International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2024; 7(3): 176-184
Received: 13-05-2024
Accepted: 21-06-2024

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The prognostic value of angiotensin I converting enzyme gene polymorphism and streptococcus pneumoniae coinfection in COVID-19 patients

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DOI: https://doi.org/10.33545/pathol.2024.v7.i3c.600

Abstract
Background: Angiotensin converting enzyme (ACE) deletion/deletion polymorphisms represent a marker of thrombosis, increasing thrombosis risk in unpredisposing individuals, especially in severe corona virus disease (COVID-19) pneumonia, and playing a crucial role in early disease progression. This work aimed to investigate the prognostic value of ACE gene polymorphism among COVID-19 individuals developing Streptococcus pneumoniae coinfection.
Methods: The retrospective observational case control study included sixty cases, suffering from COVID-19 pneumoniae hospitalized between December 2021 and March 2022. All participants were categorized equally into two groups: Group (I): cases having COVID-19 were classified into two subgroups [(Ia) (n=6): positive streptococcus pneumoniae coinfection and (Ib) (n=54): negative streptococcus pneumoniae coinfection] and group (II): apparently healthy people as a control group.
Results: Lactate dehydrogenase (LDH), ACE levels, PaO_2, bacteriological examination, genotypic distribution of the insertion (I) deletion (D) polymorphism of the ACE showed a significant variation among the analyzed groups ($p<0.05$). In comparison of demographic, clinical, laboratory data with ACE insertion deletion gene polymorphism were a significant difference regarding pulmonary embolism and ACE. ROC curve which displayed the expected outcome values of ACE I D polymorphism showed 77% sensitivity, 70% specificity. Outcome of COVID19 and outcome with ACE insertion deletion gene polymorphism were a significant difference in comparing outcome of 2 groups ($p<0.05$).
Conclusions: COVID-19 patients who have the ACE insertion deletion gene polymorphism have poor clinical outcomes. The ACE I/I genotype is protective against severe disease, since cases having the I/I genotype exhibited greater survival rates and those having the D/D genotype are more likely to die at a higher rate.

Keywords: Angiotensin I converting enzyme, polymorphism, streptococcus pneumoniae, COVID-19

Introduction

Since 2020, the corona virus disease (COVID-19) occurrence has become evident worldwide, and it was then regarded as a pandemic disease. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is thought to be the cause of this condition, causing pneumonia and potentially fatal respiratory distress syndrome, despite efforts to control the virus [1, 2].

COVID-19 likely evolved naturally within bat populations. Then, it has rapidly increased in many countries since being considered to be a pandemic by the World Health Organization in March 2020 [3, 4].

Fatality rates are influenced by infection speed, population idiosyncrasies, confinement policies, socioeconomic conditions, and health system concentration limits [5]. The high transmission rate and novelty of the virus have led to global emergencies [6].

The interaction between a cell and a virus occurs when a transmembrane glycoprotein spike (S) connects to Angiotensin converting enzyme type 2 (ACE2), resulting in the unopposed effects of Angiotensin II. This leads to vasoconstriction, endothelial damage, endovascular thrombosis, along with an elevated blood volume [7].

Additionally, ACE cleaves Angiotensin (Ang) I to produce AngII, Whilst ACE2 converts

Ang II in the protective Ang 1-7. When the equilibrium between ACE and ACE2 activities is evident in COVID-19, this could have a substantial impact on the thrombo-inflammatory process [8].

Adult Respiratory Distress Syndrome (ARDs) early stages involve increased permeability pulmonary oedema, alveolar epithelial cell loss, neutrophil infiltration, along with remodelling. Renin-angiotensin system activation affects ARD pathogenesis through its effect on vascular permeability [9], vascular tone [10], fibroblast proliferation [11], along with reducing the survival rates of alveolar epithelial cells. Therefore, Angiotensin II could be a substantial etiologic factor of ARDs [10].

Insertion/deletion polymorphisms of ACE associated with ARDS and linked to its outcome. ACE deletion/deletion polymorphisms represent a marker of thrombosis, increasing thrombosis risk in unpredisposing individuals, especially in severe COVID-19 pneumonia, and playing a crucial role in early disease progression [12].

Co-occurrence of bacterial infections remains infrequent among COVID-19 patients [13], particularly those caused by streptococcus pneumoniae [14], especially in comparison to other seasonal respiratory viruses [15].

This work was designed to investigate the prognostic value of Angiotensin I converting Enzyme gene polymorphism among COVID-19 individuals developing Streptococcus pneumoniae coinfection.

Patients and Methods

The retrospective observational case control study included 60 cases developing COVID-19 pneumoniae who were admitted to hospitals within the period from December 2021 to March 2022 and 60 healthy individuals as control. Our research got approved from the Ethical Committee Tanta University Hospitals, Tanta, Egypt with approval code 35032/11/21. We asked all the participants or their relatives to sign a written consent.

Our team excluded alternative causes of lung lesions, such as individuals diagnosed with TB, invasive pulmonary fungal infection, or suspected lung tumours (primary or metastatic) in addition to cases who had inadequate medical records along with those for whom no culture was taken.

Our study's participants underwent an equal division into two groups as follows: Group (I): included COVID-19 cases, who underwent a classification into two subgroups [(Ia) (n = 6): Positive streptococcus pneumoniae coinfection I addition to (Ib) (n=54): negative streptococcus pneumoniae coinfection]. While group (II) included healthy controls.

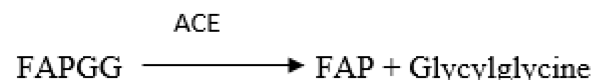
Firstly, a detailed medical history was obtained from the study's participants. Then, some laboratory tests were conducted, which involves [Complete Blood Count (CBC), C reactive protein (CRP), ferritin, interleukin 6, procalcitonin, lactate dehydrogenase, renal function test, liver function testing involving liver enzymes (Alanine amino transferase (ALT), Aspartate aminotransferase (AST)), total and direct bilirubin, prothrombin time along with INR, partial thromboplastin time (PTT), D-Dimer as well as fibrinogen, PaO_2 along with sputum and blood culture], radiological investigations [CT pulmonary angiography as well as lower limb compression ultrasonography] in addition to specific laboratory investigations [Assessment of Angiotensin I converting Enzyme (ACE I/D polymorphism) (rs1799752 SNP) by polymerase chain reaction (PCR)].

Isolation and identification of the infecting organism

Culturing was performed for all samples utilizing MacConkey, nutrient agar in addition to Blood agar plates. An anaerobic incubation was then conducted at 37 °C for 24 hours. After culturing, Gram stain smears were then produced to preserve samples from being contaminated. They were viewed thoroughly under a microscope. Identifying the isolates within the primary plates were done through: Colonial morphology: The size, shape, surface and color of the colony, characteristic feature of the growth such as type of hemolysis on blood agar plate and pigment production were evaluated and microscopic examination: Gram-stained film was prepared and examined. Identification and sensitivity were done by automated microbiology analyzer (BD phoenix) (Becton Dickinson, USA).

The Angiotensin Converting Enzyme (ACE) Evaluation was performed utilizing the Biochemical analyzer model AE-600N manufactured by Tokyo, Japan.

Initially, ACE activity was measured using Angiotensin I as the natural substrate, while the resulting products were identified with bioassay, radioimmunoassay, HPLC, or chemical techniques. Utilizing hippuryl-L-histidyl-L-leucine as a substrate resulted in creating spectrophotometric along with spectrofluorimetric assays that were more convenient to handle. Nevertheless, such methods were still not perfectly suitable for automated analysis. The infinite ACE reagent is derived from the technique first outlined by Holmquist *et al.* in 1979. This approach involves the hydrolysis of the direct substrate N-(3-(2-furyl)-acryloyl]-l-phenyl-alanylglyclglycine (FAPGG) to produce FAP and Glycylglycine as follows:



The FAPPG hydrolysis by ACE leads to an absorbance decrease at 340 nm [16].

Assessment of Angiotensin I converting Enzyme (ACE I/D polymorphism) (rs1799752 SNP) by polymerase chain reaction (PCR)

The Proteinase K dissolution was done in 1.1 ml of distilled water (ddH_2O) after which it was vortexed to achieve thorough dissolution. The bottle's box was examined. Centrifuge was done for a few seconds, which was crucial to spinning the mixture down after it had entirely dissolved. Keeping the mixture of ddH_2O and Proteinase K mixture needs to be conducted at 4 ºC for extended periods. Since ambient CO_2 could rapidly produce acidification, utilizing only fresh ddH_2O remains essential. To the Wash Buffer, adding 100 ml of absolute ethanol was conducted. Then, shaking the mixture took place for a few seconds. The bottle's box was examined. After each usage, the bottle needs to be firmly closed to prevent ethanol evaporation. The samples were allowed to reach room temperature (RT). The temperature of a heating block was raised to 56 °C. Every centrifugation step was conducted at room temperature. Cell Lysis: adding 200 µl of GSB Buffer was conducted then underwent mixing through a vigorous shaking. Incubation was carried out at 60 ºC for 5 minutes,

and tube inversion was repeated every 2 minutes. DNA binding, the sample lysate was immediately mixed by adding 200 µl of absolute ethanol and underwent a vigorous shaking for 10 seconds. If precipitate is visible, it was broken up with a pipette as much as possible. Placing a GS Column was carried out within a 2 ml collection tube. The entire mixture (including any insoluble precipitate) was transferred to the GS column. For one minute, centrifuge was carried out at 14–16,000 x g. If the combination did not permeate the GS column membrane after centrifugation, the centrifuge duration was increased till it entirely passes through. The 2 ml collection tube containing the flow-through was discarded, and GS column was then transferred to a new 2 ml collection tube. Adding 400 µl of W1 Buffer was done to the GS Column. It was centrifuged at 14–16,000 x g for 30 seconds, and the flow-through was then discarded. Placing the GS Column was done back within the two ml collection tube. The 600 µl of Wash Buffer with the addition of absolute ethanol to the GS Column then underwent centrifugation at 14-16,000 x g for thirty sec and the flow-through was discarded. The GS Column was placed back within the two ml Collection Tube. Centrifugation was then applied for three mins at 14-16,000 x g, which is essential for drying the column matrix. The dried GS Column was transferred to a clean 1.5 ml microcentrifuge tube and the 100 µl of pre-heated elution buffer was added to the column matrix center. It was stood for a minimum of three mins till a complete absorption occurs, then centrifuged at 14-16,000 x g for thirty sec, which is essential for the purified DNA elution. The DNA Elution step was repeated, which improved DNA recovery as well as the total elution volume to approximately 200 µl for higher DNA yield.
The DNA extracts were amplified using conventional Polymerase Chain Reaction (PCR). Separating the amplified products was carried out through gel electrophoresis.

DNA extracts were amplified for ACE I/D polymorphism (rs1799752 SNP) using the primers: Forward: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and Reverse: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'
Individual PCR reactions were conducted within a total volume of 33µl using 1µlof DNA extract, 1µl of each primer, 20µl nuclease free water and 10 µl master mix (containing 0.2 unit/µl nTaq-Hot DNA Polymerase, 4 mM Mg^{2+} nTaq-HOT Buffer, 0.4 mM each dNTP mixture, Stabilizer, Xylene cyanol and Orange G dyes) (2X TOP simple TM DyeMIX-HOT) to make up the volume.

Visualization of amplified material by gel electrophoresis: Two grams of agarose were dissolved by boiling in 100 ml of 1X TAE buffer till become transparent, cooling the solution was conducted to 60°C then adding 4 µl of the ethidium bromide took place for good visualization. The gel was poured on to a horizontal glass submarine to provide a gel about 3 ml in depth. After the gel had set completely for 15-20 minutes, the combs were removed. The gel was then transferred to the submarine chamber. Diluted 1x TAE buffer was poured to cover the gel thickness in the submarine chamber to allow the passage of the electric current. Mixing 1 µl of the loading buffer with 5 µl of the amplified sample was carried out within the sterile tube. Then loading the mixture was carried out to each well in the gel. A PCR marker (ladder) was used. The wells were positioned at the negative electrode to migrate from negative to positive electrode. Samples were allowed to separate for 15 min at 120 volts.

Ultraviolet Tran's illumination of the agarose Gel: placing the gel on the filter area of the UV transilluminator was conducted. Darkening the room took place, and the gel was viewed with ultraviolet shield and weared protective ultraviolet blocking eyeglasses.

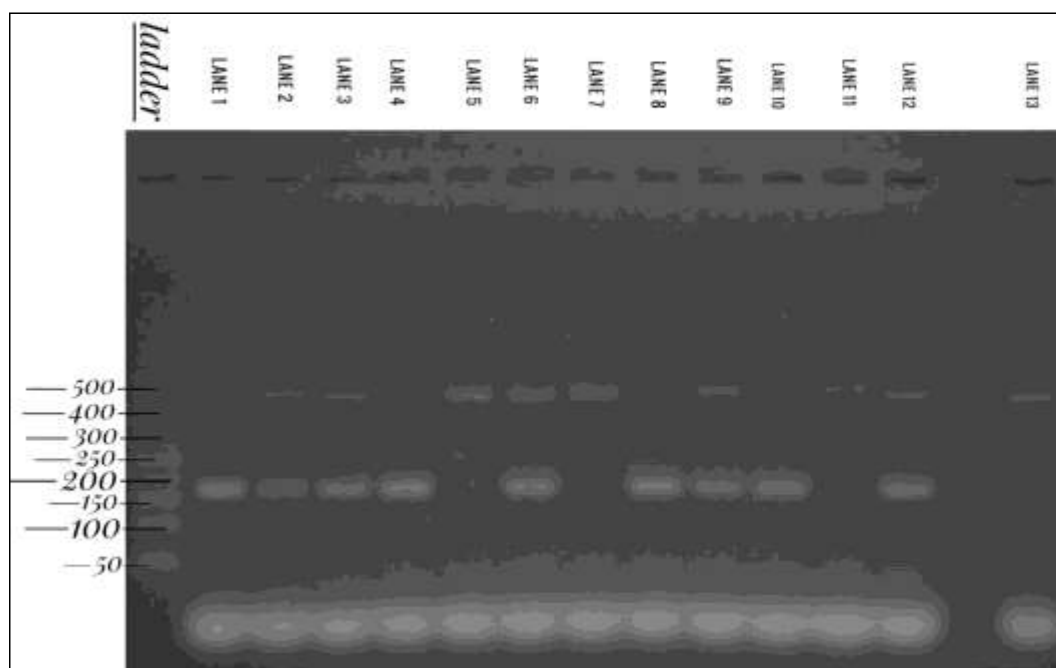


Fig 1: Gel electrophoresis result with DNA bands in lanes 1 to 13, alongside a DNA ladder for size reference

Interpretation of results

While detecting the samples, they were clear, sharp, distinct band at specific molecular weight of 481bp for insertion allele and 192bp for deletion allele. Lanes 1, 4, 8, 10 are homozygous mutant type (DD). Lanes 2, 3, 6, 9, 12 are heterozygous (ID). *In vitro* Real-Time PCR diagnostic test

for Coronavirus (COVID-19): supplied by (Primer design TM Ltd Company).

***In vitro* Real-Time PCR diagnostic test for Coronavirus (COVID-19): Supplied by (Primer design TM Ltd Company)**

Negative Extraction Control (NEC): It was prepared when extraction was carried out. This NEC served as the negative control for the entire testing system.

RNA extraction: Diluting the internal extraction control was done in 1000 µl template preparation buffer. The extraction was carried out according to the instructions provided by the extraction system's IFU. The primer design suggests adding 20 µl per sample. It is not advisable to directly include the internal extraction control into the raw sample.

Master Mix Setup: A mixture of these reagents was prepared: Component 1 × Volume required (µl) oasig qPCR OneStep MasterMix 10 Coronavirus (COVID-19) CE IVD Primer/Probe, 12µl was added into each sample well required for testing, and also one well for the positive control (PCT) is included, one well for the negative extraction control (NEC) as well as one well for negative control (NTC) were prepared. 8 µl of these was added to the suitable wells based on the plate setup. Samples [PCT; re-suspended in 800 µl kit re-suspension buffer (vortex to mix), NEC, NTC and the plate was sealed appropriately then placed in the instrument]. Prior to sample results interpretation, it remains crucial to verifying how successful the run is through these criteria: [NEC exhibits no amplification in the FAM (465-510) channel, NEC yields a CT value of less than 30 in the VIC/HEX/Yellow 555 (533-580) channel, and PCT yields a CT value ranging from 14 to 22 in the FAM (465-510) channel].

Statistical analysis

Our team analyzed the data statistically utilizing SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative variables were showcased as mean and standard deviation (SD), while their differences among the three groups were examined with ANOVA (F) test with a Tukey post hoc analysis. Qualitative variables were illustrated through frequency and percentage (%) and analyzed via the Chi-square test. The ROC curve was employed to assess diagnostic performance, evaluating sensitivity, specificity, positive predictive value (PPV), along with negative predictive value (NPV). A two-tailed P value of less than 0.05 was deemed statistically significant.

Results

Age, BMI, HTN, type 2 DM, cardiovascular illnesses, COPD, drugs that were given to individuals and lower limb compression ultrasonography showed insignificant variation among groups. Sex, smoking habit, and CT pulmonary angiography showed a significant difference among groups ($p<0.05$). Table 1

Table 1: Demographic, clinical and radiological data distribution among the studied groups

		G Ia (n=6)	G Ib (n=54)	G II (n=60)	P
Age (Years)		67.77±12.34	62.26±7.81	57.70 ± 7.66	0.174
		P1= 0.520, P2=0.135, P3=0.143			
Sex	Male (%)	4 (66.7%)	42 (77.8%)	26 (43.3%)	0.012*
	Female (%)	2 (33.3%)	12 (22.2%)	34 (56.7%)	
BMI (kg/m^2)		24.43±1.50	25.23±1.43	24.58±1.38	0.204
		P1=0.360, P2=0.864, P3=0.102			
Clinical data					
Smoking habit		4(66.7%)	24(18.5%)	20(43.3%)	0.003*
HTN		4(66.7%)	24(18.5%)	34(56.7)	0.322
Type2DM		2(33.3%)	12(22.2%)	2(3.3%)	0.065
Cardiovascular diseases		0(0.0%)	12(22.2%)	10(16.7%)	0.367
COPD		0(0.0%)	8(14.8%)	0(0.0%)	0.073
Angiotensin II receptor antagonist		2(33.3%)	14(25.9%)	18(30.0%)	0.856
ACE inhibitor		2(33.3%)	6(11.1%)	16(26.7%)	0.102
Radiological data					
CT		6(100.0%)	18(33.3%)		0.001*
Lower limb ultrasonography		0(0.0%)	2(3.7%)		0.289

Data are illustrated through mean ± SD or frequency (%). * Significant p value <0.05. P1: Group Ia versus Group Ib, P2: Group Ia versus Group II, P3: Group Ib versus Group II. BMI: Body mass index, HTN: hypertension, DM: diabetes mellitus, COPD: chronic obstructive pulmonary disease, ACE: Angiotensin-converting enzyme, CT: Computed topography.

Platelet and total leucocytic count, as well as the neutrophilic and lymphocytic percentage, renal function tests, aminotransferases levels (AST, ALT), coagulation profile, CRP, ferritin, IL6 and procalcitonin levels demonstrated a significant variance between the analyzed groups ($p<0.05$), Hb and total and direct bilirubin were insignificantly different among groups. Table 2

Table 2: Laboratory investigations in all groups

	G Ia (n=6)	G Ib (n=54)	G II (n=60)	P
CBC				
Hb (g/dl)	11.82±1.25	12.53±1.76	12.26±1.76	0.223
	P1= 0.271, P2- 0.120, P3=0.671			
Platelet(×10^3/cmm)	183.89±36.28	215±82.31	316.83±82.5	0.001*
	P1=0.439, P2=0.001*, P3= 0.013*			

TLC (×10^3/cmm)	12.67±6.04	6.11±2.05	7.85±1.49	0.001*
	P1=0.001*, P2= 0.001*, P3=0.001*			
Lymphocyte (%)	16.67±3.51	8.04±2.39	29.57±6.56	0.001*
	P1=0.001*, P2=0.001*, P3=0.001*			
Neutrophil (%)	79.67±12.74	71.22±6.25	66.77±6.75	0.003*
	P1= 0.001*, P2=0.001*, P3=0.001*			
Renal function test				
Urea (mg/dl)	36.56±7.25	54.67±22.14	27.43±6.97	0.001*
	P1=0.001*, P2=0.001*, P3=0.001*			
Creatinine (mg/dl)	1.02±0.05	1.23±0.32	0.80±0.14	0.001*
	P1= 0.006*, P2=0.001*, P3= 0.001*			
Uric acid (mg/dl)	5.37±0.59	5.93±0.90	3.67±0.98	0.001*
	P1=0.268, P2=0.038*, P3= 0.001*			
Liver function test				
AST (U/L)	154.33±66.27	102.81± 2.10	29.97±3.79	0.001*
	P1=0.114, P2= 0.001*, P3=0.001*			
ALT (U/L)	336.00±135.09	192.48±87.39	23.17±4.16	0.001*
	P1= 0.012*, P2= 0.001*, P3= 0.001*			
Total bilirubin (mg/dl)	0.63±0.22	0.46±0.16	0.53±0.20	0.200
	P1=0.140, P2=0.182, P3=0.365			
Direct bilirubin (mg/dl)	0.27±0.12	0.25±0.11	0.32±0.14	0.152
	P1=0.829, P2= 0.055, P3=0.525			
Coagulation profile				
INR	1.26±0.05	1.22±0.07	1.14±.08	0.001*
	P1=0.332, P2=0.001*, P3=0.006*			
aPTT (Second)	49.50 ± 2.78	45.78 ± 5.56	31.03 ± 3.46	0.001*
	P1=0.181, P2=0.001*, P3=0.001*			
D-Dimer (ng/ml)	3611.33±269.9	770.1±203.0	138.83±41.5	0.001*
	P1=0.001*, P2=0.001*, P3=0.001*			
Fibrinogen (mg/dl)	501 ± 69.07	449.04±44.2	317.07±59.1	0.001*
	P1= 0.114, P2=0.001*, P3= 0.001*			
CRP (mg/dl)	164.67±59.88	57.56 ± 22.63	3.64± 1.4	0.001*
	P1=0.001*, P2=0.001*, P3= 0.001*			
Ferritin (µg/l)	1465 ± 541	880.56±261.3	21.4 ± 2.7	0.001*
	P1= 0.044*, P2= 0.001*, P3=0.001*			
IL6 (ng/ml)	174.57 ±81.56	94.84±9.95	12.3 ±2.97	0.001*
	P1= 0.001*, P2= 0.001*, P3=0.001*			

Data are presented as mean ± SD. * Significant p value <0.05. P1: Group Ia versus Group Ib, P2: Group Ia versus Group II, P3: Group Ib versus Group II, CBC: complete blood count, Hb; hemoglobin, TLC: Total leucocyte count, AST: aspartate aminotransferase, ALT: Alanine aminotransferase, INR: International normalized ratio, aPTT: activated partial thromboplastin time, CRP: C-reactive protein, IL6: Interleukin 6.

LDH, ACE levels, PaO_2, bacteriological examination, genotypic distribution of the insertion deletion polymorphism of the ACE showed a significant variance among the analyzed groups ($p<0.05$). Insertion (I) as well as deletion (D) allele frequencies showed insignificant variation among groups. Table 3

Table 3: LDH, ACE, ACE and allele frequency of ACE insertion deletion genotype polymorphism in the studied groups

		G Ia (n=6)	G Ib (n=54)	G II (n=60)	P
LDH (U/L)		360.00±6.25	292.85±11.97	210.67±47.07	0.001*
		P1=0.002*, P2=0.001*, P3=0.001*			
ACE (U/L)		45.16 ± 8.36	56.07 ± 1.37	81.07 ± 10.50	0.001*
		P1=0.061, P2=0.038*, P3=0.001*			
PaO_2 (mmHg)		75.0 ± 7.21	79.43 ± 5.60	89.63 ± 0.61	0.001*
		P1=0.047*, P2=0.001*, P3=0.001*			
Bacteriological examination					
Sputum culture	Negative	4(66.7%)	54(100.0%)		0.001*
	Positive	2(33.3%)	0(0.0%)		
Blood culture	Negative	2(33.3%)	54(100.0%)	60(100.0%)	0.001*
	Positive	4(66.7%)	0(0.0%)	0(0.0%)	
ACE insertion deletion polymorphism	I / I	0(0.0%)	8(14.8%)	30(50.0%)	0.008*
	I / D	0(0.0%)	32(59.3%)	16(26.7%)	0.015*
	D / D	6(100.0%)	14(25.9%)	14(23.3%)	0.018*
D allele/ I allele	D	12(100%)	82(75.9%)	76(63.3%)	0.092
	I	0(0.0%)	26(24.1%)	44(36.7%)	

Data are illustrated through mean ± SD. * Significant p value <0.05. P1: Group Ia versus Group Ib, P2: Group Ia versus Group II, P3: Group Ib versus Group II, LDH: Lactate dehydrogenase, ACE: angiotensin converting enzyme.

While comparing demographic, clinical, laboratory data with ACE insertion deletion gene polymorphism, there were a significant difference regarding pulmonary embolism and angiotensin converting enzyme, but insignificant differences with other data Table 4.

Table 4: The comparison between the ACE insertion deletion gene polymorphism and the demographic, clinical and radiological data, routine and specific laboratory finding and bacteriological data

		I / I (n=8)	I / D (n=32)	D / D (n=20)	P
Age (years)		63.25±10.8	60.69± 6.93	59.40± 5.56	0.657
Sex	Male (%)	8 (100.0%)	24 (75.0%)	14 (70.0%)	0.475
	Female (%)	0 (0%)	8 (25.0%)	6 (30.0%)	
BMI (kg/m^2)		24.80± 1.26	24.84± 0.98	25.77 ± 1.96	0.250
Clinical data					
Smoking habit		2 (25.0%)	8 (25.0%)	2 (10.0%)	0.683
HTN		4 (50.0%)	18 (56.3%)	6 (30.0%)	0.422
Type 2DM		2 (25.0%)	10 (31.3%)	2 (10.0%)	0.458
Cardiovascular diseases		0 (0.0%)	10 (31.3%)	2 (10.0%)	0.236
COPD		0 (0.0%)	6 (18.8%)	2 (10.0%)	0.572
Angiotensin II receptor antagonist		2 (25.0%)	10 (31.3%)	4 (20.0%)	0.817
ACE inhibitor		0 (0.0%)	6 (18.8%)	2 (10.0%)	0.572
Radiological data					
C.T. pulmonary angiography (pulmonary embolism)		0 (0.0%)	8 (25.0%)	16 (80.0%)	0.004*
Lower limb ultrasonography (Deep vein thrombosis)		0 (0.0%)	2 (6.3%)	0 (0.0%)	0.636
Labotatory data					
CRP (mg/dl)		38.50±16.55	62.0±28.61	90.20±42.70	0.295
Ferritin (µg/l)		613 ±248.92	944.6±395.1	1060.4±482.94	0.551
IL6 (ng/ml)		97.75±12.1	93.67±10.52	119.47±46.77	0.277
Procalcitonin (ng/ml)		0.36±0.06	0.39± 0.08	1.86±0.75	0.072
Hb (g/dl)		11.15±1.11	11.91±1.07	12.16±1.65	0.430
Platelet ×103/cmm		167.00±27.3	183.56±39.7	200.50±48.16	0.366
TLC ×103/cmm		5.93±0.80	6.00±0.98	7.42±3.29	0.432
Lymphocyte (%)		20.50±5.32	21.69±4.56	18.10±8.90	0.394
Neutropil (%)		74.25±9.18	74.88±6.44	76.10±9.52	0.899
Urea (mg/dl)		36.75±11.30	35.56±7.54	43.50±13.33	0.168
Creatinine (mg/dl)		1.03±0.03	1.02±0.05	1.08±0.20	0.467
Uric acid (mg/dl)		4.93±0.45	5.46±0.65	5.59±0.61	0.204
AST		75.00±33.81	90.56±44.55	149 ±61.68	0.132
ALT		168 ±80.84	179.19±86.6	263.9±119.25	0. 259
Total bilirubin		0.43± 0.15	0.46± 0.18	0.53 ± 0.19	0. 542
Bilirubin direct		0.2 ± 0.08	0.27 ± 0.14	0.25 ± 0.08	0. 634
PT		14.13 ±0.81	14.47±0.63	14.91±0.43	0.067
INR		1.24±0.04	1.21±0.08	1.25± 0.04	0.302
aPTT		43.75±9.39	46.06± 4.28	47.25± 5.58	0.567
D-Dimer		379.75±136.84	838.9±245.5	1548.5±638.51	0.105
Fibrinogen		480.50±66.10	438.5±41.09	468.90±47.40	0.149
LDH		296.5±10.7	292.1± 13.5	312.7± 33.7	0.087
ACE		37.55±7.53	45.72± 9.04	50.58± 5.26	0.029*
PaO_2		84.5±2.38	82.72± 6.1	79.9± 6.19	0.337
Bacteriological data					
Sputum culture	Negative	8 (100.0%)	32 (100.0%)	18 (90.0%)	0.355
	Positive	0 (0.0%)	0 (0.0%)	2 (10.0%)	
Blood culture	Negative	8 (100.0%)	32 (100.0%)	16 (80.0%)	0.117
	Positive	0 (0.0%)	0(0.0%)	4 (20.0%)	

Data are illustrated through mean ± SD or frequency (%). * Significant p value <0.05. BMI: Body mass index, HTN: hypertension, DM: diabetes mellitus, COPD: chronic obstructive pulmonary disease, ACE: Angiotensin-converting enzyme, CT: Computed topography, Hb; hemoglobin, TLC: Total leucocyte count, AST: aspartate aminotransferase, ALT: Alanine aminotransferase, INR: International normalized ratio, aPTT: activated partial thromboplastin time, CRP: C-reactive protein, IL6: Interleukin 6, LDH: Lactate dehydrogenase, ACE: angiotensin converting enzyme.

Outcome of COVID19 and outcome with ACE insertion deletion gene polymorphism showed a significant variation when comparing outcome of 2 groups ($p<0.05$). Table 5, Figure 1.

Table 5: The outcome of COVID19, ACE insertion deletion polymorphism and the outcome of COVID19 patients with and without Streptococcus pneumoniae coinfection

		G Ia (n=6)	G Ib (n=54)	P
Outcome	Survive	2 (33.3%)	38 (70.4%)	0.047*
	Died	4 (66.7%)	16 (29.6%)	
		Survive (n=40)	Died (n=20)	

ACE insertion deletion polymorphism	I / I	8(20.0%)	0(0.0%)	
	I / D	28(70.0%)	4(20.0%)	0.001*
	D / D	4(10.0%)	16(80.0%)	

Data are illustrated through frequency (%). * Significant p value <0.05. COVID: corona virus disease, ACE: angiotensin converting enzyme.

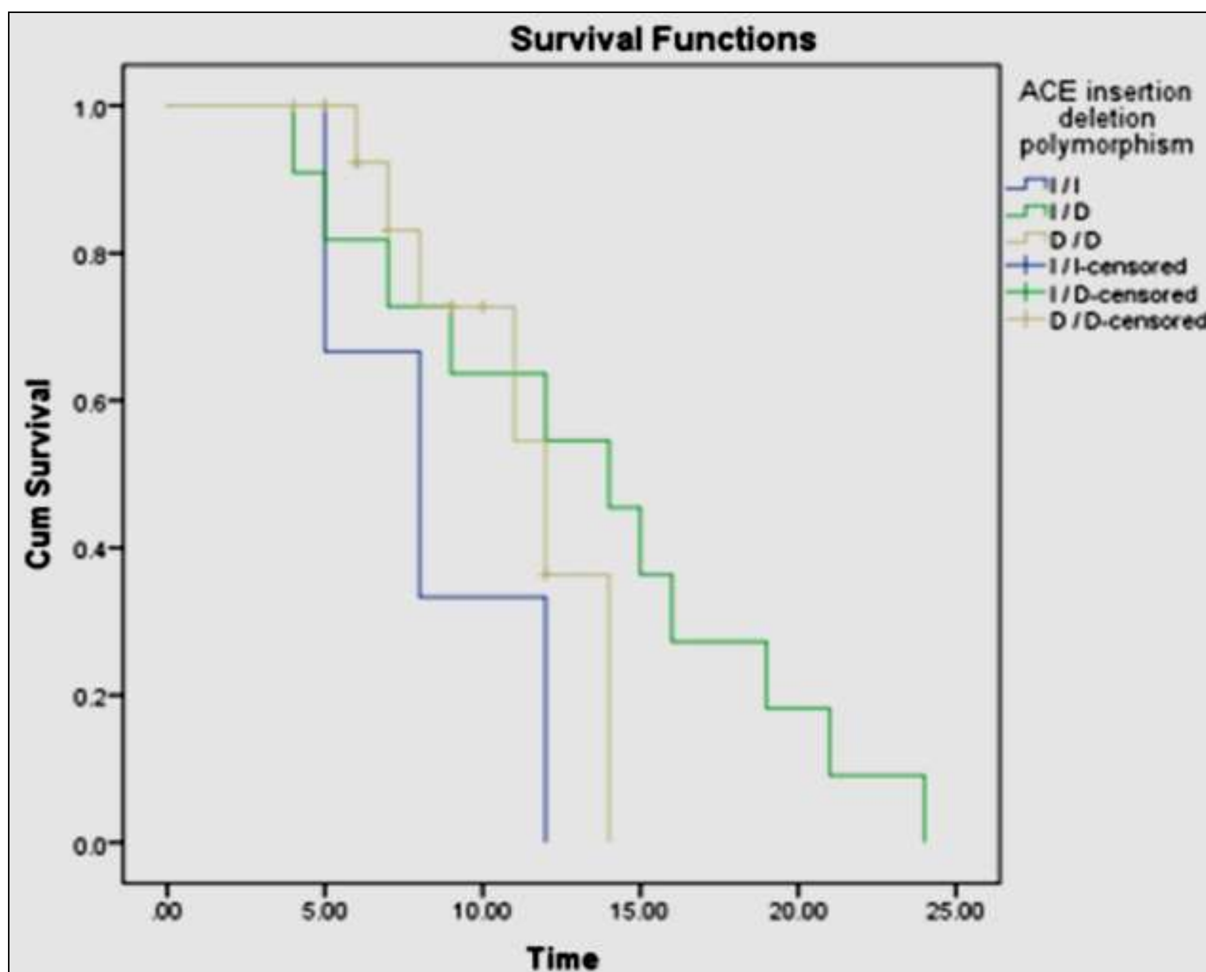


Fig 2: The Kaplan-Meier survival curve in ACE insertion deletion polymorphism and COVID19 patients

Discussion

SARS-CoV-2 remains the etiology of COVID-19 which cause a pandemic with significant medical and economic consequences [17].

In consistent with our result about radiological data, McGonagle *et al.* [18] found that the incidence of PE was higher than that of DVT due to immunothrombosis, which results from local inflammation in the pulmonary vessels.

Our research addressed a significant rise in CRP, IL6, ferritin, and procalcitonin among the COVID-19 individuals in comparison with controls, consistent with Mahat *et al.* [19] found significantly higher serum concentrations of CRP, procalcitonin, IL-6, and ferritin among individuals developing severe COVID-19 in comparison with others developing non-severe COVID-19 and among non-survivors in comparison with survivors. A significant variance was shown in PaO_2 among groups when investigated in this research, which aligned with Oliynyk *et al.* [20] who showed a significant fall in PaO_2 and a marked reduction in oxygen metabolism indices among individuals developing COVID-19 in comparison with healthy individuals and those who survived. In agreement with our result about bacteriological data, Contou *et al.* [21] indicate that a co-infection with a pathogenic bacterium was present in 28.2% of the ninety-two adult cases admitted to an ICU owing to acute respiratory failure caused by SARS-CoV-2 pneumonia. Among 32 bacteria were recovered from blood cultures and/or PCRs, 6.5% were Streptococcus pneumoniae.

As regard sputum culture, 3.3% of sixty COVID-19 patients in the present study had positive results. This aligns nearly with a retrospective study of 407 COVID-19 cases done by Cut *et al.* [22] pointed to sixty-seven cases addressed positive sputum cultures for commensal human pathogens, with Streptococcus pneumoniae being the most prevalent at 34.1%.

The present study revealed a significant decrease in ACE activity among patients with COVID-19 along with the control group, but not between COVID-19 patients who had streptococcus pneumoniae coinfection and those who do not have. Similarly, Papadopoulou *et al.* [23] retrospective case-control study demonstrated that controls exhibited significantly greater ACE activity in comparison with COVID-19 patients.

The main important findings obtained from the present work were a significant difference in ACE insertion deletion genotype. Similarly, Calabrese *et al.* [24], Papadopoulou *et al.* [23] found the significant genetic differences between cases developing COVID-19 along with controls, with COVID-19 patients having a greater occurence of D/D homozygous polymorphism.

The present study demonstrated a significant difference in ACE genotypes and pulmonary embolism between patients

under investigation. The same results were obtained by Calabrese *et al.* [24] discovered that people who had the D/D polymorphism during SARS-CoV-2 infection exhibit greater chances for pulmonary embolism.

The present study showed a positive significant association of ACE genotypes and ACE enzyme activity between the patients under investigation. This was similar to a research performed by Goren *et al.* [25] stated, patients with SARS-CoV-2 pneumonia had higher serum ACE concentrations than controls, and cases exhibiting the D/D genotype had the greatest chances, which may have contributed to endothelial dysfunction and ischemic events.

The ACE genotype and blood or sputum culture did not differ statistically significantly in this study; however, the D/D genotype was linked to a positive culture for streptococcus pneumoniae, which aligned with Ladas *et al.* revealed no correlation between the ACE I/D gene polymorphism (rs1799752) and the community-acquired pneumonia (CAP) occurence, and the rs1799752 I/I genotype of ACE was also shown to exhibit a negative correlation with a higher risk of community-acquired pneumonia.

There were insignificantly different between ACE genotypes and other demographic, clinical, and laboratory data in our research, which aligns with the results of Elbasan *et al.* [26] and Goren *et al.* [25].

Based on the present study, D/D genotype exhibited higher mortality rate than I/D and I/I genotypes, with 16/20 (80%), 4/20 (20%) and 0/20 (0%) of died COVID-19 patients have D/D, I/D and I/I genotype respectively, which supports Bellone *et al.* [27] addressing that ACE D/D polymorphism was associated with COVID-19 mortality rate higher than other genotypes.

Limitations of this study included a modest sample size. So, we recommended that larger number of patients for more comprehensive statistical analysis and better conclusions to fully understand the correlation between ACE insertion deletion gene polymorphism and poor clinical outcomes for COVID-19 patients. Testing ACE gene polymorphism in clinical trials with ACE inhibitors and ARBs could optimize treatment outcomes. Future studies should use standardized sampling and testing to study respiratory pathogens in SARS-CoV-2 cases, identifying those which are more prevalent in co-infections, and analyzing their correlation with morbidity and mortality rates. Individuals who meet the criteria should continue to get pneumococcal vaccinations in accordance with both local and national guidelines.

Conclusions

COVID-19 patients who have the ACE insertion deletion gene polymorphism have poor clinical outcomes. The ACE I/I genotype is protective against severe disease, since cases having the I/I genotype showed greater survival rates and those having the D/D genotype are more likely to die at a higher rate. Furthermore, the study suggests that a genetic predisposition to thromboembolism in COVID-19 may be due to an ACE D/D polymorphism which correlated with greater ACE levels. Streptococcus pneumoniae coinfections with COVID-19 being uncommon yet linked to high case fatalities (CFRs), especially in the elderly. About 66.6% of COVID-19 patients with streptococcus pneumoniae coinfection develop poor clinical outcomes and have the ACE D/D genotype. Therefore, when it comes to cases developing COVID-19, the ACE I/D gene polymorphism could stand as a beneficial prognostic marker for thromboembolic events along with mortality.

Financial support and sponsorship: Nil.

Conflict of Interest: Nil.

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How to Cite This Article
Abd-El-Razik SH, Abd El HMH, Hantera MS, Badr El Din NM, Abd El Halim SM. The prognostic value of angiotensin I converting enzyme gene polymorphism and streptococcus pneumoniae coinfection in COVID-19 patients. International Journal of Clinical and Diagnostic Pathology. 2024;7(3):176-184.

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