**Int. j. adv. multidisc. res. stud.** 2023; 3(6):1423-1428

**Received:** 07-11-2023
**Accepted:** 17-12-2023

International Journal of Advanced Multidisciplinary Research and Studies

**ISSN: 2583-049X**

# Exploring the Correlation between Cycle Threshold value of Coronavirus and Paraclinical Characteristics of Patients with Diabetic Nephropathy and COVID-19

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**Abstract**

**Background and objectives:** Coronavirus disease is the pandemic of the current era, affecting many patients worldwide. Underlying diseases may predispose the individual to infection, exacerbate the disease condition, or increase the risk of mortality and morbidity. Diabetes mellitus is a prevalent disease and diabetic nephropathy is a common and important vascular complication of diabetes. Evidence showed the association of diabetic glomerulopathy with COVID-19 viral infection. The present study investigated coronavirus gene expression in patients with diabetic nephropathy and its association with baseline variables.

**Materials and methods:** Sixty patients diagnosed with diabetic nephropathy and COVID-19 were enrolled. To determine their COVID-19 status, we administered real-time polymerase chain reaction (RT-PCR) tests. Based on the resulting viral load (high or low), we compared demographic factors such as age, gender, smoking status, body mass index (BMI), and duration of disease, as well as blood pressure, estimated glomerular filtration rate, protein urea, Hemoglobin (Hb)$A_1$c, and creatinine. The data was analyzed using SPSS v.24.

**Results:** The study showed based on Mann-Whitney results that patient aged 50 or above had higher viral loads than younger patients (p-value=0.0324), and also, the means HbA1c levels were higher in the high viral loads group and statistically were significant (p-value=0.029). The linear regression model also confirmed a significant correlation between gene expression and age(r= -0.401; p-value = 0.001), $HbA_1$c percentages (r=-0.364; p-value=0.04), and BMI (r=0.259; P=0.046). However, the study did not establish any association between gene expression and other variables.

**Conclusion**: The study found a notable correlation between the viral loads of patients with diabetic nephropathy and specific baseline features, including age, BMI, and Hemoglobin A1c levels. Therefore, it is important to monitor these subgroups of patients with diabetic nephropathy closely.

**Keywords:** Diabetes Mellitus, Kidney Diseases, Coronavirus, Real-Time Polymerase Chain Reaction

## Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, is the current health crisis, affecting the whole world. It has resulted in great mortality and morbidity rates, especially in the early phases of its emergence, resulting in about 7 million deaths until August 2023 [1]. In severe cases, it attacks critical organs and may result in multiorgan failure. The virus attacks the human cells, mainly through angiotensin-converting enzyme (ACE) receptors, and the presence of this receptor in specific organs, including the lungs, heart, and kidneys, facilitates virus attack and progression [2]. If these organs are affected by underlying diseases, COVID-19 can make a greater impact on them and increase the risk of morbidity and mortality [3].

Diabetes mellitus (DM) is a prevalent global health problem and the co-existence of DM and its complications with COVID-19 can result in exacerbation of both conditions, COVID-19 and DM; resulting in a 2.5-times greater risk of death due to COVID-19 in patients with DM [4]. One of the important complications of DM is diabetic nephropathy (DN), which affects about one-third of patients with DM and is a common cause of kidney replacement therapy [5]. Patients with DN are at a higher risk of developing COVID-19 pneumonia, being admitted, intubated, and fetal outcome [6]. The lung changes, caused by DM,

including reduced forced vital capacity and forced expiratory volume and promoted glycosylation of ACE2 by poor glucose control are considered the main causes of the worse prognosis [7]. The kidney injury in these patients may worsen by the exacerbated COVID-19 and the resulting inflammation and the deteriorated kidney function may end in end-stage renal disease (ESRD) that increases the mortality rate in these patients [8, 9]. Cellular investigation has also shown that the diabetic kidney disease in $ACE_2$-positive proximal tubular epithelial cells overlaps the expression pattern in cells infected with SARS-COV-2, which suggests $ACE_2$ coregulated proximal tubular epithelial cell expression program that interacts with COVID-19 infection [10].

Real-time polymerase chain reaction (rtPCR), able to detect the viral RNA by amplification of 2-3 distinct genomic regions, is the recommended diagnostic test for the identification of SARS-COV-2 [11]. In addition to accurate differentiation of negative and positive infection, the numeric value of rtPCR is also a valuable clinical determinator; the cycle threshold (Ct) values of rtPCR are known as a significant risk factor of COVID-19 severity, associated with symptomatic disease, hospitalization, severe disease, and death [12, 13]. Ct values for the envelop gene and open reading frame 1b gene were also found to have a positive association with severe COVID-19 [14]. According to the significance of DN in COVID-19 and the clinical value of rtPCR, investigation of the rtPCR values in these patients may provide us with a broader perspective on this issue. Therefore, the present study aimed to investigate coronavirus gene expression in patients with DM and evaluate its association with baseline variables.

## Materials and Methods

This study was performed on 60 adult patients > 18 years old with DN, who were referred to University-affiliated Hospitals in Mashhad in 2020 and had COVID-19 infection. Patients based on the results of Covid CT values were compared; Samples with a Ct value below 40 were considered positive, and those below 20 indicated a high viral load (Table 2). The presence of DM was confirmed by an endocrinologist at the specialized diabetes clinic of Alavi Hospital, considering the serum levels of fasting blood sugar (FBS) and hemoglobin $HbA_1C$ levels (≥6.5%). Patients with a known history of type 2 DM were included in the study; DN was confirmed by verifying albuminuria of >30 mg/24h or an albumin-to-creatinin ratio of > 30 mg/g in 2 of 3 samples taken.

During the pretrial stage, individuals suspected of having COVID-19 had two swab samples collected from their nasopharynx and oropharynx for real-time polymerase chain reaction (rt-PCR) and rapid test. The Coronavirus rapid test (Diabase Sars-Cov-2 Antigen Rapid Test, Parsian GITI Co, Iran) was used to diagnose COVID-19. Patients were only enrolled in the study if they tested positive on the Rapid Test and did not have certain medical conditions such as cancer, inflammatory or autoimmune diseases, malabsorption, liver disorders, congestive heart failure, or if they were pregnant or had taken antioxidant supplements, mineral supplements, and/or vitamin D in the past three weeks or false positive rapid test.

The sample size was calculated at 60, based on the calculated effect size of 0.7 the study power of 80%, and the significance level of 0.05. Before enrollment, the study objectives were explained to the eligible participants and they were asked to read and sign the written informed consent form. Then, they were included in the study by convenient and sequential sampling method. The researcher recorded the patient's information such as age, gender, smoking status, and duration of DN (based on clinical data from endocrinologists and nephrologists), as well as the serum level of creatinine and hemoglobin (Hb)$A_1c$ (based on the latest lab test results), and history of diseases and medications, symptoms, and possible side effects in the study's checklist. Additionally, the participant's blood pressure was classified into five stages based on The Seventh Report of the Joint National Committee (JNC7) guidelines. (As shown in Table 1); patients who were in stage 1 or higher were deemed abnormal. Furthermore, their weight and height were measured to calculate their body mass index (BMI). According to the Stage formula of Cockraft-Gault (140. age) x (weight) / (72xScr) (0.85 if the patient is female), the glomerular filtration rate (GFR) was calculated in ml/min/a.$73m^2$ and the severity of nephropathy was categorized into 6 stages, according to Kidney Disease Outcomes Quality Initiative (KDOQI) classification. Subjects were compared based on the severity of nephropathy.

The Geneova kit was used to identify the level of coronavirus gene expression in three regions of the virus genome - ORF1 ab, S, and N. RNA was extracted using the Lab Prep kit. The sample was collected before administering any anti-COVID treatment.

### Statistical analysis

After gathering all the necessary information, we input the data into the statistical software SPSS version 24 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp. Released 2013). We used appropriate statistical tables and indicators such as mean and standard deviation (SD) to describe the data. To ensure accuracy, we first assessed the normality of the data using the Shapiro-Wilk test. Continuous variables were presented as median (interquartile range [IQR]) and compared using the Mann-Whitney test, while categorical variables were presented as numbers and compared using the chi-square test. We also utilized a logistic regression model to review the association of variables and calculated the incidence rate ratio (IRR) as well as the beta coefficient with a 95% confidence interval (CI).The significance level of the tests was set at less than 0.05 and all statisticall test were two-sided.

## Results

The survey included 60 patients who suffered from DN and COVID-19. Their mean age was 58.10±17.18 (ranging from 19-84) years, with the highest frequency in the age group of 50-80 years (70.1%). Among all, 63.3% (N=38) were female. Of the total patients, 41.6% (N=25) were on dialysis, while the rest were not. Smoking status showed that 30% were active smokers, 43% were non-smokers, and 26% were former smokers. The duration of DN was observed to be 5 years in 14 (23.3%) patients, 10 years in 28 (46.6%) patients, and 15 years or more in 18 (30%) patients. The mean BMI of the patients was 28.43±2.14 (ranging from 25.1-32) $kg/m^2$, with 70% being overweight and 30% being obese.

The mean systolic blood pressure was 158±12.4 mmHg, with 93.3% having abnormal readings. The mean diastolic

blood pressure was 91.7±13.3 mmHg, with 60% having abnormal readings. The mean estimated GFR was 48.62±5.27, with 11.6% of patients categorized in stage 1, 21.6% in stage 2, 18.3% in stage 3a, 15% in stage 3b, 18.3% in stage 4 and 15% in stage 5. Coronavirus Ct value showed that 56.6% had a low viral load while 43.3% had a high viral load.

The mean serum creatinine was 5.34±1.46 mg/dL and $HbA_1c$ was 7.01±4.28 scale among patients. In assessing protein urea, 26.6% were in the sub-nephrotic stage and 48.3% were in the nephrotic syndrome stage. (Table 3)

When comparing two groups of patients based on their baseline characteristics and paraclinical variables about COVID-19 viral load, it was discovered that the older group (above 50 years old) had a significantly higher viral load compared to the younger group. (p-value=0.0324). The study also confirmed a significant connection between gene expression and age using a linear regression model (r=-0.401; P=0.001; see Table 4). The findings showed significant differences in HbA1c percentages between Ct value groups (p-value=0.029). Moreover, the study found that COVID-19 viral loads were associated with HbA1c percentages, with higher percentages indicating higher viral loads (r=-0.364, p-value=0.04). Lastly, BMI was found to be associated with rtPCR values (r=0.259, P=0.046). There were no significant relationships between DN duration, Blood pressure, the status of dialysis, protein urea, serum creatinine, and eGFR with loads of virus. Table 1 presents a comparison of baseline characteristics for patients based on viral load breakdown, while Table 3 compares paraclinical factors. Table 4 displays the findings of regression analysis that examines the relationship between rtPCR value and baseline characteristics and paraclinical factors.

## Discussion

The present study investigated patients who were hospitalized with COVID-19 and had DN as the main underlying disease; the results showed that most of the study population was female. The overall incidence of DM is higher in men; however, the most prominent risk factor of DM, obesity, is more prevalent in women. Also, women have a higher risk of diabetes-related complications and a higher risk of cardiovascular diseases (CVD), myocardial infarction, and stroke [15, 16]. Also, men seem to progress DN faster than women and more males undergo dialysis therapy with kidney transplantation [17]. However, we only evaluated patients, hospitalized with confirmed COVID-19, which could influence the sex distribution of the patients. Similar to the results of the present study, a population-based study showed that more women had DN than males [18]. More population-based studies are required to investigate the sex difference in the incidence of DN in patients infected with COVID-19.

The mean age of our patients was about 58 years which was lower than that reported by the population-based study in Korea, which reported a mean age of about 66 years in patients with DN during the COVID-19 outbreak; however, they did not investigate patients with DM, a few of whom were infected with COVID-19 [18]. As we did not evaluate the general population and only evaluated the patients hospitalized, the referral pattern of the patient influences the demographics of the study population; maybe, younger patients were referred to the study center more than the elderly.

According to the results of a survey, it seems that patients who are over 50 years old may have a higher amount of the COVID-19 virus. For every year that someone gets older, there is a 0.4 decrease in the likelihood of having a low CT value. These findings suggest that older individuals may experience more severe symptoms of COVID-19 when they first become sick. The Centers for Disease Control and Prevention (CDC) report that older adults, especially those over 50 years old, are more likely to become very sick from COVID-19. The risk of severe illness increases as someone gets older, which means they may need to go to the hospital, receive intensive care, use a ventilator to breathe, or even die. [19] Another study has shown that older patients with underlying health conditions are at a higher risk of developing severe forms of COVID-19. Some older people may not have the typical symptoms of the virus, which can make it difficult to be diagnosed on time. Additionally, several factors, such as vitamin D deficiency, low levels of albumin, changes in ACE-2 expression levels, immunological profiles, sex hormone and growth hormone secretion associated with old age, as well as oxidative stress and mitochondrial dysfunction, can increase the susceptibility of older people to COVID-19.[20]

According to this survey, there was a notable difference in the amount of virus based on individuals' serum HbA1c levels. The study also found a connection between an increase in HbA1c percentage and a higher load of the virus. Previous research has demonstrated that people with poorly controlled diabetes, as reflected by high HbA1c levels, are at a greater risk of severe complications from COVID-19, such as hospitalization, intensive care unit admission, and death, compared to those with well-controlled diabetes. [21] High blood sugar levels can impair immune function and increase inflammation, making it harder for the body to fight off viral infections like COVID-19 [22].

Obesity has been associated with a higher risk of respiratory infections in general. This is thought to be due to several factors, such as impaired immune function, chronic inflammation, and reduced lung capacity. As COVID-19 primarily affects the respiratory system, individuals with obesity may be more susceptible to contracting the virus.; [23] We found a significant positive linear association between increasing BMI and viral load, Similar to the present study, a German study evaluated the risk factors of poor outcome in patients with COVID-19 and documented that DN was the far most substantial risk factor for an unfavorable outcome and reported increased mortality rate in patients with DN or the combination of obesity plus DM [24]. Obesity and DM have been considered as two health concerns that increase the risk of complications and severe infections; they both impair the host immunity [25]. Accordingly, their concordance aggravates the immune deficiency and exacerbates COVID-19 infection; a report from the USA showed that among young patients (<60 years), those with a BMI of 30-35 $kg/m^2$ and >35 $kg/m^2$ had a higher chance of ICU admission 1(26). In our study, 70% were overweight 30% were obese and none had normal weight. The chronic activation of the immune system in obese patients influence the disease severity and complications; also, adipocyte-derived leptin in obese patients regulate the hematopoiesis of bone marrow and generate T cell in the thymus and lymph nodes. Leptin signaling mediates the expression of antiviral cytokines (such as interferon) and pro-inflammatory cells (such as interleukins and tumor necrosis

factor) [27]. Further studies are required to determine the molecular mechanisms that contribute to the poorer outcome of COVID-19 in patients with DN or DN plus obesity.

Our analysis did not find any correlation between variables related to kidney disease, such as blood pressure, dialysis status, proteinuria, serum creatinine, and eGFR, with COVID-19 CT values. This may suggest that there is a low likelihood of a link between kidney disease and contracting COVID-19. One study found no difference in the odds ratio of testing positive for COVID-19 between patients with and without CKD, while another study reported a significant odds ratio of 0.50 (95% CI 0.39-0.65) [28]. A survey indicated that having a high viral load increased the likelihood of in-hospital mortality, ICU admission, and invasive ventilation, and cycle threshold value could be considered a significant predictor of mortality in renal patients [29]. Furthermore, research has suggested that hypertension is strongly associated with increased mortality in COVID-19 patients due to dysregulation of the RAAS, immune response, gastrointestinal tract, and inflammation [30]. However, since we did not follow up with patients, we cannot draw a connection between the severity of COVID-19 viral loads and the outcome of patients.

One of the limitations of the present study was the evaluation of a small sample from one study center in one city, which limited the generalizability of the results. Also, the small number of samples in specific subgroups reduced the strength of the analysis.

However, COVID-19 is a new pandemic, and information about the possible link between DN and COVID-19 is incomplete. Therefore, we investigated the patients, hospitalized with COVID-19 and evaluated the results of rtPCR to examine the disease severity in these patients.

The immune system plays an important role in clearing the coronavirus from the body and acts by invoking immune cells and igniting the inflammation, resulting in a cytokine storm [31]. The underlying diseases that impair the immune system, including DM, hypertension, and cerebrovascular disease, can predispose the patient to a more severe COVID-19 infection [32]. This is why we investigated patients with DM. Furthermore, we selected only those with DN, because kidney injury in these patients may worsen COVID-19 [8, 9]. Both genetic and environmental factors are found responsible for the predisposition of patients with comorbidities to higher disease severity, morbidity, and mortality [33]. Based on the higher disease severity observed in patients with higher values of the rtPCR, in the present study, we studied the mean values, to determine the influential factors.

## Conclusion

COVID-19 can present with different severities in different people. Considering the previous evidence on the poor outcome and greater disease severity in patients with DN, we investigated the results of rtPCR in this population and the results showed that specific subgroups, obese, older patients and high-level HbA1c, are at a higher risk of more severe COVID-19. Therefore, it is necessary to pay attention to patients at risk and implement more aggressive therapeutic strategies in these subgroups, to reduce further complications and mortality rates in these patients. Further studies with a larger sample size and follow-up of patients are required to evaluate the validity and generalizability of these results. Also, the molecular mechanisms of the susceptibility of these patients to severe infection have to be investigated in future studies.

**Financial Disclosure:** The present study's authors declare that there are no financial conflicts of interest to disclose.

**Funding/Support:** The present study was not financially supported by any organization.

**Table 1:** Comparing baseline characteristics of the patients with Diabetic Nephropathy based on Covid CT breakdown

| Variable | Categories | | N | mean±SD | p-value |
|---|---|---|---|---|---|
| **Age (years)** | <50 | | 18(29.9%) | 34.5±1.67 | 0.0324* |
| | ≥50 | | 42(70.1%) | 67±1.58 | |
| **Gender** | Male | | 22(36.6%) | - | 0.458** |
| | Female | | 38(63.3%) | | |
| **Smoking Status** | Current-smoker | | 18(30%) | - | 0.416** |
| | Non-smoker | | 26(43%) | | |
| | Ex-Smoker | | 16(26%) | | |
| **Body mass index** | Normal | | - | - | 0.852* |
| | Overweight | | 42(70%) | 26.76±1.98 | |
| | Obese | | 18(30%) | 30.21±2.26 | |
| **DN Duration** | Five years | | 14(23.3%) | - | 0.367* |
| | Ten years | | 28(46.6%) | | |
| | 15 years or more | | 18(30%) | | |
| **Blood Pressure mmHg** | Systolic | NL | 14(23%) | 10.7±0.64 | 0.248* |
| | | pre-HTN | 25(41.6%) | 13.02±0.84 | |
| | | Stage1 | 16(26.6%) | 15.24±0.36 | |
| | | Stage 2 | 5(0.08%) | 17.29±0.51 | |
| | Diastolic | NL | 32(53.3%) | 6.97±0.82 | |
| | | Pre-HTN | | | |
| | | Stage 1 | 26(43.3%) | 8.65±0.24 | |
| | | Stage 2 | 2(0.03%) | 9.28±0.1 | |
| **Dialysis** | D | | 25(41.6%) | | 0.491** |
| | N/D | | 35(58.3%) | | |

*Mann-Whitney test result, ** Chi-square results

**Table 2:** Covid viral loads based on CT values results of RT-PCR

| | | | |
|---|---|---|---|
| **Covid CT values** | x>20 | 34(56.6%) | 34.48±4.39 |
| | x<20 | 26(43.3%) | 15.08±3.47 |

**Table 3:** Paraclinical results of the patients with Diabetic Nephropathy based on Covid CT breakdown

| **Variable** | **Categories** | **N** | **mean±SD** | **p-value** |
|---|---|---|---|---|
| **Estimated Glomerular filtration rate ml/min/a.73m$^{-2}$** | G1 | 7(11.6%) | 96.27±5.31 | 0.652* |
| | G2 | 13(21.6%) | 74.19±4.25 | |
| | G3a | 11(18.3%) | 53.33±2.47 | |
| | G3b | 9(15%) | 38.12±3.69 | |
| | G4 | 11(18.3%) | 20.84±3.71 | |
| | G5 | 9(15%) | 9.02±1.58 | |
| **Creatinin** | mg/dL | - | 5.34±1.46 | 0.154* |
| **HbA1c** | percent | - | 7.01±4.28 | ***0.029**** |
| **Proteinurea mg/g or mg/day** | 30<x<300 | 26(26.6%) | | 0.735* |
| | x>300 | 34(48.3%) | | |

*Mann-Whitney test results

**Table 4:** The association of Covid CT with baseline demographics and paraclinical variables

| Variable | | Correlation coefficient | IRR | P-value* |
|---|---|---|---|---|
| Age | | -.401** | | ***0.001*** |
| Gender | | | 0.256 | |
| BMI | | .259* | | **0.046** |
| Duration of DN | | 0.117 | | 0.375 |
| Smoking Status | | 0.254 | | *0.691* |
| Blood pressure | Systolic | 0.131 | | 0.320 |
| | Diastolic | 0.211 | | 0.106 |
| Creatinine(mg/dl) | | 0.030 | | 0.822 |

| | | | |
|---|---|---|---|
| eGFR | -0.032 | | 0.811 |
| proteinurea | 0.59 | | 0.745 |
| HbA1c | -0.364 | | ***0.04*** |
| Dialysis | | 0.258 | 0.258 |

Logistic regression, Abbreviations: BMI; body mass index, eGFR; estimated glomerular filtration rate, IRR: incidence rate ratio

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