Abstract.
The recent coronavirus outbreak from Wuhan China in late 2019 caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) resulted in a global pandemic of coronavirus-19 disease (COVID-19). Understanding the underlying mechanism of the pathogenesis of coronavirus infection is important not only because it will help in accurate diagnosis and treatment of the infection but also in the production of effective vaccines. The infection begins when SARS-CoV-2 enters the cells through binding of its envelope glycoprotein to angiotensin-converting enzyme2 (ACE2). Gene variations of ACE2 and microRNA (miR)-196 are associated with viral infection and other diseases. The present study investigated the association of the ACE2 rs4343 G>A and miR-196a2 rs11614913 C>T gene polymorphisms with severity and mortality of COVID-19 disease using amplification refractory mutation system PCR in 117 COVID-19 patients and 103 healthy controls from three regions of Saudi Arabia. The results showed that ACE2 rs4343 GA genotype was associated with severity of COVID-19 (OR=2.10, P-value 0.0028) and ACE2 rs4343 GA was associated with increased mortality with OR=3.44, P-value 0.0028. A strong correlation between the ACE2 rs4343 G>A genotype distribution among COVID-19 patients was reported with respect to their comorbid conditions including sex (P<0.023), coronary artery disease (P<0.0001), oxygen saturation <60 mm Hg (P<0.0009) and antiviral therapy (0.003). The results also showed that the CT genotype and T allele of the miR-196a2 rs11614913 C>T were associated with decreased risk to COVID-19 with OR=0.76, P=0.006 and OR=0.54, P=0.005, respectively. These results need to be validated with future molecular genetic studies in a larger sample size and different populations.

Introduction
A recent and ongoing pandemic that originated from Wuhan China, caused by a new β coronavirus termed severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) results in a disease termed coronavirus-19 disease (COVID-19) (1). COVID-19 presents with varied clinical features ranging from asymptomatic course to acute respiratory distress syndrome associated with high morbidity and mortality (2,3). The majority of COVID-19 patients (~80%) recover by their own in due course of time, but the rest suffer from moderate to severe disease (4). To date, ~29 million people have been infected with COVID-19 resulting in more than 5.4 million

Differential impact of the angiotensin-converting enzyme-2 (ACE2 rs4343 G>A) and miR-196a2 rs11614913 C>T gene alterations in COVID-19 disease severity and mortality

MOHAMMAD MUZAFFAR MIR1, RASHID MIR2, MUSHABAB AYED ABDULLAH ALGHAMDI3, BADR ABDULMOHSIN ALSAYED4, IMADELDIN ELFAKI5, ALI AL BSHABSHE6, RABIA FAROOQ7, MUHANAND ALBUHAILY7, MUFFARAH HAMID ALHARTH8, MOHAMMAD MOHAMAD S. ALAMRI8 and ABDULLAH M. AL-SHAHRANI8

1Department of Basic Medical Sciences (Biochemistry), College of Medicine, University of Bisha, Bisha 61922; 2Prince Fahd Bin Sultan Research Chair, Department of MLT, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk 71491; 3Department of Internal Medicine, College of Medicine, University of Bisha, Bisha 61922; Departments of 4Internal Medicine and 5Biochemistry, University of Tabuk, Tabuk 71491; 6Department of Internal Medicine/Critical Care, College of Medicine King Khalid University, Abha 61421; 7Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Bisha; 8Department of Family Medicine, College of Medicine, University of Bisha, Bisha 61922, Saudi Arabia

Received January 4, 2022; Accepted April 13, 2022

DOI: 10.3892/etm.2022.11345

Correspondence to: Professor Mohammad Muzaffar Mir, Department of Basic Medical Sciences (Biochemistry), College of Medicine, University of Bisha, 8989 King Saud Road, Bisha 61922, Saudi Arabia
E-mail: mmmir@ub.edu.sa

Dr Rashid Mir, Prince Fahd Bin Sultan Research Chair, Department of MLT, Faculty of Applied Medical Sciences, University of Tabuk, G232 Duba Road, Tabuk 71491, Saudi Arabia
E-mail: rashid@ut.edu.sa

Key words: gene polymorphism, coronavirus infection, severe acute respiratory syndrome coronavirus-2, coronavirus-19 disease, pathogenesis, angiotensin-converting enzyme2, susceptibility to SARS-CoV-2, microRNA196a2, COVID-19 severity, COVID-19 mortality
mortalities (https://www.worldome-69 ters.info/coronavirus, accessed on Jan, 02, 2022).

COVID-19 has been associated with age, blood group type and ACE-2 gene polymorphism (5-7). The severity of the disease has also been linked with some comorbidities including hypertension, obesity and diabetes (8,9). Angiotensin converting enzyme (ACE) converts angiotensin (Ang) I to Ang II and breaks down bradykinin which serves a role in the control of blood pressure (10). ACE2 converts Ang II into Ang (1-7), which is a vasodilatory peptide (11). The ACE2 gene is found on chromosome Xp22 (12). The ACE and ACE2 share 42% amino acid similarity as the ACE2 originates through duplication of genes (12). The ACE2 is a glycoprotein and consists of 805 amino acids (12). The N-terminal of ACE2 (catalytic domain) is a signal peptide region containing an HEXXH zinc binding metalloprotease motif (12,13). The C-terminus of the ACE2 is the functional transmembrane domain (12).

ACE2 is expressed in the respiratory system, renal system, lungs, heart, blood vessels, testes, gastrointestinal tract and central nervous system (12). ACE and ACE2 gene variations are associated with different diseases such as hypertension, cardiovascular disease (CVD) and diabetes mellitus (14-16). The ACE2 counterbalances the ACE to regulate the level of circulating Ang II (15). Ang II is the main effector of the classic RAS (15). RAS dysfunctions are associated with pulmonary injury and acute respiratory distress syndrome caused by a number of factors such as viral infections (17). Dysregulation of ACE2 expression is associated with CVD in experimental models (15). In humans, the levels of ACE2 are elevated in atherosclerosis and heart failure (15). The SARS-CoV-2 uses the spike glycoprotein on its envelope to bind the ACE2 and enter the host cells (18). It has been reported that the binding between the spike glycoprotein of the novel coronavirus (2019-nCoV) is stronger than the binding between the ACE2 with the spike glycoprotein of the SARS virus (19). It is suggested that the ACE2 levels correlate with SARS-CoV-2 infection susceptibility (13). Our recent work found a strong association between ACE2 DD genotype and COVID-19 mortality and also reported that two genotypes ACE2-CC and CT are associated with COVID-19 severity (20).

microRNAs (miRNAs) are short non-coding RNA molecules with 18-23 nucleotides and are involved in the regulation of the expression of their target genes (21). They serve important roles in differentiation, apoptosis, inflammation, diabetes, cardiovascular disease and also in diagnosis and prognosis of various diseases (22). The genome wide association studies uncovered the association of different miRNA loci with different diseases (23-26).

It has been reported that miR-196b inhibits the hepatitis C virus (HCV) replication (27) and is gradually upregulated following COVID-19 infection (28). In a report from Turkey, miR-1962a rs3217927 SNP was found to be a very effective prognostic marker for multiple myeloma (29) but to the best of the authors' knowledge the role of miR-1962a rs3217927 SNP in COVID-19 has not been reported. The present study investigated the association of ACE2 rs4343 G>A and miR-196a2 rs11614913 C>T gene variations with the COVID-19 disease severity and mortality in a patient population from the Asir and Tabuk regions of Saudi Arabia.

Materials and methods

Study population. The present collaborative and population-based case-control study involved 117 COVID-19 patients and 200 healthy controls. The blood specimens from 117 reverse transcription (RT) PCR confirmed positive COVID-19 patients were collected from different hospitals in Saudi Arabia (Bisha, Abha and Tabuk; Table I). The patient group included 85 males and 32 females with a male to female ratio of 2.66 and their ages ranged between 32 and 69 years. The recruitment time for the patients was between January 15, 2021 and August 31, 2021. The ethical approvals were obtained from three local institutional ethics committees of College of Medicine, University of Bisha (Ref. no. UBCOM/H-06-BH-087(05/25)), University of Tabuk (Decision no. KAEK2020/4/4) and College of Medicine, King Khalid University, Abha (Ref. no. H-06-B-091) in accordance with local guidelines which complied in essence with the principles of the Helsinki Declaration. Written informed consent was obtained before the collection of blood samples from the patients.

Data collection. A structured and bilingual (Arabic and English) questionnaire was given to all study subjects before enrolling for the present study. The subjects were interviewed for details of epidemiological/demographic data, history of co-morbid conditions such as cardiovascular diseases, type 2 diabetes mellitus (T2DM), history of addiction particularly smoking and family history of any other significant diseases.

Sample collection from COVID-19 patients. A lavender top (LT) tube containing EDTA was used for the collection of 3 ml of peripheral blood from all the COVID-19 patients. The blood specimens were immediately stored at -20°C until further analyses.

Sample collection from control subjects. Written consent was obtained from healthy and age matched controls and the purpose of their participation was explained to them using a structured bilingual questionnaire. The sample collection was timed in such a way that it coincided with the routine blood draws of such subjects who reported to the hospital for their routine health checkups. This group comprised of RTPCR confirmed negative individuals who attended hospital for general health checkups. As a matter of policy, RTPCR was conducted on all those individuals who wanted to see a physician in the outpatient departments during first wave of COVID-19 pandemic. 3 ml peripheral blood samples were collected in LT tubes containing EDTA and were immediately stored at -20°C until further analyses.

Genomic DNA extraction. A commercial kit from Qiagen GmbH (DNeasy) was used for DNA extraction according to the instructions provided by the manufacturer. The extracted DNA from patients and control group was dissolved in nuclease-free water and was stored at 4°C further analyses. NanoDrop (Thermo Fisher Scientific, Inc.) was used to establish the quality and integrity of extracted DNA samples. The ratio of optical density at 260 nm (OD260) and 280 nm (OD280) was used to verify the purity of the DNA samples. The OD260/OD280 ratios ranged from 1.83-1.99, thus confirming good quality DNA.
Genotyping of ACE2 rs4343 G>A and miR-196a2 rs11614913 C>T. ACE2 rs4343 G>A and miR-196a2 rs11614913 C>T genotyping was performed by using amplification refractory mutation system (ARMS-PCR) on T100 Thermocycler from Bio-Rad Laboratories, Inc. Primer3 software (version 4, https://primer3.ut.ee/) was used to design ARMS PCR primers and the details are given in Table II.

**Preparation of PCR cocktail.** A 25 µl ARMS-PCR cocktail, containing 50 ng DNA was prepared by adding 0.25 µl solution containing 25 pmol of Fo, Ro, FI and RI primers respectively. 10 µl PCR master mix (DreamTaq Green, Thermo Fisher Scientific, Inc.) was added and the final volume of 25 µl was made by using nuclease-free double distilled water.

**Thermocycling conditions.** The thermocycling conditions included a hot start at 95˚C for 8 min, followed by 40 amplification cycles at 95˚C for 35 sec, 60˚C for miR-196a2 rs11614913 C>T and 58˚C for ACE2 rs4343 (2350A>G) for 40 sec and 72˚C for 45 sec. This was followed by an elongation step at 72˚C for 10 min and storage at 4˚C.

**Gel electrophoresis for ACE2 rs4343 G>A.** The PCR products of ACE2 rs4343 (2350A>G) genotyping were separated by electrophoresis on 2% agarose and visualized on a UV transilluminator. GelPilot 100 bp Plus ladder (100) from Qiagen (cat. no. 239046) was used as a marker. Primers Fo and Ro flank the exon of the ACE2 rs4343 (2350A>G) gene and gave a band corresponding to 268 bp that acted as a control for quality and quantity of DNA. Primers FI and Ro that amplified T allele gave a band corresponding to 190 bp and primers Fo and R1 gave a band corresponding to 125 bp as depicted in Fig. 1.

**Gel electrophoresis for miR-196a2 rs11614913 C>T.** The ARMS-PCR products for miR-196a2 rs11614913 C>T were analyzed by electrophoresis on 2% agarose gel and visualized on a UV transilluminator. Primers Fo and Ro flanked the exon of the miR-196a2 rs11614913 C>T gene and gave a band corresponding to 297 bp that acted as a control for quality and quantity of DNA. Primers Fi and Ro that amplified T allele gave a band corresponding to 199 bp and primers Fo and R1 gave a band corresponding to 153 bp from the C allele as depicted in Fig. 2.

**Healthy controls**

For ACE2 rs4343 G>A gene polymorphism. The age matched and healthy control group comprised 103 subjects out of whom 70 (67%) were males and 33 (33%) were females. The age distribution of the control group showed that 75 (72%) patients were >40 years and 28 (27%) were ≤40 years old.

For miR-196a2 rs11614913 C>T gene polymorphism. The miR-196a2 rs11614913 was studied in 200 age matched healthy controls comprising 130 (65%) males and 70 (35%) females. The age distribution of the control group showed that 146 (73%) were >40 years and 54 (27%) were ≤40 years old.

**Statistical analysis.** Deviations from Hardy-Weinberg disequilibrium (HWD) were calculated by Chi-square ($\chi^2$) goodness-of-fit test. Group differences were compared using Student’s two-sample t-test and one-way analysis of

### Table I. Baseline characteristics of the COVID-19 patients.

| Patient characteristics | n=117 | %    |
|-------------------------|-------|------|
| Age (years)             |       |      |
| >40                     | 97    | 82.90|
| ≤40                     | 20    | 17.09|
| Sex                     |       |      |
| Male                    | 85    | 72.64|
| Female                  | 32    | 27.36|
| CKD                     |       |      |
| Yes                     | 11    | 9.40 |
| No                      | 106   | 90.60|
| T2DM                    |       |      |
| Yes                     | 47    | 40.17|
| No                      | 70    | 59.83|
| Oxygen saturation       |       |      |
| <60                     | 47    | 40.17|
| >80                     | 70    | 59.83|
| Hypertension            |       |      |
| Yes                     | 37    | 31.62|
| No                      | 80    | 68.37|
| CAD                     |       |      |
| Yes                     | 17    | 14.53|
| No                      | 100   | 85.47|
| Duration in hospital (days) |   |      |
| >30                     | 57    | 48.71|
| <30                     | 60    | 51.29|
| CRP                     |       |      |
| <10 mg/l                | 13    | 2.56 |
| ≥10 mg/l                | 104   | 97.44|
| ALT                     |       |      |
| <36 U/l                 | 72    | 61.53|
| >36 U/l                 | 45    | 38.57|
| AST                     |       |      |
| <40 U/l                 | 69    | 58.97|
| ≥40 U/l                 | 48    | 41.3 |
| Steroids therapy        |       |      |
| Yes                     | 77    | 65.81|
| No                      | 40    | 34.19|
| Antiviral therapy       |       |      |
| Yes                     | 79    | 67.52|
| No                      | 38    | 32.48|
| Survival                |       |      |
| Yes                     | 43    | 36.75|
| No                      | 74    | 63.24|

CKD, chronic kidney disease; T2DM, type 2 diabetes mellitus; CAD, coronary artery disease; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate transaminase.
Table II. ARMS primer details.

| Direction     | Sequence                                           | Product size | Annealing temperature |
|---------------|----------------------------------------------------|--------------|-----------------------|
| ACErs4343 FO  | 5'-CTGAAATTCTCTTGAGCTCCCT-3'                       | 268 bp       | 58˚C                  |
| ACErs4343 RO  | 5'-GAAATGAAAGGCACCCCAAGTGC-3'                      |              |                       |
| ACErs4343 FIA | 5'-CTGACGAATGTGATGGCCTCCCA-3'                      | 190 bp       |                       |
| ACErs4343 RIG | 5'-CATAACAGGCTTCTATATTTCCCGTAC-3'                 | 125 bp       |                       |
| miR-196a2 FO  | 5'-ACCCCTTCCTTTCTCCTCCAGATAGAT-3'                  | 297 bp       | 61˚C                  |
| miR-196a2 RO  | 5'-AAAGCAGGGTTCTCCAGACTTGTTCTGC-3'                 |              |                       |
| miR-196a2 FI (T allele): | 5'-AGTTTTGAACGCAAAAACGGT-3'                  | 199 bp       |                       |
| miR-196a2 RI (C allele) | 5'-GACGAAAACCGACTGATGTAACCCGG-3'            | 153 bp       |                       |

Figure 1. ACE2 rs4343 (2350A>G) genotyping utilizing amplification refractory mutation system (ARMS-PCR) in COVID-19 patients. M, 100 bp DNA ladder; P1, P2, P5, P6, P7 and P12, heterozygous; P3, P4, P9 and P10, homozygous GG-(190 bp); P2, P8 and P11, homozygous TT-(125 bp).

Figure 2. MicroRNA-196a2 rs11614913 C>T genotyping utilizing amplification refractory mutation system (ARMS-PCR) in COVID-19 patients. M, 100 bp DNA ladder; P1, P8 and P12, heterozygous; P3, P4, P7, P9, P10, P11, P13 and P14, homozygous CC-(153 bp); P2 and P6, homozygous TT-(199 bp).
Table III. Association of ACE2 rs4343 G>A gene variation in COVID-19 cases and controls.

| Subjects | n=  | GG %  | GA %  | AA %  | G    | A    | Degree of freedom | $\chi^2$ | P-value |
|----------|-----|-------|-------|-------|------|------|-------------------|--------|---------|
| Cases    | 117 | 57 (48.71) | 53 (45.29) | 7 (5.98) | 0.71 | 0.29 | 2 | 6.10 | 0.047 |
| Controls | 103 | 65 (63.10) | 30 (29.12) | 8 (7.76) | 0.78 | 0.22 |               |        |         |

COVID-19, coronavirus-19 disease.

Results

Demographic characteristics and baseline features. The demographic features and the baseline characteristics for 117 COVID-19 patients are given in Table I. Of the patients, 97 (82.90%) were >40 years of age and 20 (17.10%) patients were ≤40 years old. From the patients, 85 (74.64%) were male and 32 (27.36%) were female. Regarding the co-morbidities, 47 (40.17%) were T2DM patients, 37 (31.62%) had hypertension and 11 (9.40%) had chronic kidney disease. A total of 47 (40.17%) patients had low oxygen saturation (<60 mm Hg) at the time of admission and 57 (48.71%) patients stayed >30 days in hospital. In the COVID-19 patient group, 79 (67.52%) patients received antiviral therapy whereas 77 (65.81%) received steroid therapy. Out of 117 COVID-19 patients, 43 (36.75%) patients succumbed and 74 (63.24%) survived and were discharged from the hospital. As can be seen in Table I, out of 117 COVID-19 patients, 45 (38.57%) had elevated levels of alanine aminotransferase, 104 (91.44%) had high levels of C-reactive protein and 48 (41.3%) had high levels of aspartate transaminase (AST).

Association of ACE2 rs4343 G>A SNP between COVID-19 patients and controls. The present study found the frequency of ACE2 rs4343 G>A in compliance to the Hard-Weinberg equation (HWE) in all the study subjects and randomly chose only 10% samples from control group to analyze genotyping results, ensuring an accuracy rate of more than 99%. The GG, GA and AA genotype frequencies were 48.71, 45.29 and 5.98% in COVID-19 patients respectively, whereas in healthy controls GG, GA and AA genotype frequencies were 63.10, 29.12 and 7.76% respectively (Table III). The difference in the distribution of ACE2 rs2323G>A genotypes in COVID-19 patients and healthy controls was significant (P<0.047). The frequency of G allele (fG) was also found to be significantly higher in COVID-19 patients as compared with the control group (0.71 vs. 0.29; Table III).

Association between ACE2 rs4343G>A genotypes and COVID-19 severity. Table IV summarizes the data on the association between ACE2 rs4343G>A genotypes and risk to COVID-19. These data were obtained by using a multivariate analysis model based on logistic regression such as odds ratio (OR) and risk ratio (RR) with 95% confidence intervals (CI). The results indicated that the COVID-19 disease severity correlated significantly with ACE2 genotypes (GG vs. GA) in the codominant model with OR 2.10 CI=1.13-3.56, RR=1.47 (1.05-2.05) and P<0.016. A strong association was also observed between ACE2 GG vs. ACE2 (GA+AA) genotype in dominant inheritance model that leads to increased COVID-19 severity with OR=1.80, 95% CI=1.04-3.08, RR=1.37 (1.01-1.85) and P<0.032 as depicted in Table IV. The A allele was not associated with COVID-19 severity with an OR 1.39, 95% CI=0.90-2.15, RR=1.20 (0.93-1.54) and P-value=0.131 on making allelic comparisons. No significance was observed between different alleles and COVID-19 severity in over dominant inheritance model. The results indicated a potential dominant effect of ACE2-AA genotype but not A allele on COVID-19 severity in the patients from Asir and Tabuk regions of KSA. The results also showed that in case of overdominant inheritance model, the ACE2 rs4343- GG+AA vs. GA genotype of the ACE2 rs4343 G>A was not associated with susceptibility to COVID-19 with OR=1.89 (1.29-1.90) and P=0.170.

Association of ACE2 rs4343 G>A genotypes with gender and comorbid conditions and COVID-19 severity. Table V summarizes the statistical comparisons (P-values) of ACE2 rs4343 G>A genotypes with comorbid conditions of COVID-19 patients and disease severity. A multivariate analysis based on logistic regression such as OD and RR with 95% CI was correlated significantly with ACE2 genotypes (GG vs. GA) in codominant model with OR 2.10 CI=1.13-3.56, RR=1.47 (1.05-2.05) and P<0.016. A strong association was also observed between ACE2 GG vs. ACE2 (GA+AA) genotype in dominant inheritance model that leads to increased COVID-19 severity with OR=1.80, 95% CI=1.04-3.08, RR=1.37 (1.01-1.85) and P<0.032 as depicted in Table IV. The A allele was not associated with COVID-19 severity with an OR 1.39, 95% CI=0.90-2.15, RR=1.20 (0.93-1.54) and P-value=0.131 on making allelic comparisons. No significance was observed between different alleles and COVID-19 severity in over dominant inheritance model. The results indicated a potential dominant effect of ACE2-AA genotype but not A allele on COVID-19 severity in the patients from Asir and Tabuk regions of KSA. The results also showed that in case of overdominant inheritance model, the ACE2 rs4343- GG+AA vs. GA genotype of the ACE2 rs4343 G>A was not associated with susceptibility to COVID-19 with OR=1.89 (1.29-1.90) and P=0.170.
Correlation of ACE2 rs4343 G>A genotypes with mortality of COVID-19 patients. In a co-dominant model, ACE2-DD genotype heterozygosity showed a strong association with increased COVID-19 mortality with OR 3.44, 95% CI=1.53-7.72 and P=0.0028 as depicted in Table VI. However, ACE2-AA genotype (GG vs. AA) was not associated with COVID-19 mortality with OR 0.51 95% CI=0.056-4.62 and P=0.55 as depicted in Table VI. In dominant inheritance model, ACE2-GA+AA genotype (GG vs. GA+AA) was strongly associated with increased COVID-19 mortality with OR 2.87, 95% CI=1.30 to 6.31 and P<0.008. However, in recessive inheritance model, ACE2-genotype (AA vs. GG+GA) was not associated with increased COVID-19 mortality with OR 0.75 95% CI=0.26-2.16 and P=0.86 (0.52-1.42) 0.60. In allelic comparisons, the A allele too did not show any association with COVID-19 mortality, with OR 1.60, 95% CI=0.90-2.86 and P=0.108, on allelic comparisons. In overdominant inheritance model, ACE2-genotype (GA vs. GG+AA) was strongly associated with increased COVID-19 mortality with OR 1.89, 95% CI=1.004 to 3.58 and P<0.040.

Potential association of miR-196a2 rs11614913 C>T genotypes with COVID-19. A multivariate analysis based on logistic regression such as OD and RR with 95% CI was used to determine the association between miR-196a rs11614913 C>T genotypes and risk to COVID-19 and the data are summarized in Table VIII. The results showed that the CT genotype of the miR-196a2 rs11614913 was associated with decreased susceptibility to COVID-19 with OR=0.452 (0.26-0.79), RR=0.76 (0.64-0.91) and P=0.006. The T allele of the miR-196a2 rs11614913 was also associated with decreased susceptibility to COVID-19 with OR=0.54 (0.35-0.84), RR=0.81 (0.71-0.92) and P=0.005 (Table VIII). The results showed that in case of the overdominant model, the miR-196-CC+TT vs. CT genotype of the miR-196a2 rs11614913 was associated with decreased susceptibility to COVID-19 with OR=0.49 (0.28-0.85), RR=0.79 (0.67-0.93) and P=0.0016.

Association of miR-196a2 rs11614913 C>T genotypes with gender, comorbid conditions and COVID-19 severity. A multivariate analysis was used to elucidate the association of miR-196a2 rs11614913 C>T genotypes with sex, comorbid conditions and COVID-19 severity and the results are summarized in Table IX. The results indicated that there was a significant difference (P=0.006) in rs11614913 genotype distribution between patients >40 years old and patients ≤40 years old (Table IX). The results also showed that there...
was a significant difference (P=0.035) in rs11614913 genotype distribution between male and female patients (Table IX). The results showed that there were significant differences in patients with hypertension and coronary artery disease.

| Patient characteristics | n=117 | GG | GA | AA | Degree of freedom | $\chi^2$ | P-value |
|-------------------------|-------|----|----|----|-------------------|--------|---------|
| Age (years)             |       |    |    |    |                   |        |         |
| >40                     | 97    | 46 | 44 | 07 | 1.64              | 2      |         |
| ≤40                     | 20    | 11 | 09 | 00 |                   |        |         |
| Sex                     |       |    |    |    |                   |        |         |
| Male                    | 85    | 42 | 41 | 02 | 7.47              | 2      |         |
| Female                  | 32    | 15 | 12 | 05 |                   |        |         |
| T2DM                    |       |    |    |    |                   |        |         |
| Yes                     | 47    | 23 | 20 | 04 | 0.97              | 2      |         |
| No                      | 70    | 34 | 33 | 03 |                   |        |         |
| CKD                     |       |    |    |    |                   |        |         |
| Yes                     | 11    | 07 | 04 | 00 | 1.5               | 2      |         |
| No                      | 106   | 50 | 49 | 07 |                   |        |         |
| Hypertension            |       |    |    |    |                   |        |         |
| Yes                     | 37    | 14 | 21 | 02 | 4.37              | 2      |         |
| No                      | 80    | 43 | 32 | 05 |                   |        |         |
| CAD                     |       |    |    |    |                   |        |         |
| Yes                     | 17    | 06 | 05 | 06 | 30.41             | 2      | 0.0001  |
| No                      | 100   | 51 | 48 | 01 |                   |        |         |
| Oxygen saturation       |       |    |    |    |                   |        |         |
| Yes                     | 47    | 15 | 29 | 03 | 9.24              | 2      |         |
| No                      | 70    | 42 | 24 | 04 |                   |        |         |
| Duration in hospital (days) |     |    |    |    |                   |        |         |
| >30                     | 57    | 25 | 29 | 03 | 1.4               | 2      |         |
| <30                     | 60    | 32 | 24 | 04 |                   |        |         |
| ALT                     |       |    |    |    |                   |        |         |
| <36 U/l                 | 45    | 23 | 20 | 02 | 0.39              | 2      |         |
| >36 U/l                 | 72    | 34 | 33 | 05 |                   |        |         |
| CRP                     |       |    |    |    |                   |        |         |
| <10 mg/l                | 13    | 51 | 47 | 06 | 0.09              | 2      | 0.956   |
| ≥10 mg/l                | 104   | 06 | 06 | 01 |                   |        |         |
| AST                     |       |    |    |    |                   |        |         |
| <40 U/l                 | 48    | 30 | 18 | 0  | 9.14              | 2      |         |
| >40 U/l                 | 69    | 27 | 35 | 07 |                   |        |         |
| Antiviral therapy       |       |    |    |    |                   |        |         |
| Yes                     | 79    | 30 | 43 | 06 | 3.87              | 2      |         |
| No                      | 38    | 27 | 10 | 01 |                   |        |         |
| Steroids therapy        |       |    |    |    |                   |        |         |
| Yes                     | 77    | 35 | 36 | 06 | 1.82              | 2      |         |
| No                      | 40    | 22 | 17 | 01 |                   |        |         |
| Survival                |       |    |    |    |                   |        |         |
| Yes                     | 43    | 14 | 28 | 1  | 11.6              | 2      |         |
| No                      | 74    | 43 | 25 | 6  |                   |        |         |

COVID-19, coronavirus-19 disease; T2DM, type 2 diabetes mellitus; CKD, chronic kidney disease; CAD, coronary artery disease; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate transaminase; NS, non-significant; SG, significant.
MIR et al: DIFFERENTIAL IMPACT OF ACE2 rs4343 G>A AND miR‑196a2 rs11614913 C>T IN COVID‑19

Table VI. Statistical comparisons (P‑values) of ACE2 rs4343 G>A genotypes with mortality of COVID‑19 patients.

| Model     | Genotype | Survival n=74 | Mortality n=43 | OR (95% CI) P‑value |
|-----------|----------|---------------|----------------|--------------------|
| Codominant| GG       | 43            | 14             | 1 (ref.)           |
|           | GA       | 25            | 28             | 3.44 (1.53‑7.72)   | 0.0028 SG          |
|           | AA       | 06            | 01             | 0.51 (0.056‑4.62)  | 0.55 NS            |
| Dominant  | GG       | 43            | 14             | 1 (ref.)           |
|           | GA+AA    | 31            | 29             | 2.87 (1.30‑6.31)   | 0.008 SG           |
| Recessive | AA       | 06            | 01             | 1 (ref.)           |
|           | GG+GA    | 68            | 42             | 3.7 (0.43‑31.86)   | 0.23 NS            |
| Allele    | G        | 111           | 56             | 1 (ref.)           |
|           | A        | 37            | 30             | 1.60 (0.90‑2.86)   | 0.108 NS           |
| Overdominant | GG+AA  | 49            | 15             | 1 (ref.)           |
|           | GA       | 148           | 86             | 1.89 (1.004‑3.58)  | 0.040 SG           |

Table VII. Association of miR‑196a2 rs11614913 C>T gene variation in COVID‑19 cases and controls.

| Subjects | n  | CC          | CT          | TT          | Degree of freedom | $\chi^2$ | C    | T    | P‑value |
|----------|----|-------------|-------------|-------------|-------------------|---------|------|------|---------|
| Cases    | 117| 90 (76.92%) | 22 (18.80%) | 05 (4.27%)  | 2                 | 9.48    | 0.86 | 0.14 | 0.008   |
| Controls | 200| 120 (60%)   | 64 (32%)    | 16 (8%)     |                   | 0.76    | 0.24 |      |         |

COVID‑19, coronavirus‑19 disease.

(CAD) compared with patients without hypertension and CAD (P=0.044 and 0.035, respectively; Table IX). Results also indicated that there was a significant difference (P=0.01) in patients with oxygen saturation <60 and those with oxygen saturation >80. Furthermore, the results showed that there was a significant difference (P=0.01) in rs11614913 genotype distribution between the patients who survived from the COVID‑19 and the patients who succumbed (Table IX).

Discussion

The diverse clinical manifestations of the SARS-CoV-2 infection vary from no symptoms to severe disease (ICU admission) and mortality in COVID-19 patients. The results of the present study indicated that there was a significant difference in the ACE2 rs4343 G>A genotype distribution between the patient and the control groups (P<0.05; Table III). Results also showed that the GA genotype of the rs4343 G>A was associated with increased susceptibility to COVID-19 (9) (Table IV). rs4343 G>A influences the activity and the levels of ACE and increases susceptibility to hypertension, T2DM, obesity, renal disease, CVD and autoimmune diseases (30). The results of the present study are consistent with a recent study that reported the association of the G allele with the SARS-CoV-2 severity in the presence or absence of metabolic and other comorbidities (30). Furthermore, it has been suggested that GG genotype of the rs4343 SNP is associated with increased circulating ACE levels and its activity (31,32). The increased activity and levels of the ACE2 are reported to increase the susceptibility to COVID-19 (33). The results of the present study seem to be in agreement with these studies (31‑33) as rs4343 GA genotype increases the activity and levels of ACE2 (30) which may increase the susceptibility to COVID-19 (9) (Table IV). The results also showed that there was a significant difference in ACE2 rs4343 G>A SNPs between male and female patients (P<0.023; Table V). This result is in agreement with earlier studies that report higher expression of ACE2 in males compared with females and the increased expression of ACE2 is reported to promote the entry of SARS-CoV-2 (9,13). It is suggested that the reduced expression of ACE2 in females renders them less sensitive to severe adverse effects of COVID-19 (13). The results of the present study also indicated that there were significant differences (P<0.05) in the rs4343 G>A genotype distribution between the patients with CAD and reduced oxygen saturation and patients without CAD and with normal oxygen saturation (Table V). This result is in agreement with a study that reports the association of the ACE2 rs4343 G>A with dyslipidemia and severity of COVID-19 (30).
Moreover, the results of the present study indicated that there was a significant difference (P<0.05) of rs4343 G>A genotype distribution with elevated patient AST levels (Table V). This result is in agreement with an earlier study that reports the association of SARS‑COV‑2 with liver dysfunction (34). As ACE2 is expressed in the hepatic tissues (9), it is possible that the rs4343 G>A SNP modulates the SARS‑COV‑2 infection and increases the liver damage but this need further validation.

The results of the present study also indicated that there was no significant difference (P>0.05) in rs4343 G>A genotype distribution between diabetic and non‑diabetic subjects (Table V). This result was rather unexpected and was inconsistent with a study that reported diabetes to increase the susceptibility to coronavirus infection since ACE2 is highly expressed in T2DM patients (9). This inconsistency may be because the sample size used in the present study was relatively small (n=117). In addition, the results showed that the genotype distribution of rs4343 was significantly different (P<0.05) between patients who needed antiviral therapy and those who did not (Table V). This result is in agreement with Íñiguez et al (30), who report that the G allele of the rs4343 increases the severity of COVID‑19.

The results of the present study indicated that the miR‑196a rs1164913 C>T genotype distribution was significantly different (P<0.05) between patients and controls (Table VII). The results also showed that the CT genotype and the T allele of the miR‑196a rs1164913 C>T were associated with the decreased risk to COVID‑19 (Table VIII). It is reported that miR‑196 is among interferon‑induced miRNAs and that miR‑196 directly targets the CORE and NS5A coding region of genomic RNA of the HCV and thereby suppresses the replication of the virus by ≤80% (45). In addition, it has been demonstrated that miR196 inhibits the expression of the HCV (46) by repressing the expression of the Bach‑1 protein (46). Bach‑1 is an inhibitor of the anti‑oxidative and anti‑inflammatory heme oxygenase 1 (HMOX1) (46,47). miR‑196 mimics significantly repress the expression of the protein Bach1 and upregulate the gene expression of HMOX1 and thereby inhibit the HCV expression (46).

In an experiment conducted in lung tissues of hamster, it was shown that miR‑196a is among five miRNAs that commonly bind to SARS‑CoV, MERS‑CoV and SARS‑CoV‑2 viruses (48). It is reported that miR‑196a is gradually upregulated after SARS‑CoV‑2 infection (48). The present study hypothesized that rs1164913 affected the immune response against SARS‑CoV‑2 and that the T allele and CT genotype carriers became less susceptible to the SARS‑CoV‑2 infection (Table VIII). The results are in partial agreement with the result of Tian et al (47), who report that miR‑196a C>T (rs11614913) is probably

| Genotypes               | Healthy controls | Covid-19 cases | OR (95% CI)     | Risk Ratio (RR) | P-value |
|-------------------------|------------------|----------------|-----------------|-----------------|---------|
| Codominant              | (N=200)          | (N=117)        |                 |                 |         |
| miR‑196a2‑CC            | 120              | 90             | 1 (ref.)        | 1 (ref.)        |         |
| miR‑196a2‑CT            | 64               | 22             | 0.452 (0.26‑0.79) | 0.76 (0.64‑0.91) | 0.006   |
| miR‑196a2‑TT            | 16               | 05             | 0.41 (0.14‑1.17) | 0.75 (0.57‑0.97) | 0.09    |
| Dominant                |                  |                |                 |                 |         |
| miR‑196‑CC              | 120              | 90             | 1 (ref.)        | 1 (ref.)        |         |
| miR‑196‑CT+TT           | 80               | 27             | 0.45 (0.26‑0.75) | 0.76 (0.65‑0.89) | 0.001   |
| Recessive               |                  |                |                 |                 |         |
| miR‑196‑(CC+CT)         | 184              | 112            | 1 (ref.)        | 1 (ref.)        |         |
| miR‑196‑TT              | 16               | 05             | 0.51 (0.18‑1.43) | 0.81 (0.63‑1.05) | 0.20    |
| Allele                  |                  |                |                 |                 |         |
| miR‑196‑C allele        | 304              | 202            | 1 (ref.)        | 1 (ref.)        |         |
| miR‑196‑T allele        | 96               | 35             | 0.54 (0.35‑0.84) | 0.81 (0.71‑0.92) | 0.005   |
| Over dominant           |                  |                |                 |                 |         |
| miR‑196‑CC+TT           | 136              | 95             | 1 (ref.)        | 1 (ref.)        |         |
| miR‑196‑CT              | 64               | 22             | 0.49 (0.28‑0.85) | 0.79 (0.67‑0.93) | 0.0016  |

COVID‑19, coronavirus‑19 disease.
associated with reduced susceptibility of HBV and HCV-related HCC, particularly in the Chinese population.

The results of the present study further showed that the carriers of the CT genotype and the T allele of the miR-196a
rs11614913 in >40-year-old patients were at reduced risk to SARS-CoV-2 infection (Table IX). It also observed that the males who are carriers of the CT genotype and T allele of the miR-196 rs11614913 were less susceptible to the SARS-CoV-2 infection compared with females (Table IX). The results also showed that miR-196 rs11614913 significantly (P>0.05) increased the risk to SARS-CoV-2 infection in patients with hypertension and CAD (Table IX). This result is consistent with a study that indicated the association of miR-196 rs11614913 with CAD (41). The results of the present study also suggested that 69% of the patients who succumbed were miR-196 rs11614913CC genotype carriers (Table IX) suggesting that CC genotype contributed to disease severity and mortality. Limitations of the present study included the relatively small sample size. Further studies with larger sample size and on different ethnic populations are recommended.

Taken together, the present study examined the association of the ACE2 rs4343 G>A and miR-196a rs11614913 C>T with the severity and mortality of SARS-CoV-2 infection in a study population from Asir and Tabuk regions of Saudi Arabia. The results clearly indicated that the GA genotype of the ACE2 rs4343 was associated with increased severity and mortality of COVID-19. The results also showed that the CT genotype and T allele of the miR-196a rs11614913 C>T were associated with decreased susceptibility of COVID-19. More studies in different ethnic populations and bigger sample sizes are necessary to further investigate the roles of genetic alterations of ACE2 and miR-196a in the molecular pathogenesis of SARS-CoV-2 and COVID-19.

Acknowledgements

The authors extend their appreciation to Dr Suhail Ahmed of the English Department, University of Bisha, for language review and editing and Dr Mohammed Jeelani of UBCOM for his technical assistance.

Funding

The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number UB-47-1442.

Availability of data and materials

The datasets used during the present study are available from the corresponding author upon a reasonable request.

Authors’ contributions

All the authors were involved in the conception and planning of the study. MMM, RM, MAAA, RF, MMSA and MA designed the study. MAAA, BAA, AAB, MMSA and AMA were involved in the recruitment of patients. BAA, AAB and AMA collected the patient data and analyzed the clinical outcomes of patients with COVID-19. MHA, RM and IE performed the experiments. MMM, RM and IE wrote the initial draft which was revised and edited by all the authors. MMM and MAAA were involved in the acquisition of grants and project administration. RM, MMM and IE confirm the authenticity of all raw data. All the authors read and approved the final version of the manuscript for publication and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The ethical approvals were obtained from three local institutional ethics committees of College of Medicine, University of Bisha (Ref. no. UBCOM/H-06-BH-087(05/25), University of Tabuk (Decision No: KAEK2020/4/4) and College of Medicine, King Khalid University, Abha (Ref. no. H-06-B-091) in accordance with local guidelines which complied in essence with the principles of the Helsinki Declaration. Written informed consent was obtained before the collection of blood samples from the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information (optional)

Prof. Mohammad Muzaffar Mir (corresponding author) is currently working as Professor and Chairman, Medical Biochemistry at College of Medicine, University of Bisha. He is actively involved in biomedical research in molecular genetics, signal transduction and cancer biology, in addition to his commitment to teaching of medical students in a problem-based and SPICES model curriculum.

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, et al: A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382:727-733, 2020.
2. Wang Z, Ye D, Wang M, Zhao M, Li D, Ye J, Liu J, Xu Y, Zhang J, Pan W, et al: Clinical features of COVID-19 patients with different outcomes in Wuhan: A retrospective observational study. Biomed Res Int 2020:2138387, 2020.
3. Alsofayan YM, Althunayyan SM, Khan AA, Hakawi AM and Assiri AM: Clinical characteristics of COVID-19 in Saudi Arabia: A national retrospective study. J Infect Public Health 13: 920-925, 2020.
4. Mahase E: Coronavirus covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. BMJ 368: m641, 2020.
5. Chegni H, Pakravan N, Saadati M, Ghaffari AD, Shirzad H and Hassan ZM: Is there a link between COVID-19 mortality with genus, age, ABO blood group type, and ACE2 gene polymorphism? Iran J Public Health 49: 1582-1584, 2020.
6. Zietz M, Zucker J and Tatonetti NP: Associations between blood type and COVID-19 infection, intubation, and death. Nat Commun 11: 5761, 2020.
7. Suryamohan K, Diwanji D, Stawiski EW, Gupta R, Miersch S, Liu J, Chen C, Jiang YP, Fellouse FA, Sathirapongsasuti JF, et al: Human ACE2 receptor polymorphisms and altered susceptibility to SARS-CoV-2. Commun Biol 4: 475, 2021.
8. Shah H, Khan MS, Dhurandhar NV and Hegde V: The triumvirate: Why hypertension, obesity, and diabetes are risk factors for adverse effects in patients with COVID-19. Acta Diabetol 58: 831-843, 2021.
9. Soldo J, Heni M, Königsrainer A, Häring HU, Birkenfeld AL and Peter A: Increased hepatic ACE2 expression in NAFL and diabetes-risk for COVID-19 patients? Diabetes Care 43: e136‑e143, 2020.

10. Wong MK: Angiotensin converting enzymes, subchapter 29D. In: Handbook of Hormones. Comparative Endocrinology for Basic and Clinical Research. Takei Y, Ando H and Tsutsui K (eds). Elsevier. pp263‑265, e291‑e295, 2016.

11. Clarke NE and Turner AJ: Angiotensin‑converting enzyme 2: The first decade. Int J Hypertens 2012: 307315, 2012.

12. Samavati L and Uhal BD: ACE2. Much more than just a receptor for SARS‑COV‑2. Front Cell Infect Microbiol 10: 317, 2020.

13. Devaux CA, Rolain JM and Raoult D: ACE2 receptor polymorphism: Susceptibility to SARS‑COV‑2, hypertension, multi-organ failure, and COVID‑19 disease outcome. J Microbiol Immunol Infect 53: 425‑435, 2020.

14. Ellfaki I, Mir R, Duhier FMA, Alotaibi MA, Alalawy AI, Barnawi J, Babakr AT, Mir MM, Altayeb F, Mirghani H and Frah EA: Clinical implications of Mir128, angiotensin I converting enzyme and vascular endothelial growth factor gene abnormalities and their association with T2D. Curr Issues Mol Biol 43: 1859‑1875, 2021.

15. Burrell LM, Harrap SB, Velkoska E and Patel SK: The ACE2 gene: Its potential as a functional candidate for cardiovascular disease. Clin Sci (Lond) 124: 65‑76, 2013.

16. Loo J, Li L, Li T, Li Y, Liu Y, Li F, Zhao H, Mainmait T and Zeyaweeding A: Association of ACE2 genetic polymorphisms with hypertension‑related target organ damages in south Xinjiang. Hypertens Res 42: 681‑689, 2019.

17. Sarzani R, Giulietti F, Di Pentima C, Giordano P and Spannella D: Disequilibria between the classic renin‑angiotensin system and its opposing arm in SARS‑CoV‑2‑related lung injury. Am J Physiol Lung Cell Mol Physiol 319: L325‑L336, 2020.

18. Ni W, Yang X, Yang D, Bao J, Li R, Xiao Y, Hou C, Wang H, Liu J, Yang D, et al: Role of angiotensin-converting enzyme 2 (ACE2) in COVID‑19. Crit Care 24: 422, 2020.

19. Wrapp D, Wang S, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al: Structural and functional characterization of SARS‑CoV‑2 spike in the prefusion conformation. Science 367: 1260‑1263, 2020.

20. Mouaddib M, Al‑Shahrani AM, Al‑Shahrani AM: Effects of ACE2 gene polymorphism with elevated serum ACE activity and major depression in an Iranian population. Psychiatry Res 200: 336‑342, 2012.

21. Fugay M, Kertész A, Siket IM, Bánhegyi V, Krácskó B, Szegedi A, Szolko M, Vajda G, Rácz I, Gulyás H, et al: Level of the SARS‑CoV‑2 receptor ACE2 activity is highly elevated in old‑aged patients with aortic stenosis: Implications for ACE2 as a biomarker for the severity of COVID‑19. GeroScience 43: 19‑29, 2021.

22. Yu D, Du Q, Yan S, Gao XG, He Y, Zhu G, Zhao K and Ouyang S: Liver injury in COVID‑19: Clinical features and treatment management. Viril J 18: 121, 2021.

23. Bensen JT, Graft M, Young KL, Sethapathy P, Parker J, Pecot CV, Crosby K, Haddad SA, Zick D, Oyarzúa‑Navarre EA, Haiman CA, et al: A survey of miRNA single nucleotide polymorphisms identifies novel breast cancer susceptibility loci in a case‑control, population‑based study of African‑American women. Breast Cancer Res 20: 45, 2018.

24. Foroudi J, Jakobik D, Jarosz‑Popek J, Wick Z, Eyleten C, De Rosa S, Indelicato C, Siller‑Matula JM, Czajka P and Postula M: Significance of circulating microRNAs in diabetes mellitus type 2 and platelet activity: Bioinformatic analysis and review. Cardiovasc Diabetol 18: 113, 2019.

25. Zhou SS, Jin JP, Wang QJ, Zhang ZG, Freedman JH, Zheng Y and Cui L: miRNAs in cardiovascular diseases: Potential biomarkers, therapeutic targets and challenges. Acta Pharmacol Sin 39: 1073‑1084, 2018.

26. Peng Y and Croe CM: The role of MicroRNAs in human cancer. Signal Transduct Target Ther 1: 15004, 2016.

27. Gholami M, Asgarbeik S, Razi F, Esfahani EN, Zoughi M, Vahidi A, Larjiani B and Amoli MM: Association of microRNA gene polymorphisms with type 2 diabetes mellitus: A systematic review and meta‑analysis. J Res Med Sci 25: 56, 2020.

28. Gafouri‑Fard S, Gholipour M and Taheri M: Role of MicroRNAs in the pathogenesis of coronary artery disease. Front Cardiovasc Med 8: 632292, 2021.

29. Sung JH, Kim SH, Yang WI, Kim JW, Moon JJ, Kim CHA, Cho SY, Kim J, Kim KA, et al: miRNA polymorphisms (miR‑146a, miR‑149, miR‑196a2 and miR‑499) are associated with the risk of coronary artery disease. Mol Med Rep 14: 2328‑2342, 2016.

30. Elwaniger JH, Zambrana‑FMB, Guzmíes RL and Chies JA: MicroRNA‑related polymorphisms in infectious diseases‑tiny changes with a huge impact on viral infections and potential clinical applications. Front Immunol 9: 1316, 2018.

31. Drury RE, O'Conor D and Pollard AF: The clinical application of MicroRNA's in infectious disease. Front Immunol 8: 1192, 2017.

32. Chen C, Zhang Y, Zhang L, Weakley SM and Yao Q: MicroRNA‑196: Critical roles and clinical applications in development and cancer. J Cell Mol Med 15: 14‑23, 2011.

33. Gupta P, Cairns MJ and Saksema NK: Regulation of gene expression by microRNA in HCV infection and HCV‑mediated hepatocellular carcinoma. Viril J 11: 60‑78, 2018.

34. Hou W, Tian Q, Zheng J and Bonkovsky HL: MicroRNA‑196 represses bcl1 protein and hepatitis C virus gene expression in human hepatoma cells expressing hepatitis C viral proteins. Hepatology 51: 1494‑1504, 2010.

35. Tian T, Wang M, Zhu W, Dui ZM, Lin S, Yang PT, Liu XH, Liu K, Zhu YY, Zheng Y, et al: miR‑146a and miR‑196‑2 polymorphisms are associated with hepatitis virus‑related hepatocellular carcinoma risk: a meta‑analysis. Aging (Albany NY) 9: 381‑392, 2017.

36. Kim WR, Park EG, Kang KW, Lee SM, Kim B and Kim HS: Expression analyses of MicroRNAs in hamster lung tissues infected by SARS‑CoV. Mol Cells 43: 953‑960, 2020. This work is licensed under a Creative Commons Attribution‑NonCommercial‑NoDerivatives 4.0 International (CC BY‑NC‑ND 4.0) License.