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Original Research Article

Differential Expression of ACE2 Receptor in Buccal Mucosa and Blood of COVID-19 Patients Correlation with Clinical Severity

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Abstract: ***Background:*** SARS-CoV-2 is a virus that enters human cells through the ACE2 receptor. The ACE2 transcript profile in oral and systemic tissues can affect the degree of COVID-19 infection and its spread. ***Objectives:*** This study aimed to determine the difference in ACE2 expression between the buccal mucosa and blood of COVID-19 patients and to compare it with demographic, clinical, and severity variables. ***Study Design:*** experiment, laboratory-based study. ***Place and Duration of Study***: Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar, in collaboration with Hayatabad Medical Complex (HMC), Peshawar from January 2021 to July 2024. ***Methods:*** This study was conducted at the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU), Peshawar, in collaboration with Hayatabad Medical Complex (HMC), Peshawar, where sample collection was performed. The study duration spanned from January 2021 to July 2024.A cross-sectional study with a laboratory background was conducted among 54 unvaccinated COVID-19 patients. Tissue samples of buccal mucosa were examined for ACE2 protein detection using the immunohistochemically method, and blood samples were analyzed for ACE2 mRNA via quantitative PCR (qPCR). Logistic regression identified risk factors of disease severity. ***Results***: The mean age was 45.24 years SD 12.2 yrs; 70.4% were men. The most common were fever, fatigue, and taste disorders. ACE2 was present universally in buccal mucosa but not in blood. ACE2 levels were did not differ significantly between moderate and severe cases. Logistic regression analysis revealed that age was a significant predictor (OR = 1.41, p = 0.005). ***Conclusion:*** ACE2 could be consistently detected in buccal mucosa, thus confirming its role in viral entry, whereas no correlation was observed between the expression level and severity. Age was the sole independent predictor of the disease severity.

Keywords: COVID-19, ACE2 receptor, buccal mucosa, blood, severity predictors

INTRODUCTION

The current pandemic of coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a widespread effort to identify biomarkers and understand the pathophysiological processes that contribute to disease severity. One of the molecules that play an important role in the process of viral entry is the angiotensin-converting enzyme 2 (ACE2) receptor [1,2]. ACE2 is broadly expressed in the human tissues, in pulmonary alveoli, heart, kidneys, gastrointestinal tract, as well as in the buccal mucosa and the oral epithelium[3-5]The SARS-CoV-2 spike (S) protein interacts with ACE2 with high affinity inducing cell entry and viral replication[6].The entrance of the cell downregulates ACE2 receptors leading to disruption of the renin-angiotensin-aldosterone system (RAAS) and poor production of cytokines, all of which aid in inflammatory processes and vascular dysfunction of the body[7,8]. Therefore, understanding how the ACE2 gene is expressed in tissues may enhance risk stratification and the targeting of therapeutics in COVID-19.This is because emerging evidence indicates that ACE2 is not only highly expressed in lung alveolar cells but also expressed in the stratified squamous epithelial cells in

the oral cavity [tongue, gingiva and buccal mucosa [9-10]. The sites are suggested to act as a SARS-CoV-2 reservoir, particularly during early infection, and could be the cause of frequent oral symptoms, including dysgeusia, xerostomia, and mucosal lesions [11,13]. Moreover, person-to-person transmission through saliva particles and contact with contaminated surfaces could be explained by oral ACE2 expression [14].Although the ACE2 expression profile in respiratory tissues has been widely investigated, little is known about its occurrence within buccal mucosa in clinical COVID-19. Still, fewer have examined ACE2 expression in circulating blood cells, which, in principle, provides a systemic measure of infection or disease progression [15-18]. This deficit is more noteworthy in the patient population with poor access to high-resolution diagnostic tools, where simple oral or blood-derived biomarkers can play a helpful role in pre-evaluating and predicting severity. Old age, specifically, has been repeatedly found to be a powerful predictor of poorer clinical outcomes, possibly due to immunosenescence and underlying comorbidities [19]. Since severe COVID-19 is associated with high morbidity and early predictors of disease severity are needed, the study at hand examines ACE2 receptor expression in the buccal mucosa and blood of COVID-19 patients using immunohistochemistry and qPCR, respectively. This study aims to determine whether the levels of these expressions are correlated with the severity of the diseases and to examine their association with demographic and clinical factors in a Pakistani population cohort. The study can enrich our understanding of oral and systemic ACE2 dynamics in COVID-19 and inform specific measures related to disease management and community health protection.

MATERIAL AND METHODS

The study is experiment, laboratory-based study conducted at the Institute of Basic Medical Sciences (IBMS), Khyber Medical University, Peshawar, whereby samples were collected between January 2021 and July 2024 at the Hayatabad Medical Complex.54 PCR-confirmed COVID-19 patients were chosen through convenience sampling, but they had not been vaccinated. Buccal mucosal tissue specimens were obtained via oral swabs and analyzed using immunohistochemistry to assess the development of ACE2 receptors. Blood specimens were analyzed using quantitative PCR (qPCR) to detect ACE2 mRNA. A predesigned pro forma was used to record demographic data, symptom profiles, and comorbidities. The calculation of sample size was performed using G * power software, assuming an effect size of 0.5 and a power of 95%. Criteria. Adults aged 18 years and older with unvaccinated Adults aged ≥18 years with PCR-confirmed COVID-19, unvaccinated, categorized as moderate or severe, and who provided written informed consent.

Exclusion Criteria

The study excluded patients with a history of oral diseases, cancer, immunosuppressive treatment, and those who were already vaccinated against COVID-19 or refused to participate.

Ethical Approval Statement

This study was approved by the Graduate Studies Committee and Advanced Studies and Research Board of Khyber Medical University (**Ref: DIR/KMU-ASRB001290/DE/IBMS**), and the Institutional Review and Ethical Board of Hayatabad Medical Complex **(Ref: 353/HEC/B&PSC/2020).** Written informed consent was obtained from all participants prior to sample collection.

Data Collection

The data was acquired by clinical examination, patient history, and laboratory tests. Blood samples and buccal smears were analyzed in the IBMS laboratories. Anti-ACE2 monoclonal antibodies were used in immunohistochemistry, while ACE2 mRNA expression was determined by quantitative PCR (qPCR). The demographic and clinical variables were noted with structured data sheets.

Statistical Analysis

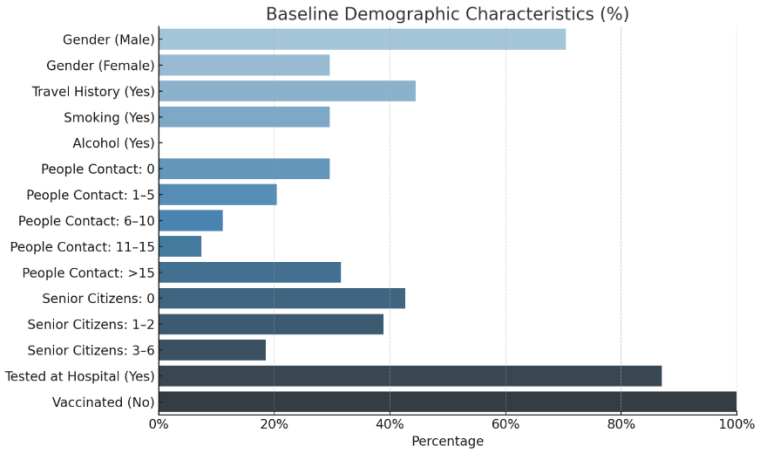
The data analysis was performed using SPSS 24.0. Quantitative variables were reported as means ± SD, and qualitative variables were expressed as frequencies and percentages. To extract predictors of severity, logistic regression was used. The level of statistical significance was $p < 0.05$. Normality was assessed using the Shapiro–Wilk test.

RESULTS

Out of 54 COVID-19 patients was 45.24 ± 12.20 years, and 70.4% of the population was male. None of the patients was vaccinated. The incidence of fever was 100%, whereas fatigue (98.1%), taste disorders (92.6%), and shortness of breath (90.7%) remained highly prevalent. The most frequent comorbidity was diabetes (35.2%) and hypertension (29.6%).ACE2 receptor expression was observed in all samples of buccal mucosa with mean optical density (OD) values of 0.16, 0.51 (moderate cases), and 0.17 0.78 (severe cases) (p=0.852). Severe cases had a reduced number of ACE2-positive cells compared to moderate and severe although the difference was not significant (224.2 vs. 283.8; p = 0.067). There was no ACE2 expression in the qPCR of the blood samples. Binary logistic regression revealed that only age was a significant predictor of disease severity (OR = 1.41, p = 0.005). ACE2 with consistent ± SD format and cell counts were not significant predictors, and neither was gender. The accuracy of the model in classification

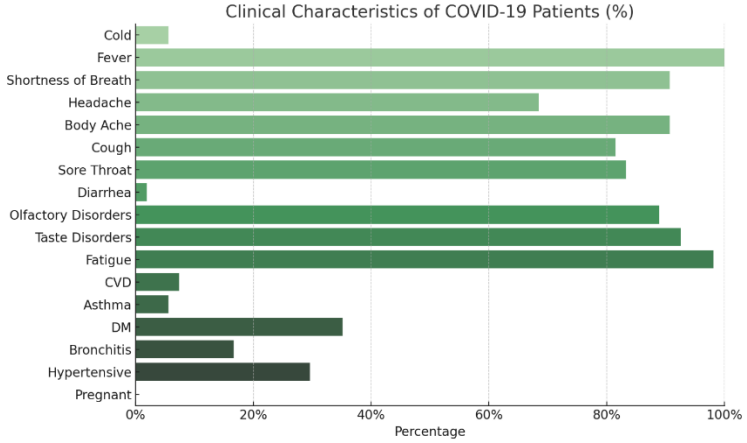
was high (92.6% of cases correctly predicted), indicating that age may have a good predictive value itself. These results indicate that although the receptor ACE2 is essential for the entry of SARS-CoV-2, its abundance in mucosal tissue is not a prognostic marker of disease severity, regardless of the presence of other receptors.

Figure 01: Baseline demographic and exposure characteristics of COVID-19 patients.



This figure illustrates the percentage distribution of baseline demographic variables and exposure-related characteristics among the study participants, including gender, travel history, smoking status, alcohol use, contact history, household senior citizen exposure, COVID-19 testing status, and vaccination status.

Figure 2. Clinical characteristics and comorbidities of COVID-19 patients.



This figure depicts the percentage distribution of presenting clinical symptoms and underlying comorbid conditions among the study participants, including respiratory, gastrointestinal, sensory symptoms, and chronic medical illnesses

Table 1: Baseline Demographic Characteristics

Characteristic	Frequency	Percentage
Gender (Male)	38	70.4
Gender (Female)	16	29.6
Travel History (Yes)	24	44.4
Smoking (Yes)	16	29.6
Alcohol (Yes)	0	0.0
People Contact: 0	16	29.6

This table summarizes the demographic profile and selected exposure-related variables of COVID-19 patients included in the study, including gender distribution, travel history, smoking status, alcohol use, and reported contact history.

Table 2: COVID Status Vs Demographics

Factor	Moderate (%)	Severe (%)
Gender (Female)	30	29.5
Gender (Male)	70	70.5
Travel History (No)	60	54.5
Travel History (Yes)	40	45.5
People Contact: 0	10	34.1
People Contact: 1–5	30	18.2

This table presents the distribution of demographic variables according to disease severity (moderate and severe COVID-19), including gender, travel history, and reported contact exposure.

Table 3: Outcome finding of Clinical Characteristics

Characteristic	Frequency	Percentage
CVD	4	7.4
Asthma	3	5.6
DM	19	35.2
Bronchitis	9	16.7
Hypertensive	16	29.6
Pregnant	0	0.0

This table outlines the frequency and percentage of major clinical comorbidities observed among the study participants, including cardiovascular disease, asthma, diabetes mellitus, bronchitis, hypertension, and pregnancy status.

DISCUSSION

ACE2 receptor was examined in the buccal mucosa and blood of COVID-19 patients, and its correlation with disease severity was investigated. Although ACE2 was expressed in buccal mucosa in every case, there was a lack of any statistically significant difference between moderate and severe cases, which suggests that tissue-level ACE2 expression may not be a determining factor of COVID-19 severity. Furthermore, ACE2 was not found in the blood of any patient. Specifically, only age was found to have a significant effect on disease severity, as verified through logistic regression analysis.The results also correspond to those of Wrapp et al. and Hoffmann et al., which identified ACE2 as the entry receptor of SARS-CoV-2 but also suggested that viral entry relies on host proteases such as TMPRSS2 [20,21]. Nevertheless, ACE2 is required for infection with SARS-CoV-2; however, the levels of its expression may not be the only factors that correlate with clinical severity. This is supported by findings from Li et al., who showed that ACE2 expression is high in most tissues. However, they did not relate the expression of this protein in non-respiratory locations to increased morbidity or mortality of disease[22].Some studies have also reported low ACE2 expression in the blood. Li and colleagues found a strong downregulation of ACE2 mRNA in the peripheral blood mononuclear cells of COVID-19 patients, in line with our qPCR result of undetectable ACE2 expression in human blood [23]. On the same note, Sharif-Askari et al. pointed to a lack of ACE2 expression in immune cells and the low value of ACE2 as a blood-based severity marker[24].The correlation between age and severe COVID-19 has also been well-established in our study. Bourgonje et al. report age-related changes to immune responses and baseline inflammation that are sufficiently pronounced to prime elderly individuals to experience severe outcomes, regardless of ACE2 expression [25].defined age-related immune dysregulation as a significant factor leading to the cytokine storm and organ failure in COVID-19, which again fully supports our results. Interestingly, although a steady ACE2 expression was measured in biopsies of buccal mucosa, its diagnostic or prognostic significance remains doubtful. Based on _in vitro_ expression, Park et al. recently confirmed the presence of ACE2 in buccal epithelial cells by immunolocalization. However, they did not demonstrate its relation to the severity of symptoms or viral load level[26]. This implies that although the buccal tissue might play a role as an early point of viral attack, the overall disease outcome is primarily determined by the systemic immune and inflammatory responses of the host. Finally, our findings contribute to a growing body of literature suggesting that ACE2 expression is a prerequisite for SARS-CoV-2 infection, but it is not in itself a sufficient explanation for clinical differences. It is recommended that further research focus on panels of multiple markers that involve immunity and inflammation markers, which will be more helpful in forecasting COVID-19 outcomes.

CONCLUSION

ACE2 receptor expression was consistently detected in the buccal mucosa but was absent in peripheral blood. Although ACE2 is essential for SARS-CoV-2

cellular entry, its expression levels did not correlate with disease severity. Age emerged as the strongest independent predictor of severity. These findings underscore the multifactorial nature of COVID-19 pathogenesis beyond tissue-specific receptor distribution.

Limitations

The study's small sample size limits statistical power and generalizability. All participants were unvaccinated, which may influence results compared to vaccinated cohorts. Single-center design and lack of viral load quantification further restrict conclusions. Broader, multicenter studies with vaccinated controls are necessary to validate these findings.

Future Directions

Future studies should explore ACE2 co-factors like TMPRSS2 and furin in buccal tissues. Including vaccinated populations and diverse age groups will enhance understanding of receptor-related severity. Longitudinal studies assessing ACE2 expression pre- and post-infection may provide deeper insights into transmission, symptomatology, and clinical outcomes in COVID-19.

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