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Comparative immunohistochemical evaluation of variable expression of ACE2 and TMPRSS2 in different age groups

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Abstract. Background and objectives: COVID-19 pandemic declared by World Health Organisation has drastically upsurged the death rate in the past three years. The specific host cell receptors for viral spike protein have been identified as Angiotensin Converting Enzyme 2 (ACE2) and Transmembrane Serine Protease 2 (TMPRSS2). The study aimed to evaluate the variation in the pattern of expression of ACE2 and TMPRSS2 by immunohistochemistry in the oral and nasopharyngeal mucosa of different age groups. **Methods**: Total of 40 patients were recruited for the study and segregated to four groups. Oral tissue samples from patients of age 18-40 years and 41-70 years were grouped as group I, group II respectively. Nasal tissue from 18-40 years was grouped as III and 41-70 years old as group IV. Immunohistochemical expression of ACE and TMPRSS2 were studied in the tissue samples. Scoring was done based on the intensity and percentage of staining and quantitative image analysis using Fiji image analysis software. Independent sample t-test was done to compare the mean difference in pattern of expression among the age groups studied. Pearson correlation coefficient was done to correlate the expression with age. Statistical significance was set at value less than 0.05. **Results**: The mean difference in expression was significant for ACE2 ($p=0.01$) & TMPRSS2 ($p=0.02$) expression in oral tissue. Both ACE2 and TMPRSS2 expression showed positive correlation between the groups. **Conclusion**: Age-specific variation might provide deeper understanding of clinical severity and elaborate the validation of therapeutic targets.

Keywords: ACE2, age, nasopharyngeal, oral, TMPRSS2

INTRODUCTION

Viruses are obligate intracellular parasites, which hijacks the host synthesising machinery for replication. Receptor mediated tropism of certain viruses for specific host tissue mediates viral pathogenesis. Virus-receptor interaction depends on the expression pattern of the receptors, which contributes to the clinical severity of the disease. Both enveloped and non-enveloped viruses have specific viral attachment proteins. Enveloped viruses have spike-like extensions from the virion, which serve as the first point of contact with the host receptor. Non-enveloped viruses bind to the host receptors through the components of the capsid protein (Maginnis, 2018).

Corona viruses are positive-sense RNA viruses in the size range of 8nm to 160nm and are enveloped. SARS-CoV2 belongs to the β corona virus family and has crown-like morphology. The virus consists of four structural, six non-structural, and five to eight accessory proteins. Envelope, membrane, nucleocapsid, and spike (E, M, N, and S respectively) proteins constitute the

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structural proteins. As far as location is concerned, N protein is found in association with the genomic RNA and M, S, E proteins are present in the envelope (Parodi & Liu, 2020; Zhu *et al.,* 2020; Vasanthi *et al*., 2020). The S protein is a transmembrane glycoprotein whose function is to bind to the specific receptors present on the host cell, namely angiotensin-converting enzyme 2 (ACE2). SARS pandemic in 2002-2003 from was also proven to be from similar receptor-protein attachment. Literature has proven that SARS-CoV and SARS-CoV2 have 76% similarity with respect to their S protein, and hence ACE2 was claimed to be the specific receptor for the SARS-CoV2 pandemic (Walls *et al.,* 2020; Wan *et al.,* 2020; Letko *et al.,* 2020).

The pathogenesis of any viral infection includes attachment to the specific host receptors (tropism), proteolysis and conformational change in the viral structural proteins, fusion with the receptor on the host cell and penetration to take over the host synthesising machinery. The structural configuration of the S protein has 3 domains, namely ectodomain/extracellular, transmembrane region, and cytoplasmic intracellular tail. The structure of S protein is clove-shaped morphology with three heads (S1) and one stalk (S2). The extracellular domain has aminoterminal and carboxyterminal S1 domains (Walls *et al.,* 2020; Wan *et al.,* 2020; Letko *et al.,* 2020; Li, 2016). The receptor binding domain of S1 attaches to the ACE2 receptor and S2 fuses with the membrane of the host cell. Protease from the host cell cleaves S at two sites: S1/S2 interface and S2. The transmembrane protease serine 2 (TMPRSS2), which has structural resemblance to ACE2, cleaves the S at S1-S2 interface, after which a conformational change occurs at the S2 to facilitate fusion (Shang *et al.,* 2020; Hoffman *et al.,* 2020; Shereen *et al.,* 2020; Verdecchia *et al.,* 2020).

The ACE2 receptor is a transmembrane glycoprotein expressed in the respiratory epithelium (trachea-bronchial epithelial cells, macrophages, type 2 pneumocytes), oral tissues (epithelium of salivary gland, tongue, lips, buccal mucosa, and gingiva), the digestive system (gut and colon enterocytes), the cardiovascular system (smooth muscles and vascular endothelial cells), the testis (Sertoli cells, Leydig cells), the brain (glial cells and neurons) and the kidney (epithelial cells of renal tubules). Gene coding for ACE2 is present on chromosome Xp22.2. It belongs to the family of dipeptidylcarboxypeptidases and is the master regulator of the renin-angiotensin-aldosterone system. ACE is a monocarboxypeptidase responsible of cleavage of angiotensin I to angiotensin (1–9) and angiotensin II to angiotensin (1-7). There exist two forms: full-length ACE2 and soluble ACE2 present in the transmembrane location for binding and in the circulation, respectively (Verdecchia *et al.,* 2020; Donoghue *et al.,* 2000; Salamanna *et al.,* 2020; Tipnis *et al.,* 2000).

TMPRSS2 is a member of type II transmembrane serine protease (TTSP) family. The gene coding for TMPRSS2 is present on chromosome 21 with 14 exons and 13 introns. The structure of TMPRSS2 includes an active ectodomain, transmembrane domain, and an intracellular domain. The ectodomain has three subdomains, namely protease domain, receptor type A domain, receptor cysteine-rich domain, and carboxy teminal trypsin-like serine peptidase domain (Fraser *et al.,* 2022; Afar *et al.,* 2001). TMPRSS2 is normally expressed in the ocular epithelium, olfactory epithelium, gastrointestinal tract, respiratory epithelium, and prostate (Sarker *et al.,* 2021).

As the pathogenesis of any viral infection is determined by tropism, specific receptor binding and fusion, tissues with over expression of ACE2 and TMPRSS2 are the portal of viral entry. COVID-19 infected patients were reported to experience hyposmia and dysgeusia. The potential cause for the decreased sense of smell and taste was attributed to the presence of ACE2 and TMPRSS2 receptors in the epithelial cells of olfactory and oral mucosa. As oral and nasopharyngeal mucosa are the portal of entry of SARS-CoV2, ACE2 and TMPRSS2 receptors at these sites might augment the understanding the pathogenesis of the prevailing pandemic. The severity of clinical manifestation is thought to vary due to co-morbid conditions and age-dependant pattern of expression of these receptors (Zheng & Song, 2021). Therefore, a study aiming to assess the expression pattern of ACE2 and TMPRSS2 receptors might enable deeper understanding of the distribution and mechanism of replication of the virus, as well as provide insight into the possibility of shed epithelium contributing to

asymptomatic transmission. The present study aimed to identify the variation in the pattern of ACE2 and TMPRSS2 expression in the tissues of oral cavity and nasopharynx of population belonging to extreme age groups.

MATERIALS AND METHODS

Ethics and informed consent

The objectives, protocol, risks, and benefits of the study were explained to the study participants and consent was obtained. Institutional ethical board approved the study (SRMDC/IRB/2020/MDS/No.603).

Patient selection

Patients undergoing minor oral surgical procedures such as crown lengthening, surgical extraction, surgical impaction, operculectomy, mucocele excision, ulcer of the tongue were recruited for obtaining oral tissues and grouped as Group I (18-40 years -10 patients) and Group II (41-70 years-10 patients) respectively (Figure 1a, 1b). Patients undergoing surgical procedures such as tonsillectomy, adenoidectomy, nasal polyp excision, and septoplasty were grouped as Group III (18-40 years of age) and Group IV (41-70 years of age) in the study for obtaining nasopharyngeal tissues (Figures 1c, 1d).

Immunohistochemical staining

Tissue processing was done and embedded in paraffin blocks. 4µm thick sections were made from Formalin-fixed paraffin embedded (FFPE) blocks on coated slides. The sections were air dried and incubated at 37ºC overnight. The sections were then deparaffinized at 50-60ºC for 1 hour, placed in 3 changes of xylene, rehydrated through 3 changes of absolute alcohol for 5 minutes each and immersed in running tap water for 5 minutes. The reagents for the procedure were brought to room temperature. The tissue sections were refrained from drying during the entire procedure. After blocking for peroxidase activity and antigen retrieval, about 25µl of primary antibody [ACE2 Monoclonal antibody (MA5-31395) (CL4035) (Thermo-Invitrogen)] was added. Later horse radish peroxidase conjugated secondary antibody [Horse Radish Peroxidase HRP/ 3,3'- Diaminobenzidine DAB (DAKO)] was added to the section. Counter staining was done with Hematoxylin for 5 minutes and the slides were mounted. The same steps were followed for TMPRSS2 primary antibody [TMPRSS2 Polyclonal antibody (PA5-14264) (Thermo-Invitrogen)] also. Lung tissue was used as positive control (Figure 2c) and breast tissue (Figure 2d) was used as negative control for both ACE2 and TMPRSS2 expression.

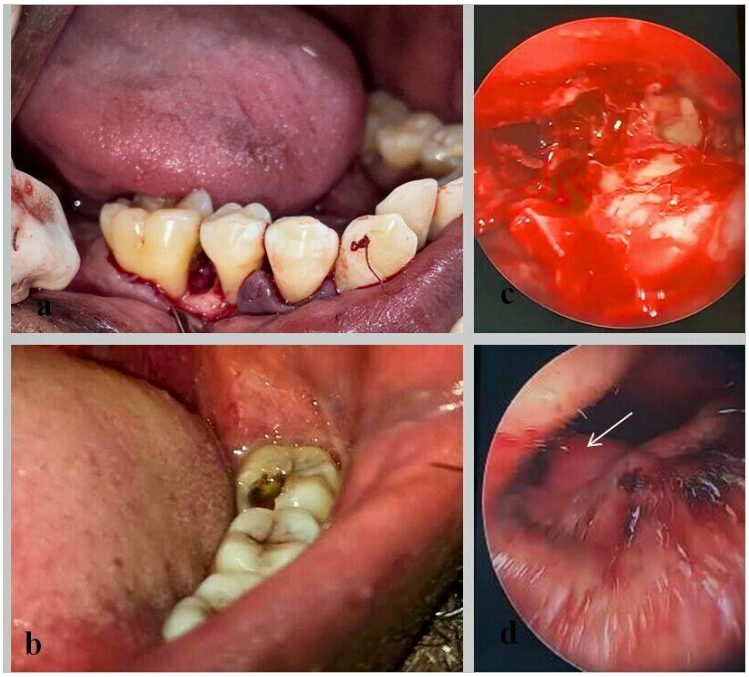

Figure 1.
a-Clinical image of patient from group I;
b-clinical image of group II patient;
c-endoscopic image of patient from group III undergoing septoplasty;
d-endoscopy of patient from group IV undergoing excision of nasal polyp

Scoring criteria for semi-quantitative analysis

Hematoxylin and eosin staining was also done for the sections from the FFPE blocks. Slides were evaluated for positive staining by two individual pathologists. In case of inter-observer variability, a third observer finalised the staining score. Scoring for positive staining was done as per the protocol followed by Allawi and Abdullah (2022) and Sakaguchi *et al.* (2020). Intensity of the staining was scored as 0 (no stain), 1 (mild - light brown color), 2 (moderate-brown color), 3 (intense-dark brown color). Percentage of staining was observed as 0 (no stain), 1 (less than 30%), 2 (30%-60%), 3 (more than 60%). Staining index was calculated by multiplying the intensity and percentage of staining. Mean of the staining index was calculated for each group.

Quantitative analysis for immuno-histochemical staining

The most representative areas were selected for analysing the area of positive immunostaining. Photomicrographs of the ACE and TMPRSS2 stained sections were taken from five high power fields and the images were standardized to uniform pixel. Immunostaining was analysed quantitatively using Fiji image analysis software (Johannes Schindelin, Albert Cardona, Mark Longair, Benjamin Schmid, and others - version 1.2). H-DAB vector was selected, images were deconvoluted and converted to RGB images. Color 2 was selected for DAB image. Threshold tool was adjusted to modify the area of interest. Binary image was created, processed and particles were analysed using following specifications: size: 0.0002-infinity, circularity: 0.00-1.00, show: outlines. Mean percentage area of expression of ACE (Figures 4 and 6) and TMPRSS2 (Figures 5 and 7) was calculated (Murphy *et al.,*2021).

Statistical analysis

IBM SPSS statistics 21 software was used to check for statistical significance of the study. Independent sample t-test was used to compare the mean difference between the study groups. Pearson correlation coefficient was done to correlate ACE2 and TMPRSS2 expression with age. p value less than 0.05 was considered as statistically significant.

RESULTS

Demographics

Among the 40 patients recruited for the study, 50% of the patients were 18-40 years old and 50% of the patients were 41-70 years old. Among the groups, group I and III patients were aged 18-40 years and group II and IV patients were 41-70 years old. The mean age was 30.5±7.1, 53.4±8.8, 28.1±6.4, 51.3±8.4 for group I, II, III, IV respectively.

55% of the patients were males and 45% of them were females. In group I, 60% of the patients were males and 40% were females. In group II, male and female were both 50%. In group III, males and females were 60% and 40% respectively. In group IV, both males and female were 50%.

Staining pattern in oral epithelium

Both the keratinised and non-keratinised epithelium were positive for ACE2 and TMPRSS2. It was observed that cytoplasmic and membranous expression was mostly found for ACE2 with sparse nuclear expression in the epithelial cells. TMPRSS2 expression was also cytoplasmic and membranous to a major extent similar to ACE2.

ACE2 and TMPRSS2 expression in buccal mucosa

Basal and parabasal layers of the non keratinised epithelium stained positive for ACE2 and TMPRSS2. Stratum superficiale was negative for ACE2 staining. Most of the sections showed intense staining. Fibroblasts and vascular endothelial cells in the underlying connective tissue stroma were positive. Adipocytes from buccal mucosa also stained positive which was membranous with moderate intensity.

ACE2 and TMPRSS2 expression in salivary gland (Figure 2a, 2b)

Submandibular salivary gland tissue and few labial minor salivary glands were studied. Serous salivary acini showed moderate staining whereas ducts in the interlobular septa showed intense staining for ACE2. Only mild staining was observed for TMPRSS2 as compared to the intensity of ACE2.

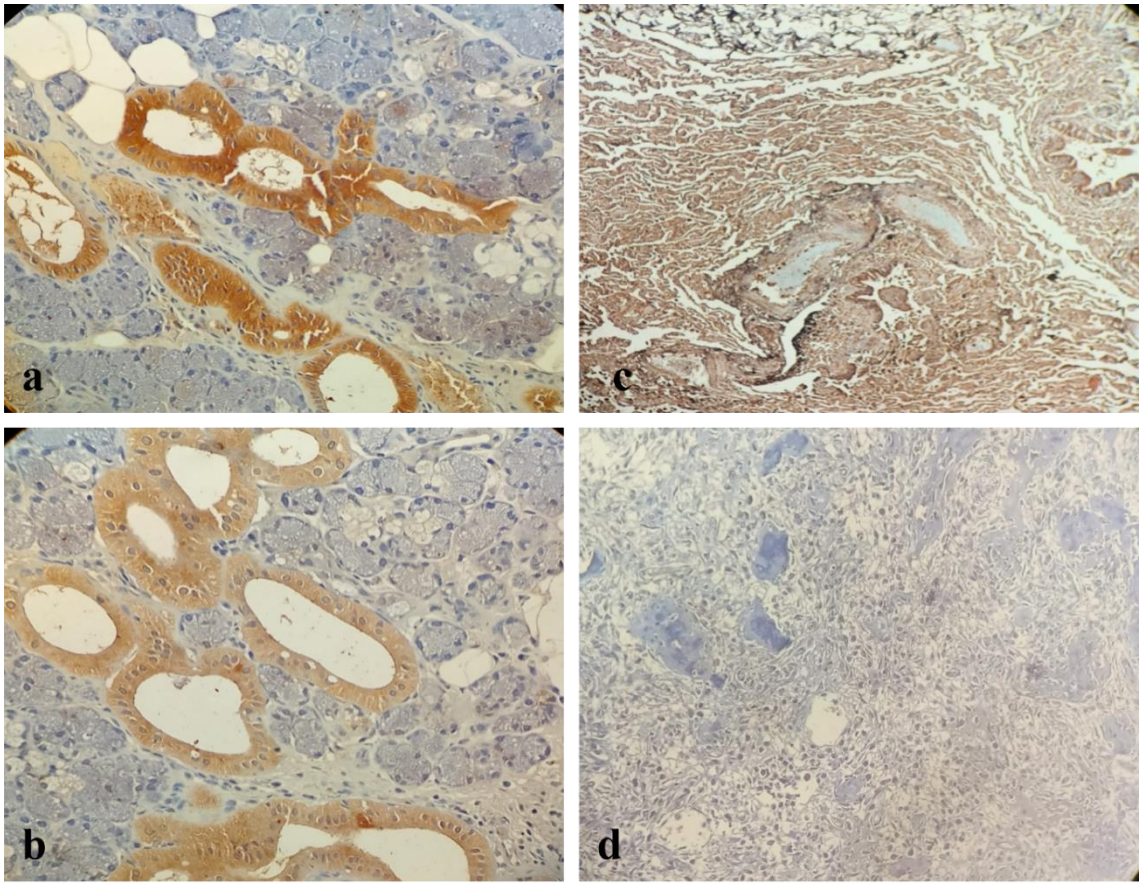

Figure 2. a-ACE2 positive staining in salivary duct; b-TMPRSS2 positive staining in salivary duct; c-positive control lung tissue; d-negative control breast tissue.

ACE2 and TMPRSS2 expression in gingiva (Figure 3a, 3b)

Stratum basale, stratum spinosum of the gingival epithelium exhibited positive staining for ACE2 and TMPRSS2 expression. Stratum granulosum was negative for ACE2 in all the studied sections. Stratum corneum was positive in few cases and negative in the other sections. TMPRSS2 staining was observed in the superficial layers of the keratinised gingiva also in few individuals. Intensity of the staining was severe in most of the studied sections. Only the blood vessels and few fibroblasts were positive for ACE2 and TMPRSS2 in the underlying connective tissue of the gingival tissues.

ACE2 and TMPRSS2 expression in tongue

Basal cells of the keratinised epithelium of the dorsum of the tongue showed intense positive staining for ACE2 and TMPRSS2. Muscles of the tongue were also positive for ACE2 and TMPRSS2 but not as intense as the epithelium. Comparatively ACE2 on was more intense when compared to TMPRSS2 in the tongue.

Staining pattern in nasopharyngeal epithelium (Figure 3c, 3d)

ACE2 & TMPRSS2 expression was mostly membranous. The epithelial cells were positive for the proteins in the basal and parabasal layers. Expression of ACE2 and TMPRSS2 were comparatively higher in the oral mucosa than the nasopharyngeal mucosa.

Comparison of immunohistochemical expression of ACE2 and TMPRSS2 among study groups: semi-quantitative analysis (Table 1)

The mean expression of ACE2 in group I was 2.5±1.8 and group II was 5.3±2.5. On comparison, the difference was statistically significant (p=0.01). The mean expression of TMPRSS2 in group I was 2.9±2.5 and group II was 5.4±2.9. On comparison, the difference was

statistically significant (p=0.02). Mean ACE2 expression was 1.7± 1.3 in group III and 3.7 ±2.1 in group IV. The difference was not statistically significant (p=0.05). Mean TMPRSS2 expression was 2.5± 1.5 in group III and 3.8 ±1.9 in group IV. The difference was not statistically significant (p=0.11).

Comparison of mean percentage area of expression of ACE2 and TMPRSS2 among study groups: quantitative analysis

ACE2 mean percentage expression was 44.73 and 48.42 in oral tissue among group I and II respectively. The difference between the groups was statistically significant (p=0.02). Group III and IV showed a mean percentage area of 39.32 and 43.27 respectively in nasal tissues respectively with a statistically significant p value of 0.03. Mean percentage area of TMPRSS2 expression in group I, group II, group III and group IV were 38.24, 42.36, 35.76 and 39.37 respectively. The difference was statistically significant in oral tissues (p=0.01) but not in nasopharyngeal region (p=0.07).

Pearson correlation for age specific variable expression of ACE2 and TMPRSS2 in oral and nasopharyngeal mucosa (Table 2)

Both ACE2 and TMPRSS2 expression showed positive correlation between the groups though statistical significance was found only for ACE2 oral expression in the oral tissues. (p=0.01).

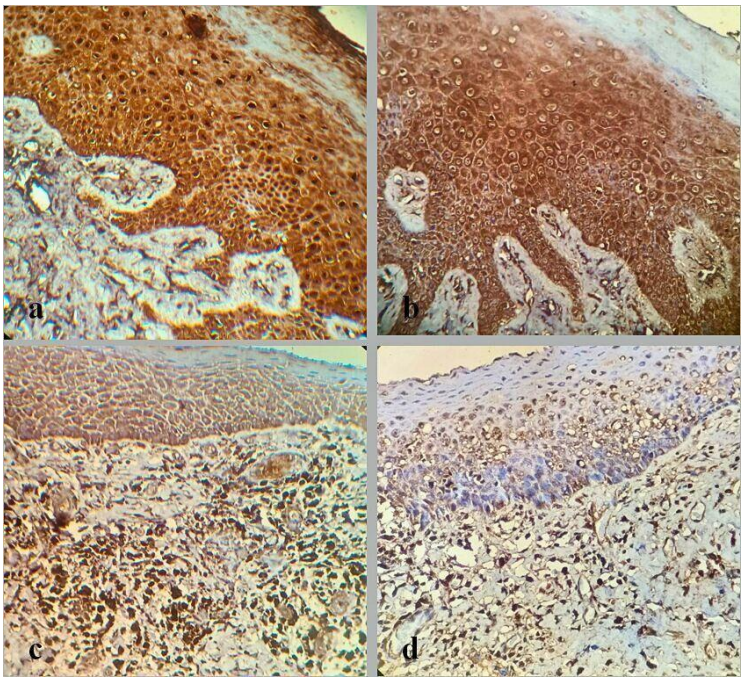

Figure 3.
a-ACE2 expression in gingiva (group II);
b-TMPRSS2 expression in gingiva (group II);
c-ACE2 expression in nasopharyngeal tissue (group IV);
d-TMPRSS2 staining in nasopharyngeal tissue (group IV)

Table 1. Comparison of semi-quantitative immunohistochemical expression of ACE2 and TMPRSS2 among the study groups.

Groups	Protein expression	Mean±SD	p value
Group I (n=10)	ACE2	2.5±1.8	0.01
Group II (n=10)	ACE2	5.3±2.5	
Group I (n=10)	TMPRSS2	2.9±2.5	0.02
Group II (n=10)	TMPRSS2	5.4±2.9	
Group III (n=10)	ACE2	1.7± 1.3	0.05
Group IV (n=10)	ACE2	3.7 ±2.1	
Group III (n=10)	TMPRSS2	2.5± 1.5	0.11
Group IV (n=10)	TMPRSS2	3.8 ±1.9	

ACE 2 - Angiotensin Converting Enzyme 2; TMPRSS2 - Transmembrane Serine Protease 2
*Independent sample t-test. P value < 0.05 - considered significant

Table 2. Pearson correlation for age-specific variable expression of ACE2 and TMPRSS2.

Pearson correlation	Age groups	Pearson correlation	p value
ACE2 expression	Group I (n=10) Group II (n=10)	0.607	0.01*
TMPRSS2 expression	Group I (n=10) Group II (n=10)	0.192	0.41
ACE2 expression	Group III (n-10) Group IV (n=10)	0.442	0.05
TMPRSS2 expression	Group III (n-10) Group IV (n=10)	0.26	0.27

ACE 2 - Angiotensin Converting Enzyme 2; TMPRSS2 - Transmembrane Serine Protease 2
*Independent sample t-test. P value < 0.05 – considered significant

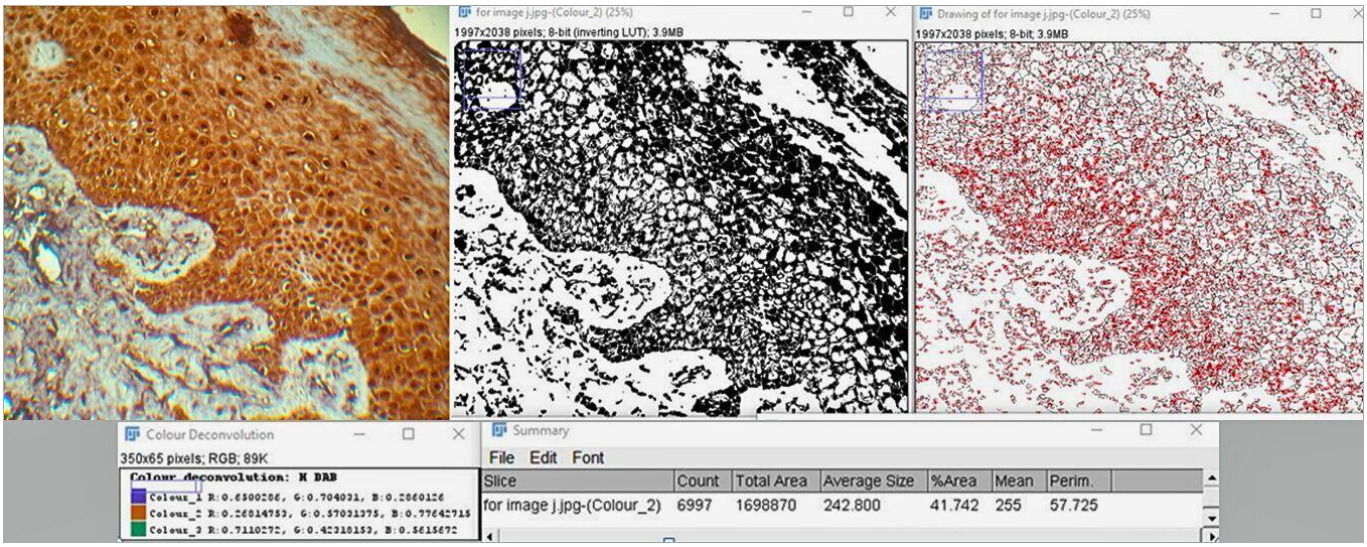

Figure 4. Fiji Image J quantitative analysis of ACE2 staining of gingival epithelium.

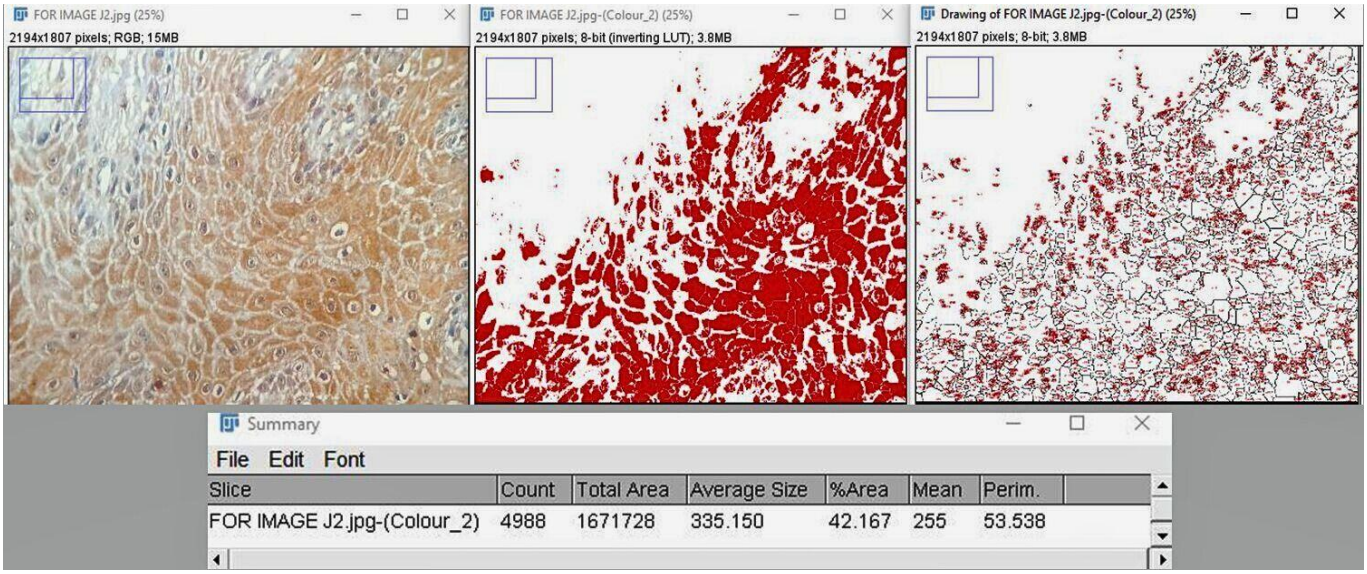

Figure 5. Fiji Image J quantitative analysis of TMPRSS2 staining of gingival epithelium.

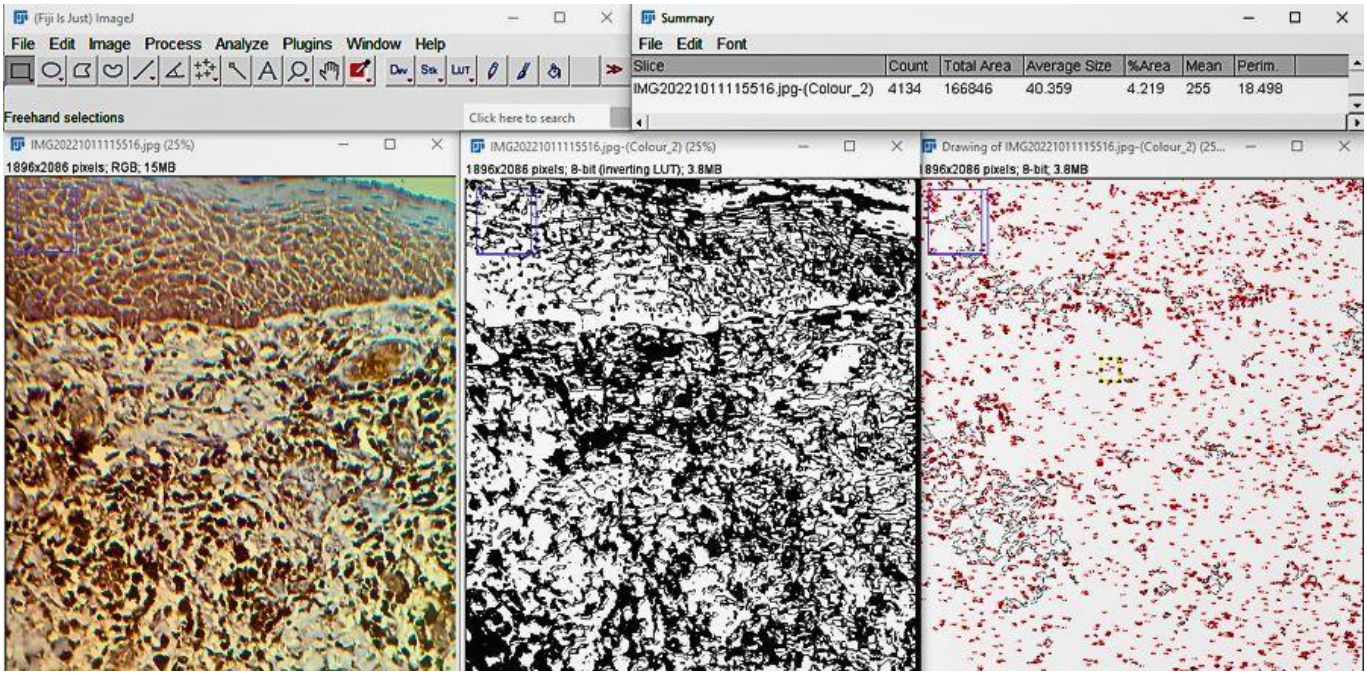

Figure 6. Fiji Image J quantitative analysis of ACE2 staining of nasopharyngeal mucosa.

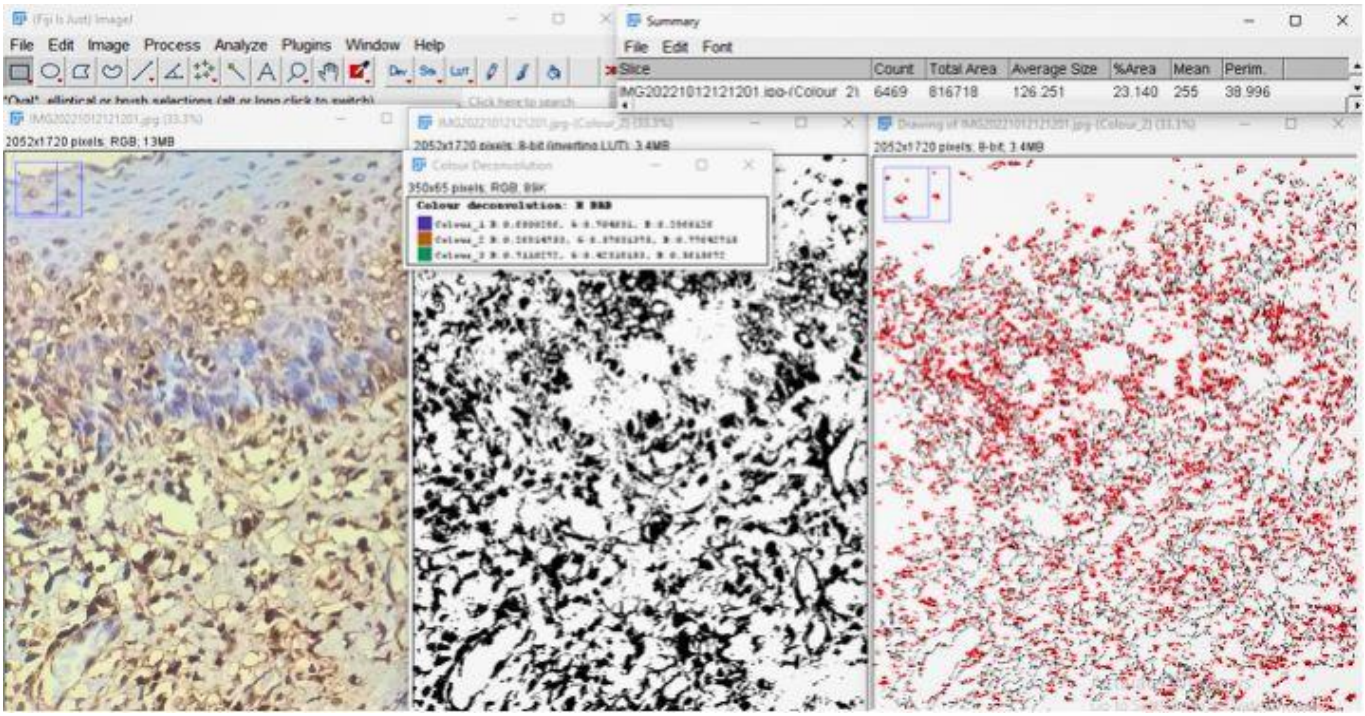

Figure 7. Fiji Image J quantitative analysis of TMPRSS2 staining of nasopharyngeal mucosa.

DISCUSSION

Receptors are the key players in viral infection as they determine the cellular or tissue tropism of the virus. The exquisite role of the receptor in progression of the viral replication should be well understood. Only upon binding to the specific receptors, the obligate intracellular parasites hijack the host cell and makes use of the host's protein synthesizing machinery. COVID-19 pandemic virus is also not an exception, as even nCoV (novel Corona Virus) like any other virus is dependent on binding to host cell receptors.

The expression of potential receptors, ACE2 and TMPRSS2 has been documented to have an

age-dependant expression correlating with the symptoms in elderly individuals infected with Covid-19. Bunyavanich and Vincenzo (2020) conducted a retrospective analysis in children and adults (4 to 60 years) to observe the pattern of ACE 2 gene expression in the nasal tissue. The study reported that ACE2 gene expression increased with age and was lowest in younger children. Peng *et al.* (2021) also analysed similar expression of ACE2 and TMPRSS2 in elderly people than the younger adults of both sexes ranging from 27 to 77 years of age. The study also detected the co-expression of both proteins by immunofluorescence in the epithelial cells of normal oral tissues from floor of the mouth, tongue, buccal mucosa, gingiva and palate (Peng *et al.,* 2021). Saheb *et al.* (2020) also reported a similar observation on the reduced gene expression of both the receptors in the upper airway epithelium of children than adults. Chen *et al.* (2020) evaluated ACE2 expression in adrenal gland, blood, nervous system, colon, adipose tissue, salivary gland and reported that the age dependent expression of ACE 2 receptor was tissue specific. Bille *et al.* (2020) studied ACE2 in the duodenal tissues of healthy individuals and found that the expression increased with aging. In a molecular study by Bilinska *et al.* (2020), RNA sequencing was done to study the gene expression of ACE2 and TMPRSS2 and found that the expression increased with aging in the olfactory epithelium. However, Schouten *et al* evaluated the bronchoalveolar lavage and found similar expression of ACE2 in all age groups. This may be attributed to the fact that the shedding of protein varies in the bronchoalveolar lavage (Schouten *et al.,* 2019*).*

In a review, Getachew and Tizabi (2021) stated that with increasing age, the expression of ACE2 decreases in both the sexes. Our findings were inconsistent with the findings of Xie *et al.* (2006), with high ACE2 in the lungs of younger rats than older of both genders. Similarly, Gu *et al.* (2021) also reported that high expression of ACE2 was seen in the lungs of newborn mice as compared to older ones. Age-specific variation was also reported extensively in the literature in 2002 outbreak of SARS-CoV and as the receptor for both SARS-CoV2 & SARS-CoV are alike, the present study was done to identify the age-specific variation in SARS-CoV2.

With respect to gender, Lukassen *et al.* (2021) documented increased ACE2 in males of 40-50 years than age-matched females. In a study by Peng *et al.* (2021), older males showed increased expression of TMPRSS2 in the oral epithelium than females. Zhao *et al.* (2020) also concluded that the expression of ACE by single-cell RNA sequencing was increased in Asian males than females. Piva *et al.* (2021) also observed that TMPRSS2 and ACE2 co-expression was exaggerated in the lungs of males than females. These observations are in conjecture with our results with enhanced ACE2 and TMPRSS2 expression in males. The existing literature reports that males with the habit of smoking showed exaggerated ACE2 expression. However, individuals with the habit of smoking were not included in our study. In our study, we found the expression levels of ACE2 and TMPRSS2 in relatively elderly people and males were higher than that of females. The increased expression in males may be attributed to the fact that estrogen in females contributes to the difference in protein expression. The oral tissue samples included in our study were taken from tongue, gingiva, buccal mucosa, and salivary glands. Nasopharyngeal tissues were taken from patients undergoing procedures such as adenoidectomy, tonsillectomy. Limited sample size, different tissue sites, less samples from females as compared to males might be related to the difference in receptor expression between both genders.

The findings of Xu *et al.* (2020) concluded that the expression was more in the tongue than other sites of the oral cavity in concordance with our findings of increased ACE2 in the oral epithelium. Study by Hamming *et al.* (2004) found that the basal cells of non-keratinising epithelium also expressed ACE2 receptors. The findings of a study by Peng *et al.* (2021) were also similar to our present study in terms of ACE2 and TMPRSS2 expression in the epithelial cells rather than the submucosa. Vinayachandran and Balasubramaniam (2021) also stated that the taste buds of the tongue showed greater expression of ACE2 contributing to the altered taste in COVID-19. Sakaguchi *et al.* (2020) explained that the epithelium of tongue showed increased expression of ACE2 and TMPRSS2. However, in our study, gingiva and salivary gland expression of

the receptor was more than tongue which may be a consequence of reduced tissue samples from tongue.

Chen *et al.* (2020) also detected these receptors in the secretory salivary glands. Immunopositivity for both the receptors was also confirmed in both the major and minor salivary gland acini by Xu *et al.* (2020). Usami *et al.* (2020) in contrast, found the expression of ACE2 considerably in the salivary duct than the acinar system. Zhu *et al.* (2022) studied these receptors in parotid, submandibular and sublingual salivary glands and validated the expression in the serous acini (cytoplasmic and membranous), endothelial cells, and intercalated duct. The findings of Allawi and Abdullah (2022) were also similar to the present study which showed protein expression in salivary ducts and acini. TMPRSS2 was also detected by Vaarala *et al* in the salivary glands (Vaarala *et al.,* 2001). These findings suggested that coronavirus might infiltrate the salivary glands by binding to the ACE2 and TMPRSS2 receptors.

Our study showed immunopositivity for both the receptors in the tonsillar epithelium, which was in concordance with the observations of Sakaguchi *et al.* (2020). Saheb *et al.* (2020) mentioned the receptors were increased in the epithelium of the nasal mucosa of adults than saliva and blood. Similarly, their detection was higher in the epithelium of oral mucosa as compared to the nasopharyngeal epithelium in our study, which was similar to the findings of increased expression in salivary glands than the lungs in the study by Song *et al.* (2020*)*.

The current pandemic had varying degrees of disease severity in different age groups Elderly individuals were the most infected in the first wave in India, which could be due to the fact that older people have increased expression of SARS-CoV2 receptors, namely ACE2 and TMPRSS2. Immunosenescence was also stated to be the cause for disease severity in elderly patients with co-morbidities (Witkowski *et al.,* 2022). Subsequently, during the second wave even younger adults had severe clinical manifestations. Similar differences in age in the consecutive waves of the pandemic were reported in studies from different countries. Iftimie *et al.* (2021) reported that patients who reported being positive for COVID-19 infection during the second wave were younger than those infected during the first wave in Spain. An Indian study by Kumar *et al.* (2021) based on the National COVID-19 registry observed that the second surge had lesser mean age values than the first wave. This variation may be because of the vaccination drive for elderly adults following the first wave of SARS-CoV2. However, Verduri *et al.* (2022) observed no such difference in age in European cohorts in the consecutive outbreaks. Similarly, Reddy *et al.* (2021) acknowledged that patients affected were younger in the first than the second surge in eastern parts of Uttar Pradesh. Other risk factors than receptor expression for COVID-19 infection may also be accountable for such age disparities in subsequent waves.

The present study has a few limitations, such as limited sample size, limited tongue samples and immunohistochemical analysis as the onsly method to analyse the expression. As furin is also involved in fusion and binding of the receptors, the expression of furin could have also been included in the study. Future studies with further co-localisation of the receptors, a wider cohort, mRNA analysis, single-cell sequencing, and therapeutic receptor blocking may provide better insight on the importance of variations in receptor expression.

CONCLUSION

As elderly people had higher oral and nasopharyngeal expression of ACE2 and TMPRSS2 than younger adults, we observed age-specific variation in the expression of potential SARS-CoV2 receptors in the present study. Our study identified the expression of ACE2, TMPRSS2 in oral and nasopharyngeal tissues and explained the correlation of expression with age. As both the oral and nasal mucosa are the initial gateway for any infectious disease, studying the difference in expression pattern of receptor in these two sites may provide a perceptive idea into the role of receptors in disease severity. The findings revealed that ACE2 and TMPRSS2 expression levels were greater in the comparatively old group than in the relatively younger group, and male oral epithelial cells expressed higher levels of TMPRSS2. As a result, the oral mucosa may be at risk of infection by

SARS-CoV-2, particularly in male or older individuals. Age-specific variation might provide an insight into better understanding of clinical expression of the disease and elaborate the validation of therapeutic target. Specific targeting at the receptor binding site might pave the way for localized or systemic therapeutic blockage at the receptor site.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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