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ASSESSMENT OF ELEVATED LEVELS OF ANGIOTENSIN CONVERTING ENZYME II (ACE-II) IN SARS-COV-2 INFECTED PATIENTS IN PAKISTAN

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ABSTRACT

Several factors influence the prevalence of SARS-CoV-2 infection and the severity of symptoms. The host functional receptor for the SARS-CoV-2 that causes coronavirus disease 2019 (COVID-19) is angiotensin-converting enzyme 2 (ACE II). The ACE II enzyme negatively regulates Renin-Angiotensin System (RAS) found in different organs. This study was aimed to screen covid-19 patients for the elevated level of ACE II and determine the relationship between ACE II and disease severity. The results show equal disease prevalence 50.85, 49.14%, and recovery in males and females, i.e., 51.21 and 48.78%, respectively. After Enzyme-linked immunosorbent assay (ELISA), ACE II concentration was higher in COVID-19 patients compared to COVID-19 cured and healthy control. The ACE II concentration increased with age, and the highest (41.6 ±2.7 and 40.86 ± 5.78 ng/ml) were in 51-60 age groups of males and females. ACE II concentration was (40.8±1.4, 34.1 ± 4.1, 31.6 ± 9.6, 31.6 ± 9.6) in obese, overweight, underweight, and normal COVID-19 patients, respectively. Similarly, ACE II concentration was higher in patients with hypertension (43.6 ± 3.2), obesity (40.8 ± 1.4), diabetes (43.3 ± 6.7), and combination (43.3 ± 6.7) of these diseases. The baseline serum ACE II activities in COVID-19 patients increased compared healthy control group. However, the serum ACE II activities were decreased steadily in the recovery phase, and there was a significant difference among the control, COVID-19 cured, and COVID-19 groups. It is concluded that ACE II activity might be utilized as a marker to assess the clinical condition of COVID-19 as enhanced production was linked to disease severity.

KEY WORD: ACE II, COVID-19, SARS-CoV-2, hypertension, obesity, diabetes

1. INTRODUCTION

COVID-19 is caused by a new coronavirus (SARS-COV-2) that has spread at an unprecedented rate and scale to become the most severe healthcare challenge of the 21st century. Multiple SARS-CoV-2 variants have been identified according to the world health organization (WHO), including B.1.1.7 UK, B.1.351 South African, B.1.525 Nigerian, Cal.20C Californian, P.1 Brazilian, and B.1.617.2 of Indian origin.

The ACE II receptor allows SARS-CoV-2 to enter human cells.[1] SARS-CoV-2 binds strongly to the ACE II receptor in human cells, establishing a connection between COVID-19 and the Renin-Angiotensin System (RAS).[2] The ACE2-receptors are found in various tissues, including vascular endothelial, smooth muscle cells, nasal and oral mucosa, enterocytes in the intestines, and kidneys.[3, 4] It is also found in type II alveolar pneumocytes in the lungs.[5]

SARS-CoV-2 enters through endocytosis or membrane fusion and is mediated by the viral spike S glycoprotein, which binds to the ACE II receptor after activation of the spike protein by transmembrane protease serine 2 . This distribution explains how the virus can enter the body and why target cells like pneumocytes are so vulnerable to infection.[5, 6]

ACE II, a component of the Renin-Angiotensin System (RAS), is the major network regulating systemic arterial pressure. The RAS has a local or paracrine function in addition to its well-known systemic regulation of circulatory homeostasis. RAS is a complicated system that plays a role in a variety of cellular processes. Inflammation, angiogenesis, cell proliferation, memory, sodium and water reabsorption, thrombosis, and plaque rupture are some of the antagonistic functions of the system.[7, 8]

The significance of assessing circulating ACE II in pathologic conditions is uncertain because ACE II is a tissue enzyme with low circulating levels.[9, 10] Men and patients with aortic stenosis had greater plasma levels of ACE II than women patients with heart failure.[11, 12] ACE II levels in the blood have also been linked to a higher risk of major cardiovascular events.[13] Similarly, ACE II levels have recently been discovered to be much higher in the serum of smokers, obese people, and diabetics.[14]

This study aimed to screen the COVID-19 patients for elevated ACE II and determine the plasma concentrations of ACE II in COVID-19, COVID-19 cured patients, and healthy control subjects to find the relationship between COVID-19 and elevated ACE II concentrations.

2. MATERIAL AND METHODS

2.1. Selection of Subjects

The patients visiting Wah General Hospital Wah Cantt, Rawalpindi, and Medsol Lab Islamabad, Pakistan, were selected for the current study with their consent. The number of selected patients was 81 (including 40 COVID-19 patients and 41 COVID-19 cured individuals). A Control group of 94 age-matched healthy individuals was also chosen. The history of the subjects with their blood pressure, body weight, and height was recorded in the proforma especially developed for this purpose.

2.2. Inclusion and Exclusion criteria of subject

The patients with shortness of breath, cough, headache, fever, and high blood pressure with concurrent diabetes were included in the present study. The patients with neurological diseases, chronic renal impairment, known psychological illnesses, alcoholics, advanced hepatic and renal insufficiency and suffering from any other endocrinological disorder were excluded from the study.

2.3. Calculation of Body Mass Index (BMI)

The BMI was calculated as described by Lake et al. (1997), Where weight was measured using a digital weight scale with a precision of 0.1 Kg, and height was measured by using Harpenden stadiometer with an accuracy of 0.1 cm.

$$\mathbf{BMI} = \frac{\mathbf{Weight\ in\ kilogram}}{(\mathbf{Height\ in\ meter})\mathbf{2}}$$

2.4. Blood Sampling and Storage

Approximately 3 ml blood sample from the antecubital vein of each patient and control subject was collected in a 22-24 G (gauge) disposable syringe and placed in a gel tube to extract serum for hormonal analysis. The blood samples in the gel tube were centrifuged immediately after collection. The plasma was separated and kept frozen at -20°C.

2.5. Hormonal Analysis

The plasma ACE II concentration was measured by commercially available human Enzyme-Linked ImmunoSorbent Assay (ELISA) (Human ACE2 ELISA kit of Elabscience: Catalog No.E-EL-H0281) at Medsol Lab, Islamabad, Pakistan.

Statistical analysis

Statistical analysis was done through IBM SPSS version 25 and MS Excel. ANOVA and Pearson correlation (r) was carried out for hormonal analysis.

3. RESULTS

3.1. Prevalence of COVID-19 in Male and Females

The prevalence of the disease was found to be equal in males and females (50.85, 49.14%). Similarly, the recovery ratio from COVID-19 was also almost equal, i.e., 51.21 and 48.78%, in males and females, respectively, as shown in Fig.1. The percentage of various disease symptoms was higher in COVID-19 patients compared to COVID-19 cured individuals. (Fig.1)

3.2. Association between the ACE II and COVID-19

ELISA analyses showed that high ACE II level was associated with severity in COVID-19 among patients.

3.3. ACE II concentration in Males and Females

The concentration of ACE II was highest (41.6 ±2.7) in the COVID-19 male of 51-60 age group, higher (16.2 ± 1.5) in COVID-19 cured 51-60 males than healthy individuals, where it was lowest in each age group, as shown in Fig 2.

Similarly, the highest concentration (40.86±5.78) of ACE II was observed in COVID-19 females of 51-60 age groups and higher in COVID-19 cured females than normal individuals.(Fig.3) The results indicate no correlation between gender and ACE II concentration. However, a direct correlation was observed between ACE II concentration and age of patients.

3.4. ACE II and Body Mass Index

The subject, when studied according to BMI for ACE II concentration, the ACE II concentration was highest (40.8±1.4 ng/ml) in obese COVID-19 patient, followed by overweight (34.1±44.1 ng/ml) and underweight (31.6±9.6 ng/ml). The patients with these abnormalities shows a higher ACE II concentration compared to normal weight patient (Fig.4)

3.5. Association between ACE II and Hypertension, Diabetes

ACE II concentration in COVID-19 patients was higher with hypertension (43.6± 3.2 ng/ml) and without hypertension (44.5± 16.19 ng/ml) than COVID-19 cured patients either with or without hypertension. (Fig.5)

In patients with diabetes and without diabetes, the ACE II concentration was highest (43.3±6.7 and 42.1±2.3 ng/ml respectively) in the 51-60 age group, followed by other age groups. A reduction of ACE II concentration in a similar pattern was seen in COVID-19 cured individuals with or without diabetes. (Fig.6) Similarly, ACE II concentration was higher (42.8±3.1 ng/ml) in COVID-19 patients of the 51-60 group with hypertension and diabetes. A reduction in ACE II concentration was observed in COVID-19 cured individuals and healthy individual. (Fig.6)

4. DISCUSSION

Although disease prevalence and recovery are similar in both males and females, COVID-19 severity in patients with diabetes, hypertension, and other chronic diseases may be affected by activation of the RAS system in several tissues, resulting in a weak innate immune system.[16]It shows that people with these diseases are more vulnerable to COVID-19. Moreover, age has emerged as a critical factor in predicting the severity of the disease and ACE II.

In patients with active COVID-19 infection, circulating ACE II is higher than in healthy controls. [17, 18, 19] Results of our study show that ACE II concentration in COVID-19 patients gradually increases with age increases. The lowest concentration was in 21-30, higher in 31-40 followed by 41-50, and highest in 51-60. As previously established in experimental models of SARS-CoV infection, excessive ACE II production would involve in increased viral dissemination [20, 21] with a similar mechanism postulated to be relevant for SARS-CoV-2 infection.[22]

Obesity has emerged as a new risk factor for COVID-19-related hospitalization and death. Our results indicate that ACE II concentration is highest in obese individuals, and current research outcomes are in line with previously published results. COVID-19 enters the cell when ACE II is present. Increased ACE II expression promotes virus entrance into cells, resulting in more severe disease and worse clinical outcomes. ACE II expression appears to be higher in obese and overweight people.[23]Greater ACE-2 expression in COVID-19 patients who are overweight, underweight, or obese may be linked to increased disease severity.[24] Obesity's abundant adipose tissue makes it an ideal target for virus entrance and transmission, resulting in severe illness with severe clinical consequences. Further research is needed to fully comprehend the role of adipose ACE-2 and its link to obesity in COVID-19 patients.

ACE II levels were considerably higher in COVID-19 positive patients with hypertension and without hypertension compared to COVID-19 cured individuals with hypertension and without hypertension. The highest concentrations were observed between the age group of 40 to 60 years. Kragstrup et al. observed that ACE II concentration was higher in COVID-19 positive patients with hypertension than patients without hypertension.[25] In COVID-19-positive patients with hypertension; ACE II levels were considerably higher than in those without hypertension. ACE inhibitors are commonly used to treat hypertension patients. During this pandemic, there has been much discussion about whether or not these antihypertensive drugs should be stopped in COVID-19 patients. According to a study, the usage of ACE inhibitors boosted the expression of ACE II in cardiac tissue in rats.[26] Patients with hypertension treated with ACE2-modulating medicines like ACE-inhibitors may be at a higher risk of severe COVID-19 infection since it may alter the virus's entryway.[27]

COVID-19 severity in patients with diabetes may be affected by activation of the RAS system in several tissues, resulting in a weakened innate immune system. Our results indicate that ACE II concentration in COVID-19 patients with diabetes and without diabetes is higher than COVID-19 cure individuals. Furthermore, it is observed that ACE II concentration was higher in patients with both diabetes and hypertension than recovered individuals. Kragstrup et al. reported no significant difference in ACE II concentration in patients with and without diabetes.[25]

CONCLUSION

Overall, this research reveals that ACE II concentration may be linked to severe COVID-19 disease and related risk factors, such as hypertension, diabetes, obesity, and old age. ACE II concentration is higher in COVID-19 patients either they are obese, diabetic, hypertensive, or normal compared to healthy individuals.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interests.

AUTHOR CONTRIBUTIONS?????

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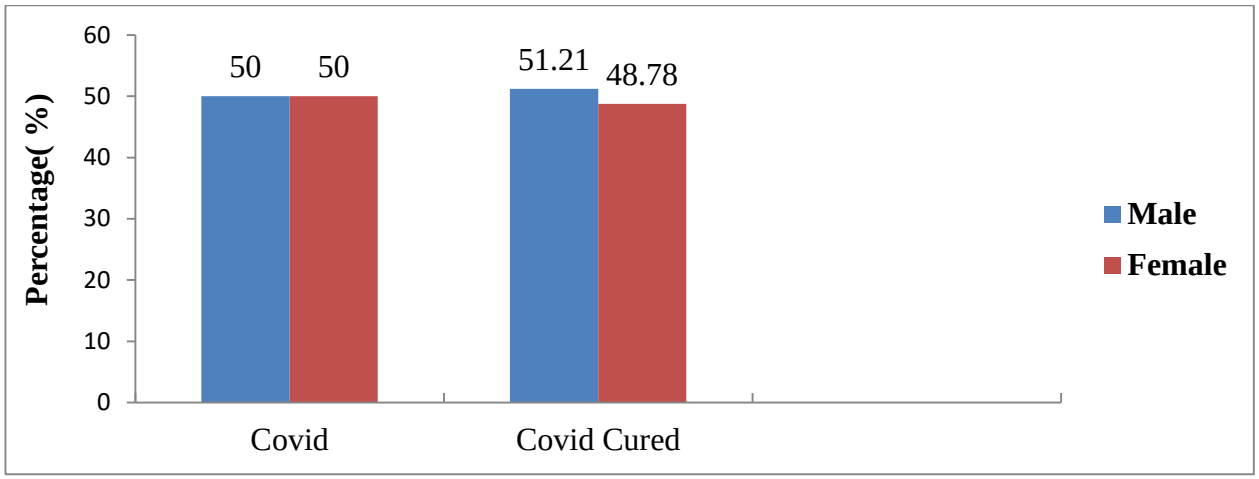


Fig. 1 Percentage of Males and Females in COVID-19 and COVID-19 Cured state

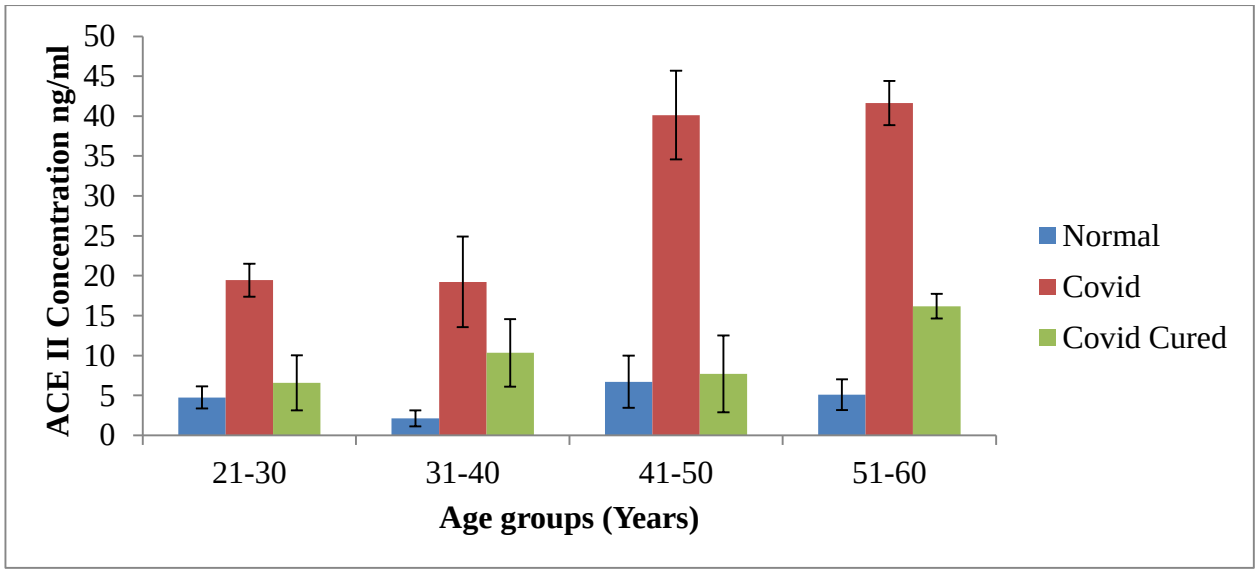


Fig.2 ACE II Concentration in COVID-19 Patients, COVID-19 cured and healthy Male of different age groups

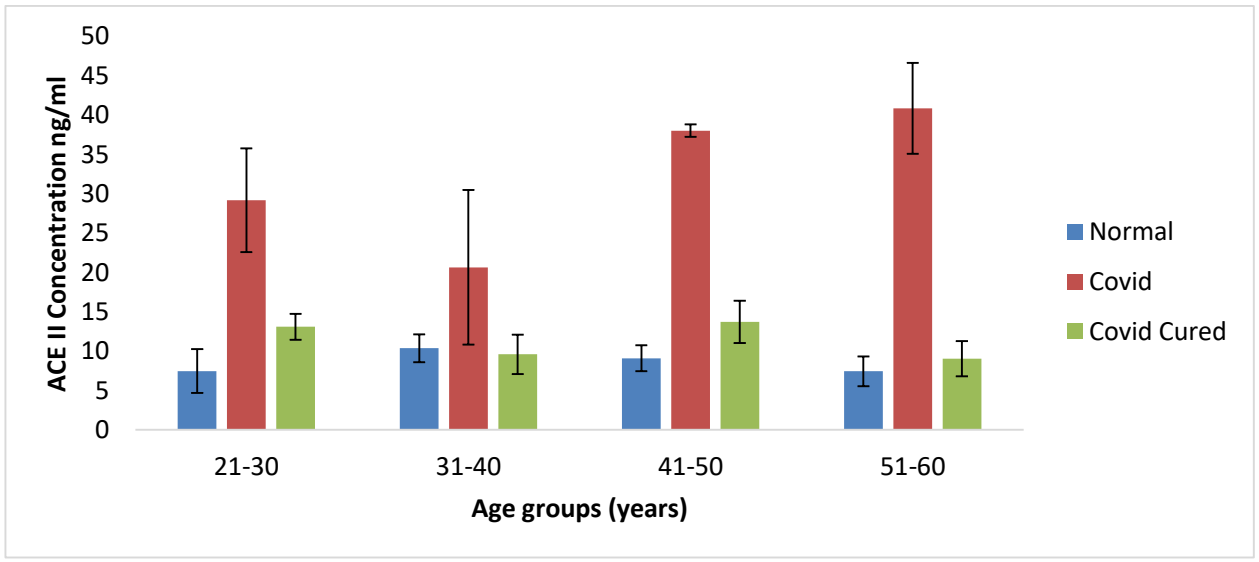


Fig.3 ACE II Concentration in COVID-19 Patients, COVID-19 cured and healthy Females of different age groups.

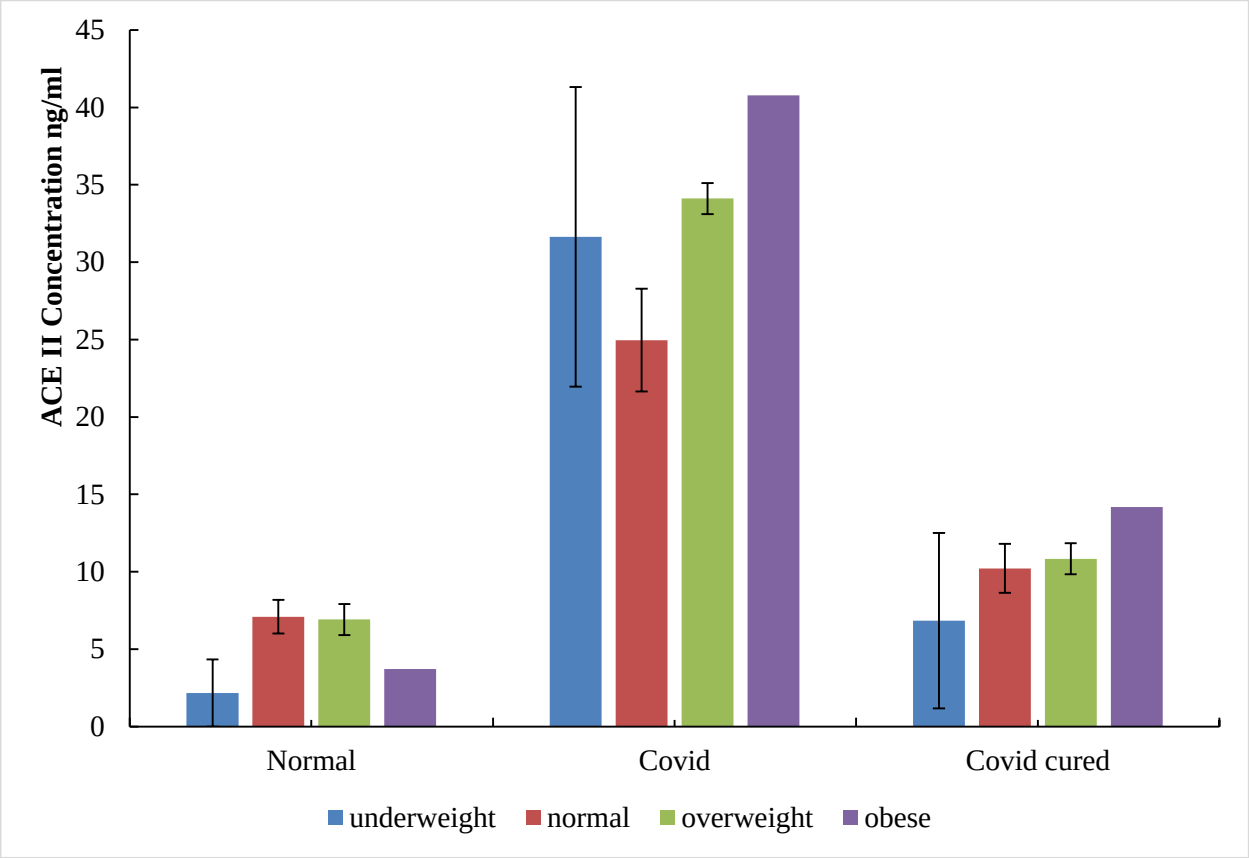


Fig.4 ACE II Concentration in COVID-19 Patients, COVID-19 cured and healthy individuals under BMI Categories

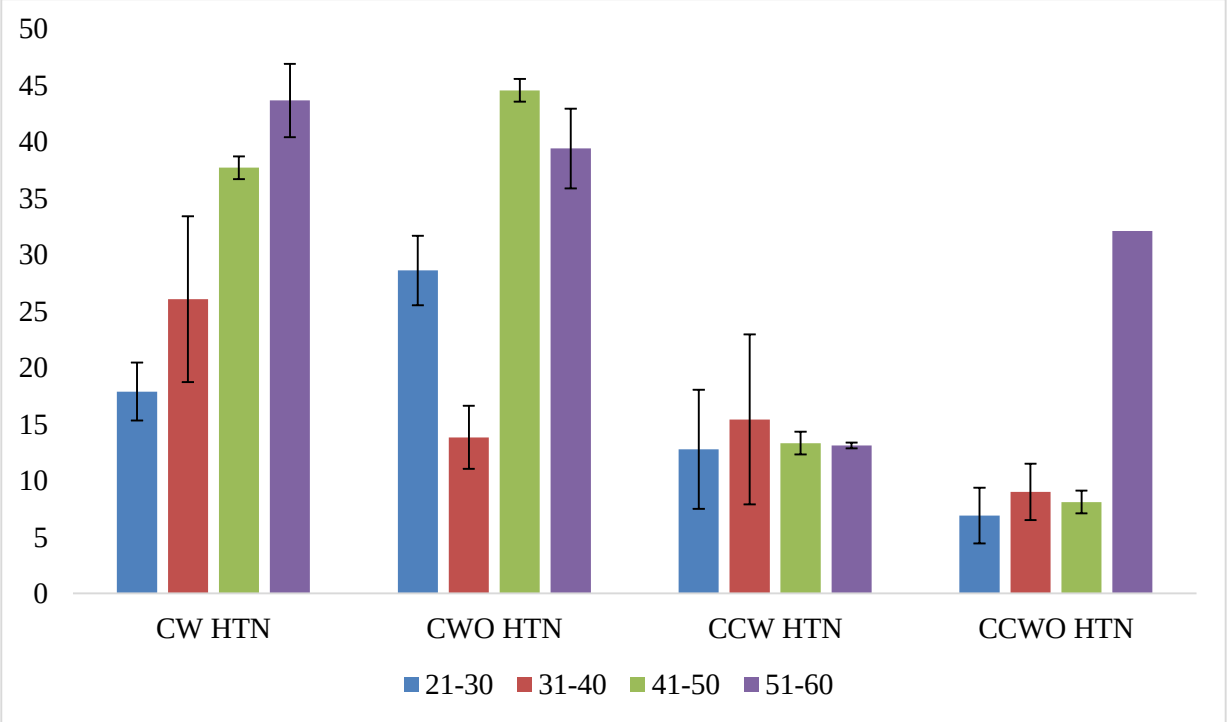
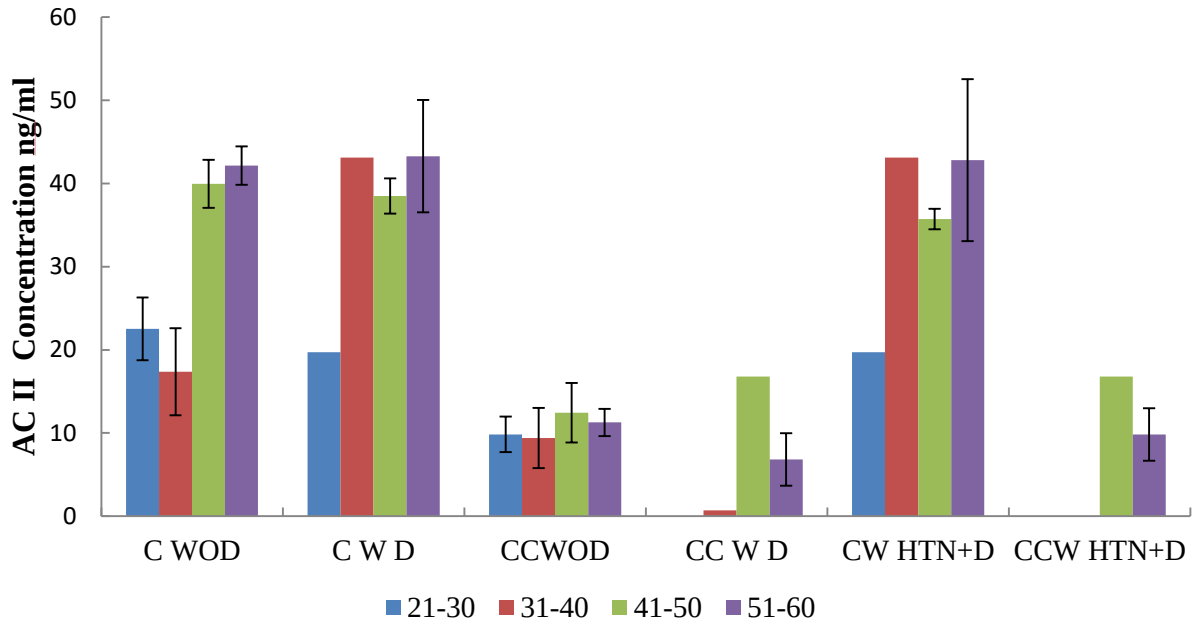


Fig. 5 ACEII Concentration in COVID-19 Patients and COVID-19 cured with and without hypertension in different age groups. (CWHTN: COVID-19 with hypertension, CWHTN: COVID-19 without hypertension, CCWHTN: COVID-19 Cured with hypertension, CCWOHTN: COVID-19 Cured without hypertension)



ACE II Concentration COVID-19 Patients and COVID-19 cured with and without diabetes in different age groups. (CWOD: COVID-19 without diabetes, CWD: COVID-19 with diabetes, CCWOD: COVID-19 cured without diabetes, CCWD: COVID-19 cured with diabetes CWHTN+D: COVID-19 with hypertension and diabetes, CCWHTN+D: COVID-19 cured without hypertension and diabetes)