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TMPRSS2 Polymorphism and Its Correlation with SARS-CoV-2 Susceptibility

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Abstract: *Background:* Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus affected more people than SARS-CoV-1 and related coronaviruses. Human genetic factors, besides respiratory droplets and direct exposure to the virus, are found highly responsible for transmitting SARS-CoV-2 infection. *Aim:* The objective of this study was to determine the plasma levels of the *TMPRSS2* gene and its role in SARS-CoV-2 susceptibility. *Methodology:* A total of 100 patients, i.e.50 SARS-CoV-2 positive patients as the case group and 50 SARS-CoV-2 negative samples as the control group, were selected randomly and included in this case-control study to determine the association between *TMPRSS2* gene and susceptibility to SARS-CoV-2 infection and severity of coronavirus disease 2019 (COVID-19). The TMPRSS2 levels of case and control samples were measured through an enzyme-linked immunosorbent assay (ELISA). Following the genomic DNA extraction, a set of reverse and forward primers of human *TMPRSS2* gene primers were used for the amplification of the *TMPRSS2* gene. *Results:* In the control group, the ratio of men to women was more or less the same while in the case group 62% of the population were women. The TMPRSS2 level was found to be 4.70 ± 7.7 ng/ml in case samples while it was 4.73 ± 5.7 ng/ml in control samples. *Conclusion:* The levels of TMPRSS2 in plasma samples of both controls and cases were found to be the same indicating that the entry of SARS-CoV-2 is not dependent on the plasma levels of this protein.

Keywords: *TMPRSS2*; SARS-CoV-2; ACE2; Gene; COVID-19; Expression

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1. Introduction

The coronavirus disease 2019 (COVID-19), the most crucial disease of the 21st century so far, was declared a pandemic in 2020. The disease struck distinct ethnic/racial sub-populations and had a huge economic and health impact in 222 countries and territories worldwide. As of 11th January 2022, a total of 5,492,595 deaths with 308,458,509 confirmed COVID-19 cases worldwide have been reported by the World Health Organization (WHO).

In the USA alone, there have been 831,548 deaths with 59,848, 136 confirmed cases from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. While, in Pakistan, the highest number of confirmed cases of COVID-19 recorded was 7,678 in a day, with 125 total deaths in 10 days (from 17th January 2022 to 26th January 2022) [2].

COVID-19 including SARS-CoV-2, belongs to the largest RNA viruses that develop infection in both upper and lower respiratory tracts. There is high variation in the symptoms of COVID-19 disease, from mild (cough, fever, body aches) to severe like influenza, pneumonia, and to the life-threatening acute distress of the respiratory tract that can eventually be fatal. SARS-CoV-2 attaches to the receptors present on the surface membrane of a host cell for its infection to occur. Human coronavirus NL63, SARS-CoV, and SARS-CoV-2 are the three coronavirus strains that involve the use of a zinc metalloprotease and angiotensin-converting enzyme 2 (ACE2) receptor. It was suggested by evidence that both the variations in viral spike and host ACE2 sequences might influence the trans-species spread of viral infection [3,4].

From the single-cell RNA sequencing, it was investigated that the SARS-CoV-2 tissue tropism is likely to be dictated by both ACE2 and transmembrane protease serine 2 (TMPRSS2) expressions [5]. The systematic studies of *TMPRSS2* polymorphism can therefore facilitate the development of personalized strategies of treatment and precision medicine for COVID-19.

TMPRSS2 is an androgen-regulated transmembrane protease found at the 21q22.3 position of the human chromosome, with an approximate length of 43.59kb containing 14 exons [6]. It is expressed in tissues including the bile duct, salivary glands, ovaries, pancreas, breast, prostate, colon, kidneys, lungs, and stomach [6]. TMPRSS2 contributes to vital biological processes including blood coagulation, tumor cell invasion, tissue remodeling, digestion, fertility, apoptosis, and inflammatory responses, however, the physiological roles of TMPRSS2 are still undetermined [7]. It has been discovered recently that TMPRSS2 is used for S protein priming by SARS-CoV-2 and ACE2 is also engaged as an entry receptor [8]. It is involved in the initial phase of SARS-CoV-2 infection, therefore, targeting TMPRSS2 activity or its expression may be of potential use for interventions against COVID-19 [9].

The objective of this study was to determine the plasma levels of the *TMPRSS2* gene and its role in SARS-CoV-2 susceptibility. As TMPRSS2 plays a very crucial role in S protein priming, it may be of great importance as a therapeutic target to inhibit or modify it to intervene in or prevent COVID-19, as it blocks the entry of the virus into target cells of hosts.

2. Methods

2.1. Sample collection

SARS-CoV-2 positive patients and SARS-CoV-2 negative samples were included in this case-control study as the case group and the control group, respectively. Institutional approval of the ethical review committee (ERC) was taken from the university (Ref. No. IERB(8)/SZABIST-KHI(LIFE)/1930114/210053). Blood samples accompanied by SARS-CoV-2 polymerase chain reaction (PCR)-confirmed reports were collected from a local lab in Karachi from October 2020 to March 2021. The experimental work was planned with the collection of weekly SARS-CoV-2 positive and negative blood samples from the lab followed by the separation and storage of plasma with the extraction of their DNA. The collection comprised of total 100 samples including 50 SARS-CoV-2 positive samples and 50 control samples. OpenEpi sample size calculator was used to calculate the sample size. The plasma and extracted DNA were stored at -20ºC for further use. Individuals with Hepatitis A, B, C, and D, HIV, AIDs, or any viral disease were excluded.

2.2. Genomic DNA extraction

For the extraction of genomic DNA from whole blood of SARS-CoV-2 positive and negative samples, the

reagent DNAzol was used. After optimization of the extraction procedure, the DNA extraction of case and control groups was done using the following steps: EDTA (ethylenediaminetetraacetic acid) blood tubes were subjected to centrifugation (x800*g* for 10 minutes) and the buffy layer was accumulated. To obtain a pellet containing white blood cells, it was treated with PCR-grade water. DNAzol reagent (500 µl) was added and incubated (15 minutes) at room temperature. Then, absolute ethanol (250 µl) was added to the lysate followed by incubation (3 minutes) at room temperature. Appearance of DNA threads was observed which were pelleted down and washed with 75% ethanol (1 ml). Decanting was done to remove ethanol and the tubes were left in the vertical position with open caps in the dryer (20 minutes) at temperatures up to 55°C. Sterile water (70µl) was then added to the pellet. It was vortexed, followed by incubation (10 minutes) in the dryer with closed caps of microcentrifuge tubes. The concentration of DNA in ng/µl was decreased for every sample using NanoDrop (Thermo Scientific 2000c).

2.3. Genomic DNA amplification

The amplification of the *TMPRSS2* gene was done after the extraction of the genomic DNA of the respective number of samples (case and control). It involved the use of a set of reverse and forward primers of human *TMPRSS2* gene (*rs12329760*) primers: forward primer 5'-CGCCCGTAGTTCTCGTTCC-3' and reverse primer 3'-TTCGCCTCTACGGACCAAAC-5'. These primers amplify 200bp of the scavenger receptor cysteine-rich (SRCR) regions in the *TMPRSS2* gene.

The polymerase chain reaction was carried out for amplification of the *TMPRSS2* gene by setting the specific PCR conditions: pre-denaturation for 5 minutes at 95°C, 38 cycles of 30 seconds each of denaturation (95°C), annealing (60°C), and extension for (72°C) with a final extension at 72°C for 10 minutes and kept on hold at 4°C till use. The PCR mix recipe contents included 4 µl extracted DNA, 1 µl forward and 1 µl reverse primers, 0.5 µl HRM (high-resolution melting) mix, and 6.5 µl water. Next, DNA gel electrophoresis (2% agarose gel) was carried out to determine the size of the PCR amplicons using Tris-borate-EDTA (TBE) buffer.

2.4. Enzyme-linked immunosorbent assay (ELISA)

A TMPRSS2 sandwich ELISA kit (Bioassay Technology Laboratory) was used to detect the levels of soluble TMPRSS2 levels in case and control plasma samples. The 96-well ELISA plate was coated with human TMPRSS2 antibody which binds with TMPRSS2 antigen in the added sample. Next, biotinylated TMPRSS2 antibodies were added to the TMPRSS2 antigen in the sample, this complex binds with the added streptavidin-HRP (horseradish peroxidase) conjugate. After incubation and washing of unbound conjugate, the substrate was added and the color appeared in response to the TMPRSS2 levels present in samples. An acidic stop solution was used to stop the reaction and A450 was measured.

Necessary reagents (standard dilutions and 1X wash buffer) were prepared for the procedure. The steps involved in ELISA were: 50 µl standard diluent added to the blank well, 50 µl stock and two-fold diluted standards added to the standard wells, 10 µl anti-TMPRSS2 antibody added to only samples wells, 40 µl of sample added to sample wells, 50 µl conjugate (streptavidin-HRP) added to standard wells and sample wells (not in blank well). Next, incubation was done for 60 minutes at 37°C, and 350 µl wash buffer per well was used to wash the ELISA plate 5 times with 30 seconds of soak time in each wash. Followed by the addition of substrate in each well (50 µl substrate A + 50 µl substrate B), incubation for 10 minutes in the dark at 37°C was done. An acidic stop solution (50 µl) was used to stop the reaction. ELISA microplate reader (BioTek) was used to get the optical density (450 nm) within 10 minutes. Between the known concentrations of diluted standards and their respective optical densities, the best-fit curve graph was prepared and the TMPRSS2 levels of case and control group samples were calculated.

2.5. Statistical analysis

The age and TMPRSS2 levels were expressed as mean ± standard deviation (SD) using Microsoft Excel 2016.

3. Results and discussions

3.1. Age and gender frequencies

The 50 SARS-CoV-2 negative individuals included in the control group consisted of 23 women (46%) and 27 men (54%) with a mean age of 45 ± 9 years. The 50 SARS-CoV-2 positive patients included in the case group consisted of 31 women (62%) and 19 men (38%) with a mean age of 47 ± 10 years. The ratio of men to women was found more or less the same in the healthy control group (**Figure 1**).

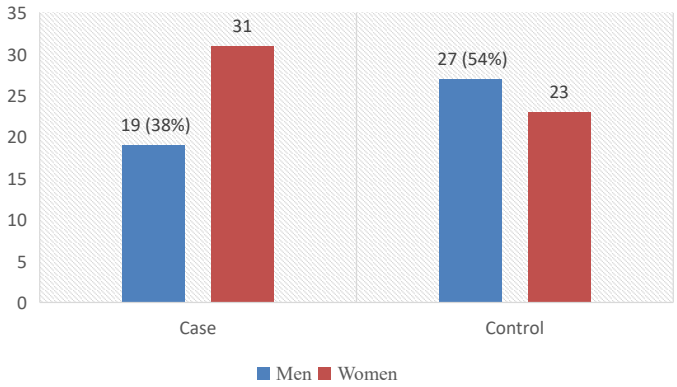

Figure 1. Graphical representation of gender frequency distribution

3.2. PCR amplification

PCR amplification was carried out after optimizing the PCR conditions. The amplification of 50 known positive SARS-CoV-2 samples (case group) and 50 SARSoV-2 known negative samples control group) was done separately in two batches. After running the conventional PCR with optimized conditions, DNA electrophoresis was carried out to run the amplicons as shown in **Figure 2**.

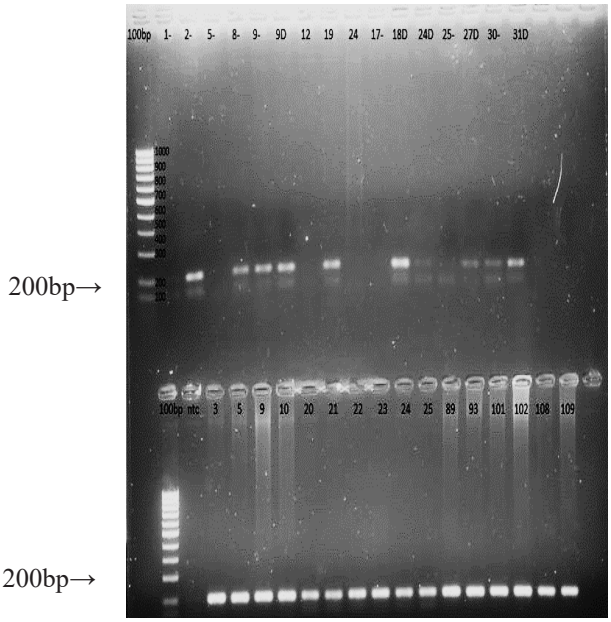

Figure 2. DNA gel electrophoresis of PCR of case (lane 1) and control (lane 2) samples. The amplicon size was 200bp according to the DNA ladder of 1kb (lane 1).

3.3. ELISA for TMPRSS2 levels measurement

The optical densities of the unknown concentration samples were plotted on the standard graph and the TMPRSS2 concentration of unknown samples from the known concentrations of standards was determined. The ELISA plate after the assay completion is shown in **Figure 3**. The TMPRSS2 level was found to be 4.70 ± 7.7 ng/ml in case samples while it was 4.73 ± 5.7 ng/ml in control samples.

Figure 3. ELISA plate after completion of the assay. Yellow color intensity shows the levels of TMPRSS2 protein in the samples.

4. Discussion

TMPRSS2 is termed as a gene that is androgen-responsive in nature [10]. Androgens, being present in low concentrations in women, might affect the expression of TMPRSS2 [11,12]. TMPRSS2 is more expressed in tissues with high sensitivity to androgen including the testis and prostate, and may considered to more likely occur in males, enhancing the chances for the acquisition of infection. This may be considered a reason behind different infection rates in different genders [13]. There is no difference in the *TMPRSS2* mRNA level expression in tissues of the lungs between men and women [9]. The susceptibility to COVID-19 disease might be reduced through the modulation of TMPRSS2 expression by the androgen-receptor-inhibition therapies, hence, the mortality rate might also be decreased. The altered profiles of resistance or susceptibility to the virus may be shown by the discrepant expression of lung-specific TMPRSS2. Generally, *TMPRSS2 rs9974589*, *rs7364083*, and *rs2070788* polymorphism have considerable roles. In males, the expression of TMPRSS2 is increased, favoring fusion of virus and membrane, by *TMPRSS2 rs8134378* polymorphism [14,15]. The missense variant *rs12329760* (c.478G>A, p.V160M), which alters the amino acid valine at position 160 to methionine, might be of interest with regard to COVID-19, because of its functional impact [16]. Thus, investigations were carried out by Wulandari *et al*. to determine the correlation between the severity of SARS-CoV-2, viral load, and the genetic variant possessed by the *TMPRSS2* gene in human host cells [17]. In lung cells and tissues, the data of TMPRSS2 expression was analyzed by correlating it to factors like exposure to pollutants, cigarette smoking habits, diabetes, sex, age, or other stimuli for extracting what factors might be responsible for altering expression of TMPRSS2, hence, impacting the infection susceptibility and prognosis of COVID-19. Additionally, a co-expression of other genes with *TMPRSS2*, specific to gender, relating to entry of SARS-CoV-2, was also highlighted, the higher infection susceptibility observed in men was explained by Piva *et al*. in 2021 [18]. TMPRSS2 allele frequency is found to be lower in East Asians [19]. In this study, the levels of TMPRSS2 in plasma samples of both controls and cases were found to be the same indicating that the entry of SARS-CoV-2 is not dependent on the plasma levels of TMPRSS2. The polymorphism can play a role in detecting the susceptibility marker for SARS-CoV-2 but it was found to be independent of TMPRSS2 plasma levels. The priming of the spike protein of SARS-CoV-2 is dependent on TMPRSS2 which can be respective of the genetic susceptibility but the plasma levels were not having any impact on viral spike protein priming for enhancing the entry of virus into the target cells.

5. Conclusions

This is the first time that the Pakistani population was examined for having *TMPRSS2* polymorphism and its association with SARS-CoV-2 susceptibility. The study aimed to determine the role of *TMPRSS2* polymorphism and its correlation with SARS-CoV-2 entry and susceptibility. Although, the results from this study showed that SARS-CoV-2 susceptibility is not associated with TMPRSS2 plasma levels and its polymorphism. The role of TMPRSS2 was not identified in SARS-CoV-2 entry in this study. The results obtained from age and gender studies of healthy and affected persons revealed that in the case group, 62% of the population were women and the ratio of men to women was found more or less the same in the healthy control group.

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Disclosure statement

The authors declare no conflict of interest.

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Experiment design: Saleem Awan, Anum Siraj, Amber Khan, Saira Yahya
Methodology and resources: Anum Siraj, Amber Khan, Arifa Anwar, Muhammad Naeem Kiani
Primer design: Amber Khan, Muhammad Naeem Kiani
Literature review, sampling, and result analyses: Saleem Awan
Principal investigator: Saira Yahya

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