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## ORIGINAL ARTICLE

# Seroprevalence of IgM and IgG in Serum of COVID-19 Diabetic Patients After Recovery

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**SUMMARY**

***Background:*** **Coronavirus disease (Covid-19) is an infection caused by SARS-CoV-2. Patients experience several symptoms in the respiratory tract following infection. Developing an immunity is essential to protect the host from future infection. In this study we will investigate the seropositivity of IgM and IgG in recovered COVID-19 diabetic patients compared to prediabetic, and non-diabetic.**
***Methods:*** **Three hundred and eighty-four recovered COVID-19 patients were enrolled in this study and subdivided according to their glycemic status, 156 diabetic, 77 prediabetic, and 151 non-diabetic included. Viva-DiagTM COVID-19 IgM/IgG Rapid Test was used to detect the IgM and IgG in the serum of the study group.**
***Results:*** **Seroprevalence of IgM and IgG was detected in the study group, IgM seroprevalence was 84% of diabetic, 60% of prediabetic, and 92% of non-diabetic. IgG seroprevalence was 93% of diabetic, 62% of prediabetic, and 87% of non-diabetic study group. HbA1c was positively correlated with both immunoglobins indicating capability of producing one or both immunoglobins even with high HbA1c. After an additional 40 days, non-diabetic participants have double the positive immunoglobins compared to the other groups indicating optimal vaccination time for those groups is less than 50 days following recovery.**
***Conclusions:*** **Glycemic status has not affected the seroprevalence of IgM or IgG. The optimal vaccination time for diabetic patients is 40 days after the recovery from Covid-19.**
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**KEY WORDS**

COVID-19, IgG, IgM, SARS-CoV-2, diabetes

## INTRODUCTION

Most of the countries in the world have been impacted since the emergence of the highly transmissible Sever-Acute-Respiratory-Syndrome Coronavirus 2 (SARS-CoV-2) in 2019. The health services around the world were highly impacted and suffered more as many mutations have occurred in the virus since the emerging in Wuhan-Hu1 [1-3]. The virus has spread to cause more than 143 million cases with mortality rate increasing globally [4,5]. Countries have followed several policies against Covid-19 spread with vaccinations being the best strategy. Still, natural immunity was the subject of debates whether it has better protection than artificially induced immunity. The World Health Organization

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(WHO) has recommended the application of molecular testing like PCR for suspected Covid-19 cases. Many reliable antibody immunoassays have been distributed for Covid-19 diagnosis; an advantage of these methods is the quick diagnosis of suspected cases, in an average of 20 minutes.

Immunoglobins are produced by the immune system in Covid-19 patients in 1 to 14 days, IgM was detected by immunoassays techniques in 3 to 5 days, and IgG titer was four times higher in acute cases [6]. Moreover, IgG avidity and neutralization potential were confirmed after 8 months of recovery even with old age [7]. Other studies have found that the optimal for detecting these immunoglobulins is 10 days following onset of disease and up to 8 weeks after the infection of SARS-COV-2 [8,9]. Later studies used magnetic chemiluminescent immunoassay tests and have shown that IgG starts to increase a week after the peak of IgM, and IgG peaks between three to five weeks while IgM peaks in two to three weeks [10].

In the current study, the aim was to explore the presence of IgM and IgG in three groups of confirmed patients after their recovery.

## MATERIALS AND METHODS

### Study group

Three hundred and eighty-four participants were included in this study. All the patients were from Taif city and diagnosed with Covid-19 between August 2020 to January 2021 and had not yet received the vaccine. The target patients were those who were recovered at least a month and a maximum of 53 days. Patients' results were confirmed by RT-PCR in Saudi Arabia and COVID-19 positive patients were requested to isolate for 14 days. The study group is divided according to their glycemic status by the levels of HbA1c into diabetic group (HbA1c > 6.5%), pre-diabetic (HbA1c 6% to 6.4%), and non-diabetic (HbA1c < 6%). The sera of 8 participants who were not infected of SARS-COV2 previously and unvaccinated were used as a control for this rapid test and no false-positive results were shown. Clinical presentation of the patients including fever, loss of taste, diarrhea, nausea, and chest pain were included in this study. This study was approved by the directorate of health affairs in Taif IRB number HAP-02-T-067.

### Anti-SARS-CoV-2 immunoassay

VivaDiagTM COVID-19 IgM/IgG Rapid Test was used for qualitative detection and differentiation of IgM and IgG. The test protocol is available at the manufacturer's website:
(https://www.vivachek.com/en/prods/prod-rapidtest.html).

This test has been validated in Saudi Arabia, the specificity is 100% and sensitivity is 97% according to the manufacturer, and according to an article published in Nature Biotechnology, VivaDiagTM has detected IgM and IgG in 9 out 10 cases after more than 20 days following Covid-19 diagnoses [11]. The test is an *in vitro* diagnostic tool to detect anti-SARS-CoV-2 antibody in patient whole blood, serum, or plasma. The results appear in 15 minutes and requires only 10 µL of serum. The test can be performed at room temperature, steps of the test start by applying the sample, followed by 2 drops of buffer, and the reading is recorded in a maximum of 20 minutes. The conjugated pad is labeled with recombinant SARS-CoV-2 antigen, and IgM or IgG well will bind to the viral antigen showing a purplish red color. Invalid results are detected in the absence of the control line. During sample collection periods, patients' blood was collected in yellow top anticoagulant-free tubes and centrifuged at 1,500 rpm for 5 minutes, then serum was inactivated in a 56°C water bath for 50 minutes, and stored at -20°C.

### Statistical analysis

Pearson's correlation coefficients were calculated by GNU PSPP (1.2.0-g0fb4db) to determine the degree of correlation between independent variables, IgG, or IgM results. Our data were evaluated by PSPP for normality test, and it has shown that our data do not follow normal distribution. Excel for Microsoft office 365 was used to prepare the bar charts. JASP version 0.14.1 was used to calculate odds ratio, confidence interval, and logistic regression which was applied to predict the effect of HbA1c levels on IgM and IgG in our study group.

## RESULTS

### Demographic of enrolled patients

Three hundred and eighty-four patients were included in the present study, including 156 diabetic, 77 pre-diabetic, and 151 non-diabetic patients. The majority were males 64% and 36% were females. Age of patients was between 17 to 98 years with median 52 years (Table 1).

### Clinical presentations and symptoms

Diarrhea was common among diabetic patients, followed by nausea, loss of taste, fever, and chest pain. Chest pain was common in prediabetic patients, followed by fever and loss of taste, diarrhea, and nausea. Nausea was common in non-diabetic patients, followed by chest pain, fever, loss of taste, and diarrhea (Table 2).

### Detecting of IgM and IgG anti-SARS-CoV-2 by immunoassay according to age

Patients with IgM+/IgG+ were significantly highly detected in the < 20 years group, followed by 20 - 34, 35 - 50, 51 - 79, and lastly ≥ 80 (p-value 0.022). IgM was highly detected in the 51 - 79 years group, followed by 20 - 34, 35 - 50, < 20, and lastly ≥ 80. IgG was highly detected in the 20 - 34 years group, followed by 51 - 79, 35 - 50, < 20, and lastly > 80 (Figure 1).

**Table 1. Demographic and clinical characteristics of all patients.**

| Characteristic | | Diabetic | Prediabetic | Non-diabetic | Total |
|---|---|---|---|---|---|
| Age (median) | | 18 to 98 (65) | 28 to 85 (58) | 17 to 98 (45) | 17 to 98 (52) |
| HbA1c (mean, median) | | 9.05, 8.2 | 6.17, 6.1 | 5.36, 5.5 | 7.5, 6.2 |
| Male | n (%) | 102 (41.46) | 50 (20.32) | 94 (38.21) | 246 (64) |
| Female | n (%) | 54 (39.31) | 27 (19.56) | 57 (41.3) | 138 (36) |
| Total | n (%) | 156 (40.6) | 77 (20.02) | 151 (39.32) | 384 |

**n - number of patients.**

**Table 2. Clinical presentations of all patients according to diabetic status.**

| Characteristic | | Diabetic | Prediabetic | Non-diabetic | Total | p-value |
|---|---|---|---|---|---|---|
| Fever | n (%) | 25 (30) | 40 (48) | 18 (22) | 83 | 0.001 |
| Loss of taste | n (%) | 25 (30) | 40 (48) | 18 (22) | 83 | 0.001 |
| Diarrhea | n (%) | 150 (71) | 36 (17) | 24 (12) | 210 | 0.001 |
| Nausea | n (%) | 156 (46) | 50 (15) | 127 (39) | 333 | 0.001 |
| Chest pain | n (%) | 22 (21) | 56 (54) | 24 (25) | 102 | 0.001 |

**n - number of patients, p-value calculated by Pearson's chi-squared test.**

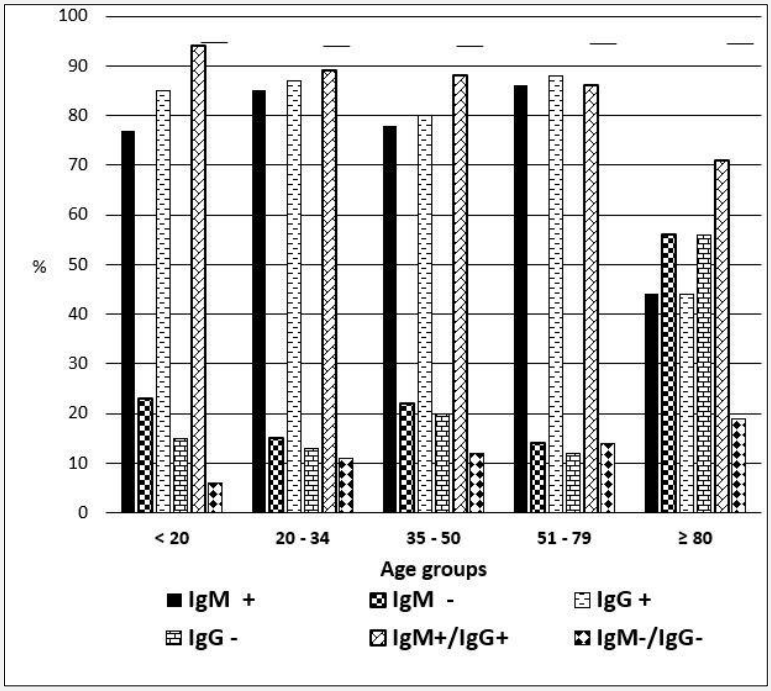


**Figure 1. Rapid detection of IgM and IgG Immunoassay of all patients according to age groups.**

**p-value calculated by Pearson's chi-squared test.**

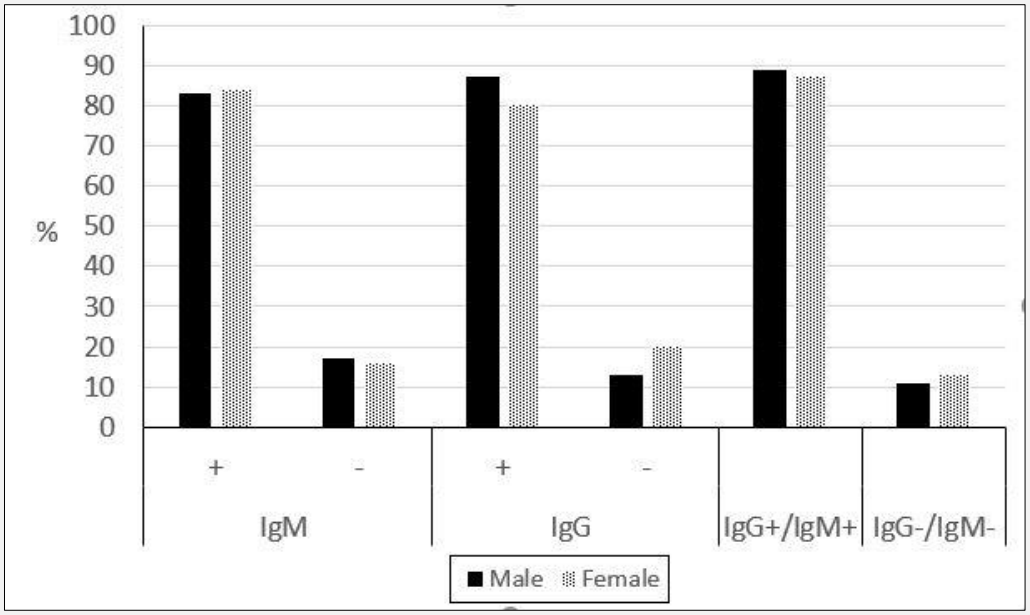


**Figure 2. Rapid detection of IgM and IgG Immunoassay of all patients according to gender.**

**p-value calculated by Pearson's chi-squared test.**

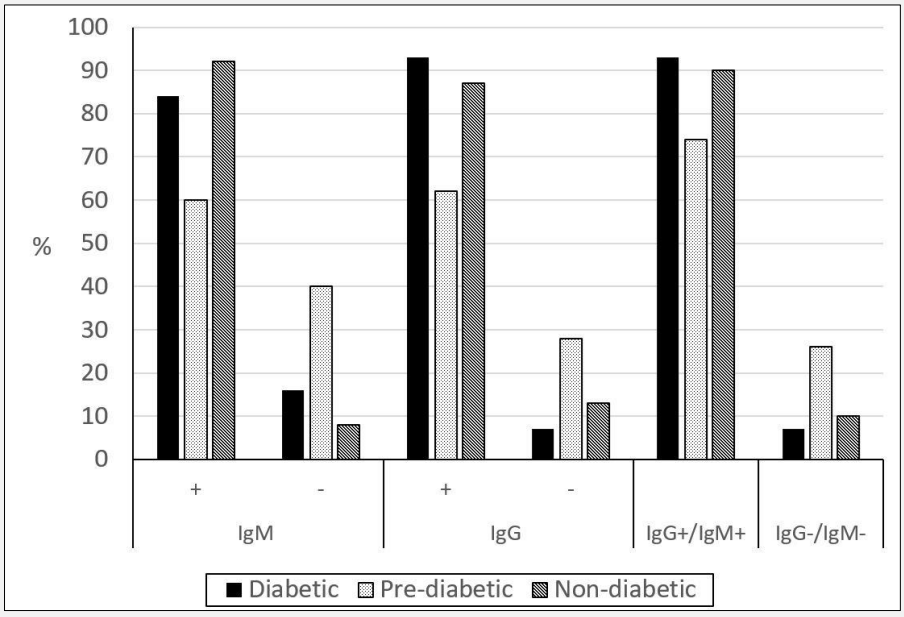


**Figure 3. Rapid detection of IgM and IgG Immunoassay of all patients according to diabetic status, all the findings were statically significant < 0.001.**

**p-value calculated by Pearson's chi-squared test.**

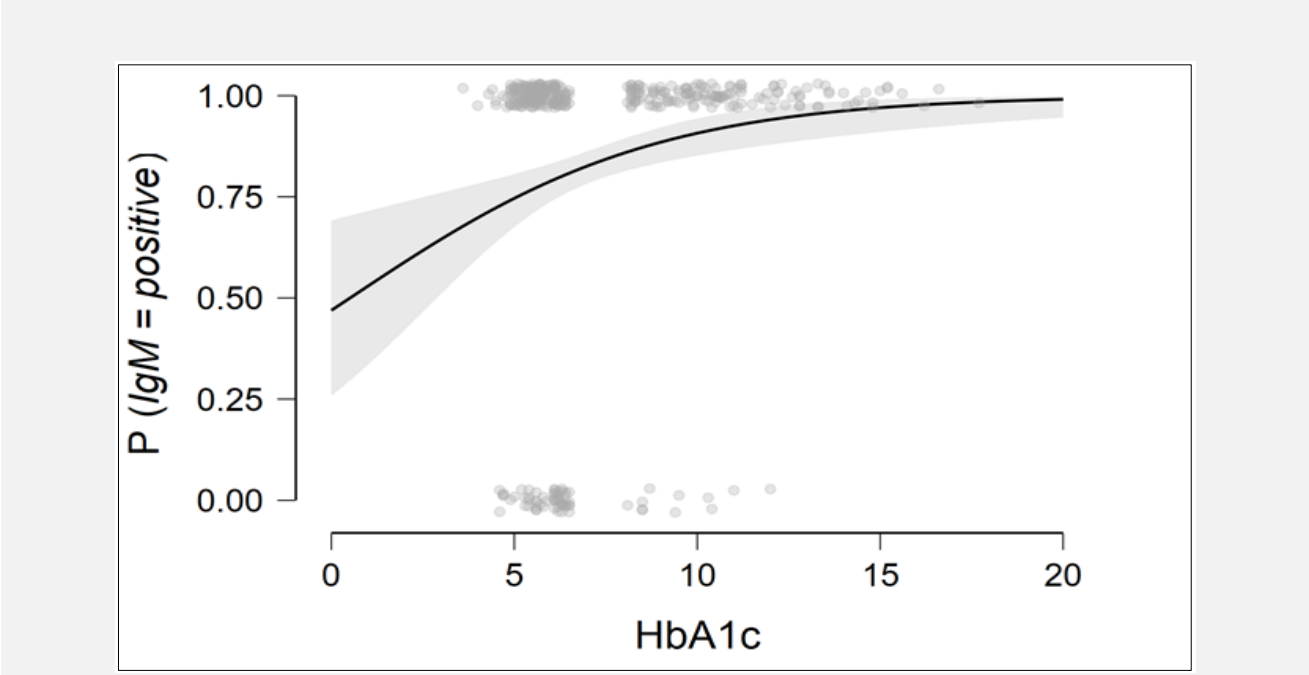


**Figure 4. Scatter plot between IgM seroprevalence and HbA1c, r = 0.045, p-value = < 0.001, OR = 1.272, 95% CI (0.105 - 0.375).**

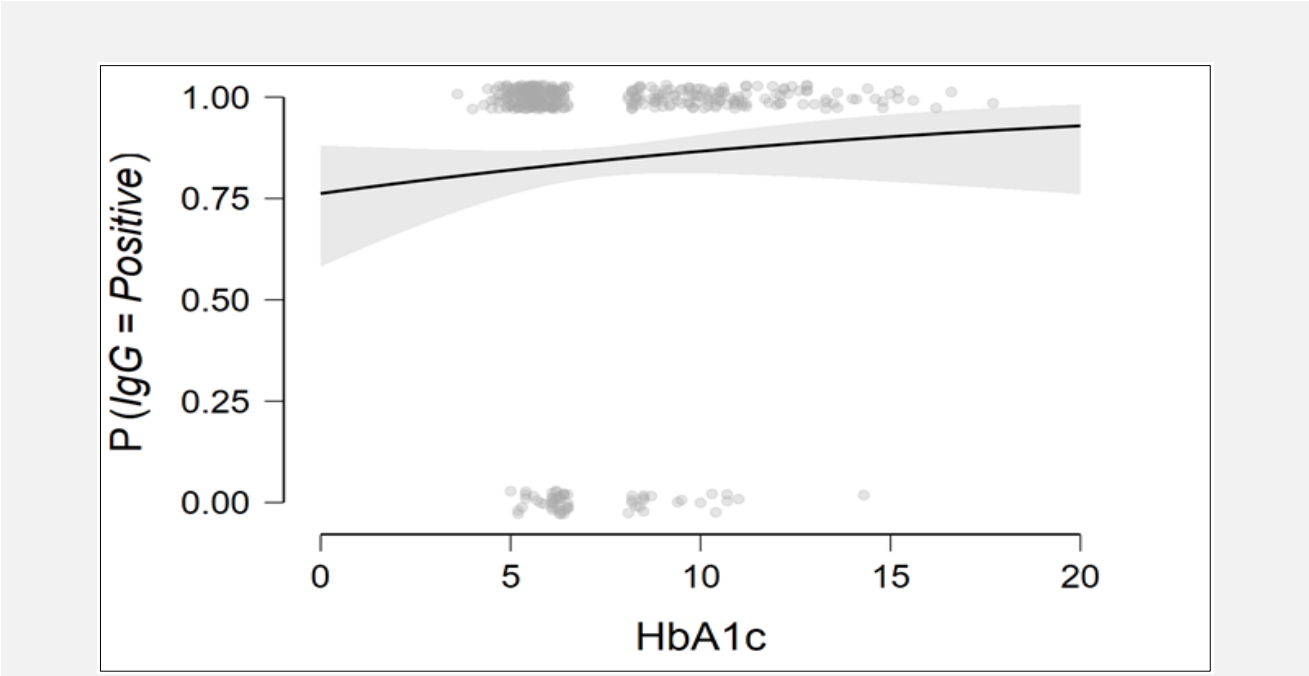


**Figure 5. Scatter plot between IgG seroprevalence and HbA1c, r = 0.005, p-value = 0.191, OR = 1.267, 95% CI (-0.039 - 0.179).**

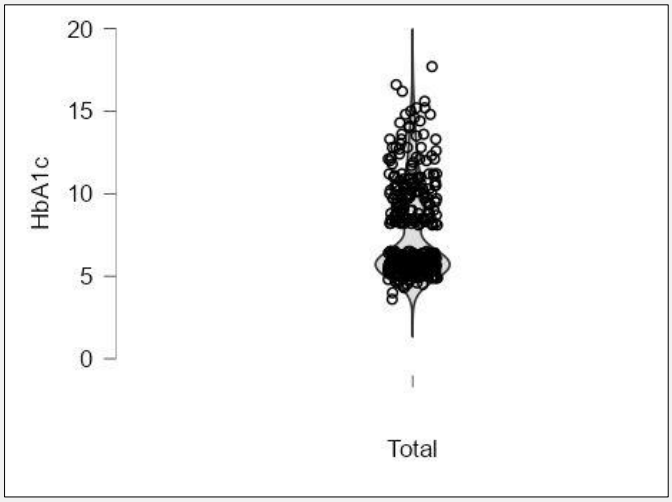


**Figure 6. Boxplot with jitter elements to correlate between HbA1c and IgM+ with IgG+ indicating seropositivity of both immunoglobins even with highest and lowest levels of HbA1c.**

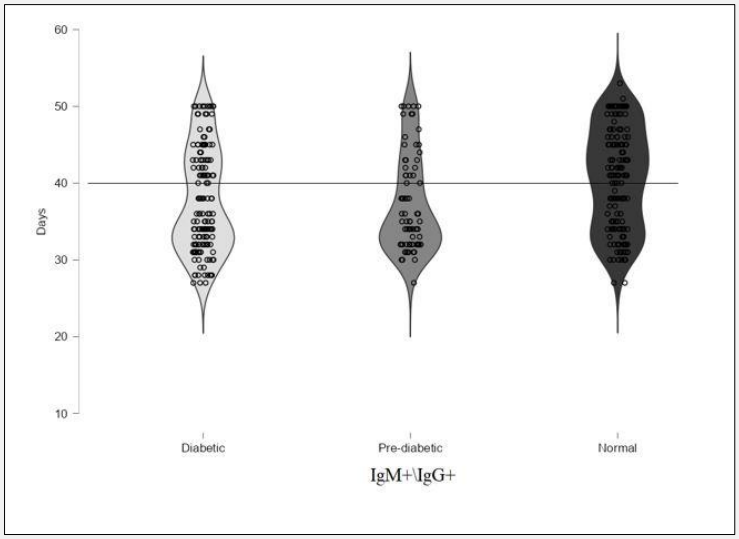


**Figure 7. Boxplot with jitter elements to correlate between days of recovery, HbA1c, and IgM+ with IgG+.**

**p-value calculated by Pearson's chi-squared test = 0.041.**

**Detecting of IgM and IgG anti-SARS-CoV-2 by immunoassay according to gender**

IgM was highly detected in male patients (83%), followed by female patients (84%). IgG was highly detected in male (87%) patients, followed by female patients (80%). Patients with IgM and IgG seropositive was highly detected in male patients (89%), followed by female patients (87%) (Figure 2).

**Detecting of IgM and IgG anti-SARS-CoV-2 by immunoassay according to diabetic status**

According to our findings presented in Figure 3, IgM seroprevalence was detected in 92% of the non-diabetic group, 84% of diabetic group, and 60% of the pre-diabetic group. IgG seroprevalence was detected in 93% of diabetic patients, 87% of non-diabetic, and 62% of pre-diabetic group. IgG+/IgM+ was detected in 93% of the diabetic, 90% of non-diabetic, and 74% of the pre-diabetic group.

Logistic regression was applied to predict the effect of HbA1c levels on seroprevalence of IgM and IgG (Figure 4, 5) which have shown positive correlation between HbA1c and IgM or IgG. Probability of seropositive status of IgM and IgG have not changed even with high and low levels of HbA1c, this was clearly illustrated in Figure 6.

**Seroprevalence of IgM and IgG anti-SARS-CoV-2 according to days of recovery**

The number of days following recovery were correlated with the diabetic status and the seropositivity of IgM and IgG (Figure 7). Our results indicated more non-diabetic patients have seropositivity of both immunoglobulins than the other groups of patients after 40 to 53 days of recovery.

## DISCUSSION

Many pandemics have occurred by viral infection of the respiratory tract. Countries have endured great losses during these pandemics due to their rapid transmission. This study is the first in the field to evaluate the seroprevalence of IgM and IgG immunoglobins in Covid-19 patients following their recovery, and one of the reasons to conduct this study was due to the high mortality rate among diabetic Covid-19 patients and the risk of development of Covid-19-pneumonia. In the current study group, we have focused on comparing diabetic, prediabetic, and non-diabetic patients following recovery, and confirmed negative molecular testing of SARS-COV-2 which is processed by government in several centers around the city. The results are shown on a mobile application called Tawakkalna that permits the person to move freely. Retaining seropositivity of IgM and IgG supports the ability of virus neutralization which was established by a preprint work regarding the neutralization capacity among recovered patients [12]. However, this work has not been peer-reviewed yet. Moreover, naturally acquired immunity was always a controversial topic in regards to inducing better immunity and resistance than vaccine or artificial induced immunity. IgM and IgG seropositivity also can induce the immune response and increase the immune cells such as NK cells and CD4+ T-cells in response to this virus [13]. Samples were collected from our study group after a minimum of 27 days after recovery and a maximum of 53 days, as seroconversion and class switching to IgG may take varying lengths of time between the patients [8] and also due to the possibility of false-negative results that may arise due to the decline of immunoglobulins titer following recovery to undetectable amounts. In our study only a small number of patients have shown IgM-/IgG-results, three random samples from each group were tested again which also showed the same results.

Our study group were mostly males 64% and female 36%, age range between 17 to 98. Fever, chest pain, and loss of taste was common among prediabetic, while diarrhea and nausea were common in the diabetic patients. There were no statically significant results among age groups, except between IgM+/IgG+ compared to IgG-/IgM-groups which was in favor of IgM+/IgG+. Prevalence of seropositivity of IgM+, IgG+, and IgM+/IgG+ was common and statistically significant. Regarding gender, more male participants were IgG+ and IgM+/IgG+ than females, while females were higher in IgM seropositivity. Retaining IgM seropositivity is essential in females as a study has detected IgG in the maternal blood of pregnant women which assisted in passive immunity of their infants [14]. A higher number of diabetic patients with IgG+ and IgG+/IgM+ than prediabetic and non-diabetic and also lower IgM than non-diabetic group, was consistent with another study by Zhou et al. that reported lower serum IgM in diabetic patients than general patients, and the mean of IgM was higher than IgG after about 28 days of infection [15]. Other studies also reported a continuous decline of serum levels of IgM after more than two to three months to reach zero in some patients [16–18]. As illustrated in Figure 7, the number of non-diabetic patients with double positive of IgM and IgG after 40 days of onset were higher than the rest of the groups. This is consistent with other studies that reported higher density of IgM+ and IgG+ after more than a month of the onset [11,15, 16,19]. Our findings were inconsistent with a pre-liminary study by Pal et al. that reported seronegative results of IgM and IgG in diabetic patients [20]; however, they had a small sample size.

Our results have shown positive correlation between serum IgG and IgM and high levels of HbA1c. This indicates that level of HbA1c has not affected the immune system to produce these immunoglobins. A previous study reported increases in IgG and IgA with poor glycemic control [21]. Another study was consistent with our findings and reported high levels of IgM in diabetic patients [22]. After 40 days of recovery, more non-diabetic patients have shown double positive IgM and IgG than the other groups, which indicates the optimal time for vaccination of diabetic patients to be less than two months after recovery to protect the patients from SARS-COV-2 reinfection.

A limitation of this study was obtaining a second serum sample from our study group in the next months. This can assist this study by testing the serum again to monitor the seropositivity of IgM and IgG and compare it with the current findings, this can be helpful as several

studies reported a decline of these immunoglobins after 7 months of onset [15].

In conclusion, this study has shown the seropositivity of both IgG and IgM in COVID-19 recovered patients. Glycemic status has not affected the prevalence of both immunoglobins. Mounting a strong immune response toward SARS-CoV-2 is essential during COVID-19. Therefore, our findings are important to determine the optimal vaccination time of diabetic patients following recovery.

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**Declaration of Interest:**
The author declares no conflict of interest.

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