascular disease occur in a significant part of the Polish population. Due to the fact that in our study, individual groups of patients were small, all comorbidity data was pooled and analyzed together. We observed that patients with comorbidities developed antibodies at a lower level compared to those without comorbidities. According to a systematic review conducted by Notarte *et al.* [31], people with more comorbidities have a reduced humoral immune response after vaccination against COVID-19. In turn, the research of Soegiarto *et al.* demonstrated the association of hypertension with lower antibody titers and breakthrough SARS infection [32].

Next, we analyzed the effects of glycemic control on the levels of all three types of antibodies. Whether hyperglycemia modulates the humoral response to a virus infection is still a subject of discussion [33]. Several defects in immunity have been associated with hyperglycemia and insulin resistance, including reduced lymphocyte proliferative response, impaired macrophage and neutrophil function, abnormal delayed-type hypersensitivity reaction, and complement activation dysfunction [16,18].

As research by Marfella *et al.* [34] showed, patients with good glycemic control (HbA1c<7%) have higher levels

of neutralizing antibodies and a better CD4+T/cytokine response than patients with poor glycemic control (HbA1c ≥7%). Related observations were reported by Cheng *et al.* [35]. Our results are similar to the above and indicate that the level of SARS IgG antibodies in the serum of well-controlled glycemia was high, as in the control group. In contrast, patients with higher HbA1c levels had significantly lower titers of all types of antibodies. Different results were obtained by Sourij *et al.* [23], who showed a similar level of antibodies against the SARS-CoV-2 S (RBD) receptor binding domain after the second vaccination in patients with diabetes and in the control group.

The control of hyperglycemia and management of glucose concentration remains the most important instrument for maintaining the health and immune status of diabetic patients [36]. Some researchers admit that well-controlled diabetes allows for an immune response against COVID-19 comparable to patients without diabetes [18]. In contrast, many infectious diseases are more prevalent among T2DM patients [4] and improved vaccination strategies are strongly recommended for them [5,18].

The presented results confirm that vaccination plays an important role in the development of adequate antibody levels, hence ensuring effective protection against the severe clinical course of the disease and/or the spread of the virus also in diabetic patients. All factors discussed here have some influence on vaccination effectiveness. Other researchers also emphasize that Type 2 diabetes is a chronic disease leading to dysregulation of the immune response [37].

A limitation of our study was too small a group of patients, especially in relation to comorbidities. Therefore, we were unable to assess the impact of other diseases on humoral immunity. In addition, the limited period of follow-up of patients made it impossible to assess changes in the dynamics of prevalence and antibody levels of patients over time, that is, how long antibodies were detectable and at what titer. We analyzed only the humoral response. In the future, it would be worth evaluating the cellular response, which may be the subject of further research.

It seems that further doses of the vaccine are recommended [38]. More research is needed to determine the best prophylaxis regimen for immunocompromised patients. This is particularly important due to the rapid spread of the new subvariant of the SARS-CoV-2 Omicron variant [8]. The vaccines available so far may not provide adequate protection against infection, which is most likely related to subsequent mutations and increased affinity for the ACE2 receptor. Moreover, further research on the role of innate and adaptive immunity may contribute to the immunotherapy of inflammation and insulin resistance.

CONCLUSIONS

These studies showed both a lower prevalence of anti-SARS antibodies and a lower level of antibodies in diabetic patients than in the control group. The level of antibodies also depended on the duration of diabetes. It was significantly lower in patients who had been ill for 10 years or more and also in obese patients. What is more, antibody levels was found to depend on HbA1c concentration and the presence of additional chronic diseases. Further research is, therefore, needed to develop the best strategy for the specific prevention of SARS-CoV-2 infection in diabetic patients.

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