Journal of Proteomics and Genomics
Volume 3 | Issue 1
ISSN: 2576-7690

Research Article Open Access

Association of ACE I/D rs4646994 and ACE2 rs2285666 Receptor Gene Polymorphisms with SARS-CoV-2 Infection in Bangladeshi People

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Citation: Md. Rezaul Karim Rana, Sumaiya Binte Hannan, Haseena Khan, A.H.M Nurun Nabi, Mohammad Riazul Islam (2023) Association of ACE I/D rs4646994 and ACE2 rs2285666 Receptor Gene Polymorphisms with SARS-CoV-2 Infection in Bangladeshi People J Proteo Genomics 3(1): 101

Received Date: August 26, 2023 **Accepted Date:** September 26, 2023 **Published Date:** September 28, 2023

Abstract

Background: Covid-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which hit the world in December 2019 is one of the worst medical emergencies people have ever encountered. Human angiotensin I converting enzyme 2 (ACE2) is the entry path of this virus to the host that regulates the renin-angiotensin-aldosterone system (RAAS).

Hypothesis: Susceptibility to SARS-CoV-2 infection may be affected by ACE rs4646994 and ACE2 rs2285666 polymorphisms.

Aim: This study aims to investigate how single nucleotide polymorphisms in the ACE and ACE2 genes, rs4646994 and rs2285666 respectively affect Covid-19 infection.

Methodologies: The study included RT-PCR-confirmed 100 Covid-19 survivors and 50 healthy controls. All samples were genotyped using PCR-based amplified fragment length polymorphism (PCR-AFLP) and PCR-based restriction fragment length polymorphism (PCR-RFLP) methods respectively for rs4646994 and rs2285666. Statistical analyses were performed with MedCalc software.

Results: The DD genotype of ACE rs4646994 was found to be significantly associated with the risk of Covid-19 infection (OR=3.85; CI: 1.26-11.76, p=0.017). This implies that the D allele is highly associated with the infection. In the case of the ACE2 gene, both the heterozygous GA (OR=3.52; CI:1.50-4.25, p=0.004) and homozygous AA (OR=11.81; CI:4.29-32.54, p<0.0001) genotypes were found to be significantly associated with the risk of the disease, demonstrating that A allele is associated with Covid-19 infection. Diabetic patients have significantly higher risk of SARS-CoV-2 infection and have strong correlation with ACE-ID and ACE2-AA genotypes.

Conclusion: These findings suggest that ACE rs4646994 and ACE2 rs2285666 gene polymorphisms may contribute to the prevalence of Covid-19 infection and serve as markers of susceptibility.

Keywords: SARS-CoV-2; Angiotensin-converting enzyme; PCR; PCR-RFLP; Polymorphism

List of Abbreviations

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; Covid-19: Coronavirus-induced disease 2019; WHO:World Health Organization; ACE: Angiotensin-converting enzyme; RAAS: Renin-angiotensin-aldosterone system; I/D: Insertion-Deletion; SNP: Single nucleotide polymorphism; RT-PCR: Reverse transcription polymerase chain reaction; NCBI:National Center for Biotechnology Information; DNA: Deoxyribonucleic acid; TE: Tris-EDTA; PCR: Polymerase chain reaction; AFLP: Amplified fragment length polymorphism; RFLP: Restriction fragment length polymorphism; OR: Odds ratio; CI: Confidence interval; S.D: Standard deviation; bp: base pair

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus-induced disease 2019 (Covid-19) which was first reported on 31 December 2019 in Wuhan, China, and thereafter spread worldwide [1,2]. Covid-19 has encountered a major threat to human health globally as it can transmit when people breathe in air contaminated by droplets and small airborne particles containing the virus [3]. The disease was declared a pandemic by World Health Organization (WHO) and has affected around 691 million people across the world and resulted in the death at around more than 6.89 million to date (https://www.worldometers.info/coronavirus/, accessed on 15 July 2023).

SARS-CoV-2 is an enveloped RNA virus containing membrane, envelope, and spike proteins [4,5]. The entry of this deadly virus occurs by binding the spike protein to the extracellular peptidase domain of human angiotensin-converting enzyme 2 (ACE2) receptors of the host membrane [6,7]. The protein encoded by this receptor gene belongs to the angiotensin-converting enzyme (ACE) family of dipeptidyl carboxypeptidase and involves in the renin-angiotensin-aldosterone system, RAAS [7]. ACE2 is expressed in various human organs, such as the small intestine, lungs, heart, kidney, and endothelium [7]. The angiotensin-converting enzyme (ACE) converts angiotensin I to angiotensin II, while angiotensin II is converted to angiotensin-(1-7) by ACE2 [8].

The ACE gene is located on chromosome 17q23 and is composed of 26 exons that encode the ACE protein [9]. The ACE gene has several polymorphisms, including the insertion/deletion (I/D) polymorphism, which is a common variation in the ACE gene that affects ACE activity [10]. The I/D polymorphism results from the presence or absence of a 287-base pair Alu repeat sequence in intron 16 of the ACE gene [11]. Individuals with the DD genotype have higher ACE activity and may be at increased risk for certain conditions, such as hypertension, cardiovascular disease, and renal disease, although the relationship between the I/D polymorphism and these conditions is complex and not fully understood [12].

On the other hand, the ACE2 gene is located on chromosome Xp22.2 and contains 18 exons [13]. Several genetic variants have been identified in the ACE2 gene, and some studies have suggested that certain variants may be associated with an increased risk of developing cardiovascular disease or other health conditions, although the evidence is not yet conclusive [14]. Males have lower ACE2 expression compared to females because they only possess one X chromosome which ultimately leads to a higher risk of cardiovascular disease, hypertension, kidney failure, and diabetes mellitus [15].

The effect of genetic diversity of RAAS pathway components is still unclear even though there have been numerous scientific reports relating susceptibility to Covid-19 and ACE, and ACE2 genes. By minimizing viral entrance into the host cell, regulating ACE2 expression may be able to properly control SARS-CoV-2 infection. Understanding how host genetic variations affect risk or

protection could offer some insights into COVID-19 results [16]. The level of ACE2 expression and its epigenetic alterations may be influenced by a number of factors, mainly environmental factors as well as drugs, endocrine disruptors, and hypoxia [17]. Recent research by Paital et al. examined a relationship between air pollution and ACE2 expression. According to this review, ACE2 expression is increased by prolonged exposure to nitrogen dioxide (NO_2) and particulate matter [18]. Another investigation confirmed a positive correlation between smoking and the expression pattern of ACE2. Epigenetic elements that regulate gene function, including DNA methylation, histone changes, and micro-RNAs can also affect how the ACE2 gene and protein are expressed [17].

The link between ACE I/D and renal disease, and myocardial infarction, left ventricular hypertrophy has been the subject of several research that investigated closely at the distribution of ACE alleles in different populations. According to the findings of these studies, the frequency recorded in numerous African communities is identical to that observed in Caucasians, however the rates observed in Asian, Amerindian, and Polynesian people are different [19]. As ACE2 polymorphism was found to be strongly associated with Diabetes Mellitus [20] and hypertension previously [21], these studies suggest that ACE I/D and ACE2 polymorphisms may be associated with SARS-CoV-2.

In addition, rs2285666 may influence ACE2 gene expression by altering mRNA splicing [22]. Some ACE2 genetic polymorphisms can cause changes in ACE2 binding affinity for the SARS-CoV-2 receptor binding domain [23]. rs2285666 is one of these single nucleotide polymorphisms (SNPs) whose wild type enhances ACE2 production with a greater affinity for SARS-CoV-2 [24]. Thus, our objective was to investigate the susceptibility to SARS-CoV-2 in relation to variations in the ACE rs4646994 and ACE2 rs2285666 genes, as well as any accompanying medical conditions in the Bangladeshi population.

Methods & Materials

Study population

This prospective cohort study was carried out on 100 Covid-19 recovered patients and 50 healthy controls residing around Dhaka city. Positive cases confirmed by real-time reverse transcription polymerase chain reaction (RT-PCR) were selected for the study. The sample collection was conducted between April to September 2021. A structured questionnaire was prepared for the subjects which included demographic data, smoking habits, and comorbidities. Informed consent was obtained from the subjects before taking interviews and blood samples. The study protocols and procedures described in this manuscript were approved by the Ethical Committee of the Faculty of Biological Sciences, University of Dhaka (ref. No. 132/Biol. Scs. Dt. 20.06.2021).

Sample collection

Approximately 4.0 mL of venous blood was collected aseptically from each study subject in an EDTA-containing vacutainer tube (Lab kits diagnostics, Spain). Specimens were transferred to the laboratory under cold conditions and preserved at −20 ^{O}C.

Genotyping

The gene sequences of ACE and ACE2 were extracted manually from NCBI. Primer pairs were selected through reviewing literatures [19,20], and their validities were checked with NCBI primer blast**.**

Genomic DNA extraction

Genomic DNA was extracted from blood serum using FavorPrep Blood Genomic DNA Extraction Mini Kit (Favorgen Biotech Corporation, Taiwan) as per the manufacturer's instructions. The extracted DNA was dissolved in a sterile 10 mM Tris-EDTA (TE) buffer (pH 8.0). The quantity and quality of the extracted DNA were checked by Nanodrop spectrophotometer (Thermo Scientific, USA). For further purification, 1.0X iso-propanol and 0.1X 3M sodium acetate were added, and after a brief incubation at

4 ^{O}C, centrifuged (Allegra X-30R, Beckman Coulter, US) at 15,000xg for 10 minutes, followed by 70% ethanol wash twice [25]. Finally, the DNA pellet was air-dried and then eluted in a 10 mM TE buffer solution.

Angiotensin Converting Enzyme rs4646994 genotyping

The ACE rs4646994 genotype was identified using the PCR-AFLP method. The primer pair 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGTCAGAT-3' were used to amplify the ACE gene using a thermal cycler (ProFlex PCR system, Thermo Fisher Scientific). The PCR was done in a reaction volume of 10 µL containing 5.0 µL GoTaq® Green Master Mix (Promega, USA), 0.5 µL 10 µM primer pair, 1 µL DNA template (~100 ng), and the final volume of 10 µL was adjusted by adding nuclease-free water. The PCR cycling conditions were carried out with an initial denaturation at 95 ^{O}C for 5 minutes, followed by 35 cycles of denaturation at 95 ^{O}C for 30 seconds, annealing at 60 ^{O}C for 40 seconds, and elongation at 72 ^{O}C for 50 seconds, followed by final elongation at 72 ^{O}C for 7 minutes.

PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide and visualized on the E-Box gel documentation imaging system (Vilber, Germany).

Angiotensin Converting Enzyme 2 rs2285666 genotyping

PCR-RFLP method was used to determine the genotypes of ACE2 rs2285666. The primer pair 5'-CATGTGGTCAAAAGGATATCT-3' and 5'-AAAGTAAGGTTGGCAGACAT-3' were used to amplify the gene. PCR composition was the same as ACE rs4646994 gene amplification. The reaction mixture was denatured at 95 ^{O}C for 5 minutes, followed by 35 cycles of denaturation at 95 ^{O}C for 30 seconds, annealing at 50.6 ^{O}C for 40 seconds, and elongation at 72 ^{O}C for 50 seconds, followed by final elongation at 72 ^{O}C for 7 minutes in the thermal cycler. PCR products were electrophoresed on 1% agarose gel stained with ethidium bromide and visualized on the E-Box gel documentation imaging system.

A 15 µL reaction mixture was prepared that consisted of 1.5 µL 10X CutSmart buffer, 5 µL PCR product, 5 U AluI (New England Biolabs), and nuclease-free water to adjust the volume and incubated at 37 ^{O}C overnight (~16 hours). The reaction mixture was run on 2% agarose gel to visualize the bands.

Statistical Analysis

Categorical variables were expressed as frequency or percentage (%), while quantitative variables were expressed as mean±sd. To estimate the association between genotypes' comorbidities and susceptibility to Covid-19, an odds ratio (OR) with a 95% confidence interval (CI) was calculated in MedCalc statistical software (ver. 22.003). P values below 0.05 were deemed to be statistically significant.

Results

Demographic features of cases and healthy controls

The demographic features of 100 Covid-19 recovered patients and 50 healthy controls are summarized in Table 1. Out of 100 cases, the age of 60 (60%) cases were below 40 years and 40 (40%) cases were equal to or above 40 years, 60 cases were male and 40 cases were female. Out of 50 healthy controls, 35 (70%) controls were below 40 years and 15 (30%) controls were equal to or above 40 years, 30 (60%) of them were male and 20 (40%) were female. Most common comorbidities in cases were renal disease (10%), cardiovascular disease (31%), asthma (39%), diabetes (56%), and hypertension (47%). With a comparison to controls, diabetes was found susceptible to Covid-19 with a 4-fold higher risk (OR=4.48; 95% CI: 1.02-19.62, p=0.046). Smoking habit (63% in cases, 38% in controls) was also found significantly susceptible (OR=2.78; 95% CI: 1.38-5.60, p=0.004).

Table 1: Baseline characteristics of controls and cases

Feature	Variable	Control	Case	OR (95% CI)	p
Age	>40	15	40	ref (1)	-
	≤ 40	35	60	0.64 (0.31-1.32)	0.232
Gender	Female	20	40	ref (1)	-
	Male	30	60	1.00 (0.50-2.00)	1
Comorbidities	Renal disease	4	10	ref (1)	-
	Cardiovascular disease	5	31	2.48 (0.55-11.06)	0.234
	Asthma	8	39	1.95 (0.48-7.40)	0.345
	Diabetes	5	56	4.48 (1.02-19.62)	0.046
	Hypertension	6	47	3.13 (0.74-13.19)	0.119
Smoking habit	No	31	37	ref (1)	-
	Yes	19	63	2.78 (1.38-5.60)	0.004

Genotype distribution of cases and healthy controls

Etiologic effects of ACE and ACE2 gene polymorphisms have been evaluated after screening the genotypes by PCR-AFLP and PCR-RFLP methods respectively. Three different fragment patterns were identified for the ACE rs4646994 polymorphism. The major allele was represented by a 490 bp fragment that contained a 287 bp Alu insertion sequence (Figure 1A). Deletion of the Alu sequence resulted in a 190 bp fragment (Figure 1A). The II genotype, which is the ancestral homozygous genotype, was represented by a single fragment at 490 bp. The DD genotype, which is the derived homozygous genotype, was represented by a single fragment at 190 bp. The ID genotype, which is the heterozygous genotype, was represented by both 490 bp and 190 bp fragments (Figure 1A).

A distinct 466 bp product was generated by PCR for the ACE2 rs2285666 polymorphism, which was then separated on a 2% agarose gel after restriction digestion. Analysis of the digested product revealed three distinct patterns. The GG genotype was characterized by a 466 bp fragment, while the GA genotype was identified by fragments of 466, 281, and 185 bp. The AA genotype was recognized by fragments of 281 and 185 bp (Figure 1B). Fragments were compared to GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher, USA).

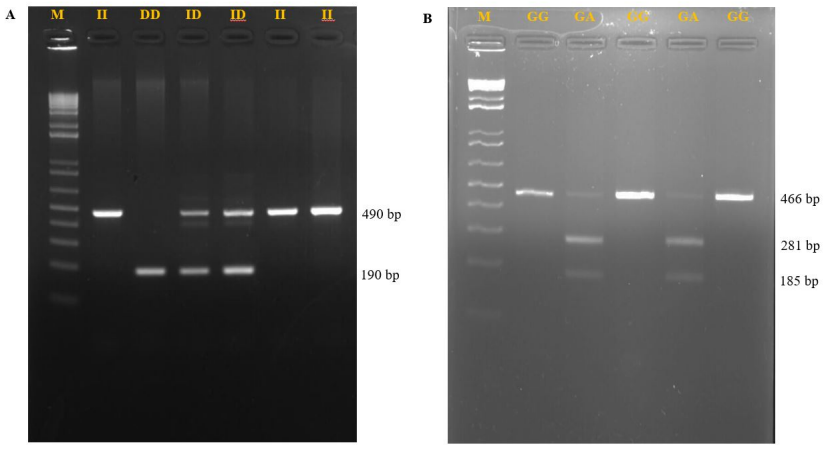

Figure 1: Representative agarose gel image of ACE and ACE2 gene PCR-RFLP. ACE Insertion-Deletion genotypes after PCR-AFLP (A) and restriction digestion product bands of ACE2 after performing PCR-RFLP (B)

Analysis of ACE rs4646994 and ACE2 rs2285666 genotypes of cases and healthy controls

Genotype distribution and analyzed data are summarized in Table 2 and indicate that, among three genotypes of ACE rs4646994, the DD genotype was found strongly associated with the risk of Covid-19 infection (OR=3.85; CI: 1.26-11.76, p=0.017). I allele frequency in the sample is lower (46%) than healthy control (60%) while D allele frequency is much higher in the sample (54%) than healthy control (40%).

Both GA (OR=3.52; CI: 1.50-4.25, p=0.004) and AA (OR=11.81; CI: 4.29-32.54, p<0.0001) genotypes from the ACE2 gene were found statistically significant and might have the association with Covid-19 infection.

Table 2: Association of ACE rs4646994 and ACE2 rs2285666 polymorphism with Covid-19 infection

Gene	Genotype	Control	Case	OR (95% CI)	p
rs4646994	II	16	18	ref (1)	
	ID	28	56	1.78 (0.79-4.00)	0.165
	DD	6	26	3.85 (1.26-11.74)	0.017
rs2285666	GG	31	21	ref (1)	
	GA	13	31	3.52 (1.50-4.25)	0.004
	AA	6	48	11.81 (4.29-32.54)	<0.0001

A correlation analysis between the genotypes of ACE and ACE2 was done to distinguish the correlation more accurately and summarized in Table 3. Our results show that Covid-19 infected cases with the ID+AA genotype were more susceptible than any other correlated genotype groups. This genotype group was found most significant with a 26-fold higher risk (OR=26.71; CI: 8.06-88.51, p<0.0001) of infection. The second highest is the ID+GA genotype with a 3-fold higher risk (OR=3.43; CI: 1.10-10.70, p=0.034).

Table 3: Correlation between rs4646994 and rs2285666 polymorphism in Covid-19 infection

rs2285666	rs4646994	No	Yes	OR (95% CI)	p
GG (N=21)	II	13	8	ref (1)	-
	ID	13	8	1.00 (0.29-3.47)	1.000
	DD	16	5	0.51 (0.13-1.93)	0.320
GA (N=31)	II	25	6	ref (1)	-
	ID	17	14	3.43 (1.10-10.70)	0.034
	DD	20	11	2.29 (0.72-7.28)	0.160
AA (N=48)	II	44	4	ref (1)	-
	ID	14	34	26.71 (8.06-88.51)	<0.0001
	DD	38	10	2.89 (0.84-9.98)	0.092

Association of ACE-ID+ACE2-AA genotype with comorbidities in Covid-19 cases

A greater vulnerability to an increased risk of Covid-19 was demonstrated by the group with the ACE-ID+ACE2-AA genotype (Table 3). As shown in Table 4, the distribution of genotypes was classified according to the comorbidities they exhibited and the length of time they had been afflicted with the condition. ACE-ID (OR=6.11; CI: 1.41-26.41, p=0.015) and ACE2-AA (OR=4.40; CI: 1.02-18.99, p=0.047) both genotypes were identified as having a significant impact on Covid-19 susceptibility in individuals with diabetes.

Table 4: Genotype distribution of the cases according to their comorbidities and disease duration

Comorbidities	Age (Mean±S.D.)	SNP	Genotype	Disease duration (Days)		OR (95% CI)	p
				<21	**>21**		
Renal disease	35.70±7.67	rs4646994	II ID DD	2 2 2	1 3 0	ref (1) 3.00 (0.1559.89) 0.33 (0.09-12.82)	- 0.472 0.555
		rs2285666	GG GA AA	2 2 3	1 0 2	ref (1) 0.33 (0.09-12.82) 1.33 (0.07-26.62)	- 0.555 0.851
Cardiovascular disease	40.61±13.36	rs4646994	II ID DD	6 7 3	3 9 1	ref (1) 2.57 (0.47-14.10) 0.67 (0.05-9.47)	- 0.277 0.765
		rs2285666	GG GA AA	3 7 8	2 5 6	ref (1) 1.08 (0.13-8.98) 1.12 (0.14-9.00)	- 0.949 0.911
Asthma	39.36±12.73	rs4646994	II ID DD	5 7 6	5 11 5	ref (1) 1.57 (0.33-7.48) 0.83 (0.15-4.64)	- 0.570 0.835
		rs2285666	GG GA AA	4 6 9	4 6 10	ref (1) 1.00 (0.17-5.98) 1.11 (0.21-5.80)	- 1.00 0.901
Diabetes	38.75±11.85	rs4646994	II ID DD	11 12 4	3 20 6	ref (1) 6.11 (1.41-26.41) 5.50 (0.91-33.18)	- 0.015 0.063
		rs2285666	GG GA AA	9 7 15	3 11 22	ref (1) 4.71 (0.94-23.68) 4.40 (1.02-18.99)	- 0.060 0.047
Hypertension	38.87±11.35	rs4646994	II ID DD	9 7 10	5 14 5	ref (1) 3.60 (0.87-14.90) 0.90 (0.19-4.16)	- 0.077 0.893
		rs2285666	GG GA AA	6 6 9	3 9 14	ref (1) 3.00 (0.53-16.90) 3.11 (0.61-15.71)	- 0.213 0.170

Discussion

This study was designed to investigate the potential association between the ACE and ACE2 gene polymorphisms and the likelihood of developing Covid-19 infection. ACE2 has been identified as the functional host receptor for SARS-CoV-2, which is responsible for the devastating Covid-19 pandemic that can cause symptoms ranging from a mild cold to potentially fatal cardio-respiratory failure. Both ACE and ACE2 receptor genes have various genetic variations, including ACE rs4646994 and ACE2 rs2285666 polymorphisms. Due to the role of this receptor gene in Covid-19 pathogenesis, researchers have become interested in ACE and ACE2 receptor gene variants.

The ACE I/D polymorphism is associated with increased levels of ACE in the blood [26], as well as adverse clinical conditions and an increased risk for severe forms of Covid-19 disease. Obesity [27], hypertension [28], and thrombophilia [29] are among the

most common conditions that have been linked to ACE I/D polymorphism, as well as an overall heightened cardiovascular risk. Our current research revealed a positive correlation between allele D and an increased rate of Covid-19 infection. D allele frequency was found higher in infected cases than in healthy controls. People with the DD genotype were shown to have a higher risk ($p=0.017$) of infection than those with other genotypes, which indicates that this allele may be considered important in assessing the risk of infection. Lee et al. found that populations in France, Italy, and Spain had a high frequency of the D allele, ranging from 82-87% [30]. This high frequency indicates that the D allele is a common variation among these populations. Reports indicate that African Americans in the US exhibit the highest frequency of the D allele, at 89%, which is notably higher than the frequency observed in white Americans, which is 69% [31]. Pati et al. proposed a significant link between the D allele of the ACE polymorphism and both the incidence of SARS-CoV-2 infection and the rate of mortality in their epidemiological study in Asian population [32]. Similarly, Gómez et al. suggested that the ACE-ID polymorphism is associated with the risk of developing severe Covid-19 depending on the hypertension status in Spanish population [33]. The meta-analysis study conducted by Hatami et al. demonstrated that the recovery rate of individuals possessing the I/D allele frequency ratio increased significantly in Iranian population [34]. Meanwhile, the findings of Delanghe et al. suggest that there may be a link between ACE I/D polymorphism and the severity of Covid-19. The D allele of the ACE rs4646994 gene was found to be associated with the lower occurrence and death rates of the virus in 33 countries [35].

This study also includes ACE2 gene polymorphic susceptibility to Covid-19 infection. Because of its location on the X chromosome, the ACE2 gene cannot be found in a heterozygous state in males because they only have one copy of the gene. Therefore, any SNPs in the gene can have more serious consequences in males than in females. This makes it important to consider the sex of a person when studying ACE-2-related conditions and treatments [36]. ACE2 is involved in the regulation of several physiological processes such as blood pressure and fluid balance. Imbalance in RAAS, resulting from the malfunctioning of ACE2, has been linked to a range of medical conditions including hypertension [37], myocardial infarction [27], acute pulmonary disease [38], and diabetes mellitus [39]. It has been reported that diabetes mellitus might promote ACE2 modifications, favoring SARS-COV-2 entry in cardio myocytes [40]. Analyzing approximately 290,000 samples constituting >400 demographic groups from public genomic datasets, Kushal et al. revealed that ACE2 gene polymorphisms are related to altered susceptibility to Covid-19 [41].

Several studies have found the relationship between SARS-CoV-2 and ACE I/D and ACE2 polymorphisms. It has been noticed that these gene polymorphisms affect differently in different geographic regions. Such as African Americans with a high D allele occurrence in the ACE gene have shown increased mortality rates in the United States [42]. On the other hand, Asian patients with a high frequency of the ACE-II genotype have demonstrated lower mortality rates compared to those with the ACE-DD genotype [43]. The occurrence of frequent polymorphic genetic variations in a population can potentially result in variations in the activity of specific proteins, which could increase the susceptibility to infection. Several studies have been conducted on the association between ACE and ACE2 with SARS-CoV-2. An Indian study worked with these two polymorphisms and found a significant association [44]. In another study, a functional insertion/deletion polymorphism-rs4646994 I/D in the ACE gene was strongly associated with SARS-CoV-2 infection and mortality in Saudi Arabia [45].

Recent studies have shown conflicting evidence on the role of various ACE2 genetic variants in Covid-19 infection rate and severity. Möhlendick et al. revealed that ACE2 rs2285666 gene polymorphism impacts the risk for SARS-CoV-2 infection [46] which is similar to our finding of this research. We found that people with the ACE2-AA genotype are more susceptible to the infection ($p<0.0001$). Srivastava et al. found a positive correlation between rs2285666 and lower infection and fatality rates among Indian populations [47]. There have been reports that the ACE2 gene may have the ability to alter the possible rate of infection in the Italian population [48]. Pati et al. and Pabalan et al. suggested that the I/D polymorphism of ACE could impact COVID-19 outcomes [32, 49]. In a subset of the Pakistani population, researchers investigated the severity of ACE2 polymorphisms and susceptibility to severe SARS-CoV-2 and found some associations [50]. On the other hand, Karakaş et al. were unable to demonstrate a link between rs2106809 and rs2285666 and the progress of Covid-19 in a group of 155 patients [51]. Similarly, three different studies in different regions of European people showed no significant relation between ACE2 polymorphism and infection of

Covid-19 and severity [33], [52], [53].

In this study, the correlation between the ACE and ACE2 gene genotypes in patients who had recovered from Covid-19 was analyzed (Table 3). The result demonstrated that individuals with the ACE-ID and ACE2-AA genotypes are at a higher risk ($p<0.0001$) of being infected by the virus when compared to other groups. Moreover, examining the link between the ACE-ID and ACE2-AA genotypes and comorbidities, it was found that people who had diabetes are at an increased likelihood of SARS-CoV-2 infection.

Conclusion

Our study findings support the hypothesis that ACE I/D rs4646994 and ACE2 rs2285666 polymorphisms are associated with the rate of SARS-CoV-2 infection. The risk factors for infection are allele D of rs4646994 and allele A of rs2285666. Individuals with ACE-ID+ACE2-AA genotype are particularly vulnerable to the virus, especially those who have diabetes and hypertension. Therefore, ACE I/D and ACE2 polymorphisms could serve as useful tools for predicting disease development and influencing treatment outcomes for Covid-19. However, limitations of the study include a small sample size of only 100 Covid-19 infected cases and 50 healthy controls, requiring further investigation with a larger cohort to gain a better understanding of the association between different genotypes of ACE and ACE2 gene and susceptibility to SARS-CoV-2.

Acknowledgments

The study was supported by the Ministry of Science and Technology, GoB project no. BS-3 (2020-2021). The authors acknowledge Covid-19 survivors and healthy controls for participating in this study.

Conflict of Interest

The authors declare no conflict of interest.

Authors' contributions

MRI, HK and AHMNN conceived the study. MRI and AHM NN and SBH designed the experiments. MRKR and SBH prepared the questionnaire. MRKR and SBH collected the data. MRKR and SBH performed the experiments and analyzed the data. MRKR wrote the original draft of the manuscript and all authors critically revised the manuscript.

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