“The role of ACE II receptor gene polymorphism in outcome of COVID 19 in Chhattisgarh population”.

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Abstract- A novel coronavirus designated as SARS-CoV-2 emerged in the city of Wuhan, China, and caused an outbreak of unusual viral pneumonia. Transmission of COVID-19 is mainly caused by respiratory droplets and prominently affect the respiratory tract (both lower and upper respiratory tract). SARS-CoV-2 infection necessitates the binding of the virus to the membrane-bound form of angiotensin-converting enzyme 2 (ACE2) receptor and internalization of the complex by the host cell so aim of study to assess ACE 2 polymorphisms can alter host susceptibility to SARS-CoV-2 by affecting this interaction and have assessed the outcome. It is Hospital based, Observational, Case Control study, in which 9 cases and 9 controls total 18 subjects were enrolled retrospectively in 10 months All subjects where COVID positive detected by RT-PCR. All cases were genotyped for ACE 2 I/D polymorphism using polymerase chain reaction We had observed that presence of genotype (ID) are exposed group of patients as these genotypes were predicted as severe form of COVID 19 infections and was found in 44% (4/9) among cases and 14% (1/9) among control groups (OR:6.40). These data suggest that the ACE 2 genotype may impact the incidence and clinical outcome of COVID-19 and serve as a predictive marker for COVID-19 risk and severity.

Keywords- ACE II receptor, COVID 19, I&D Polymorphism, RT-PCR, SARS-CoV-2.

INTRODUCTION: In December 2019, a group of pneumonia cases was reported at a wholesale seafood market in Wuhan, Hubei province, which was found to be caused by previously unknown Coronaviruses. On February 11, 2020, the International Committee for the classification of viruses designated the name of this coronavirus as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In addition, the World Health Organization has named the disease caused by the SARS-CoV-2 as coronavirus disease 2019 (COVID-19). The possible person-to-person transmission rapidly spreads to many provinces in China as well as other countries. Transmission of COVID-19 is mainly caused by respiratory droplets, direct human to human contact and fecal to oral transmission might be also associated. COVID-19 prominently affect the respiratory tract (both lower and upper respiratory tract), with the initial symptoms of common cold, fever, dry cough, fatigue, nasal congestion, sore throat and diarrhea to severe pneumonia, difficulty in breathing and ends with the patient death. The incubation period of the disease is 14 days and the time from onset of symptom to developing pneumonia is 4 days. Coronavirus are unsegmented single-stranded RNA viruses ranging from 26 to 32 kilobases in length, belonging to the subfamily Corona virinae of the family Corona viridae of the order Nidovirales. The genetic material of CoV is highly prone to frequent recombination process that results into the formation of new strains with altered virulence. ACE2 is a type I transmembrane metallo-carboxypeptidase with homology to ACE, an enzyme long-known to be a key player in the Renin-Angiotensin system (RAS), and a target for the treatment of hypertension. The mechanism for SARS-CoV-2 infection necessitates the binding of the virus to the membrane-bound form of angiotensin-converting enzyme 2 (ACE2) receptor and internalization of the complex by the host cell. A coronavirus has four structural proteins: envelope (E), membrane (M), nucleocapsid (N), and spike (S) proteins. The spike protein forms large protrusions from the virus surface, giving the appearance of a crown and, therefore, the name of coronavirus. The S protein consists of subunits S1 and S2, responsible for the attachment and membrane fusion, respectively. The spike binds to human ACE2 (hACE2) in the cell membrane through the S1 subunit of the receptor-binding domain (RBD). SARS-CoV-2 RBD binds to soluble hACE2 more strongly than SARS-CoV. This enhanced affinity for hACE2 may contribute to SARS-CoV-2’s higher infectivity. In this study, we have assessed if ACE 2 polymorphisms can alter host susceptibility to SARS-CoV-2 by affecting this interaction and have assessed the outcome.

MATERIALS AND METHODS

Study subjects
Hospital based data collection from the medical records of COVID 19 recovered patients in Dr. Bhim Rao Ambedkar Memorial Hospital (BRAM), Raipur. Online data collection from various research papers to look for most common types of ACE-II receptor gene polymorphism prevalent in India and South east Asia.
18 Patients were selected on basis of inclusion criteria. Sample was collected at least one month after patient’s recovery from Covid 19 infection diagnosed by RT-PCR to avoid infection during handling the sample. After taking consent telephonically, 2 ml venous blood sample in EDTA vial was collected at patient’s home and also from follow-up patients in respiratory medicine OPD for genetic analysis of ACE II receptor gene polymorphism in patient. Written informed consent was obtained prior to drawing of blood.

**Genotyping analysis**

High molecular weight genomic DNA was extracted from peripheral blood leucocytes using commercially available kit and quality/quantity was assessed by using spectrophotometer quantification and analyzed on gel electrophoresis. ACE2 gene polymorphisms (rs4646994) and rs2285666 were done in COVID-19 patients via polymerase chain reaction using specific primers (forward primer- 5’ CTGGAGACCCTCCCCATCTTTTCT 3’ and reverse primer- 5’ GATGTGGCCATCACATTCGTCAGA 3’) for rs4646994 and (forward primer-5’CATGTGGTCAAAAGGATATC-3’ and reverse primer 5’ AAAGTAAGGTGTCACAGCAT-3’) for rs2285666 . Initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 60 °C for 1.15 min, extension at 72 °C for 2.30 min and final extension at 72 °C for 10 min. PCR products were checked on 2.5% agarose gel and visualized by gel documentation system EZ, BIO-RAD (California), 481 bp product for allele I and 194 bp for allele D.

**Statistical analysis**

To compare between the exposed and non-exposed groups, odds ratio was used to determine ‘whether’ ACE receptor polymorphism is determining factor of severity for patient infected with COVID 19 and to compare whether outcome has any correlation with the gender and age. Staitical analysis was performed by using following softwares:[https://www.calculator.net/standard-deviation-calculator.html](https://www.calculator.net/standard-deviation-calculator.html) [https://www.medcalc.org/calc/odds_ratio.php](https://www.medcalc.org/calc/odds_ratio.php) [https://www.medcalc.org/calc/comparison_of_proportions.php](https://www.medcalc.org/calc/comparison_of_proportions.php)

**RESULTS**

The ACE 2 DD genotype, frequency of D allele were found to be high risk factors for causing disease severity among patients of COVID-19. The absence of the ‘I’ allele of ACE 2 I/D polymorphisms was significantly higher in severe COVID-19 patients

![GEL PICTURE](image)

**Table 1. Relationship between average age distribution in years between gender and study population.**

<table>
<thead>
<tr>
<th>CASE (Mean ± SD)</th>
<th>CONTROL (Mean ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 42±10</td>
<td>28.5±13.67</td>
<td>0.0294</td>
</tr>
<tr>
<td>Female 31±5.3</td>
<td>29.5±15.01</td>
<td>0.7810</td>
</tr>
<tr>
<td>Total 37±10</td>
<td>29±14</td>
<td>0.1821</td>
</tr>
</tbody>
</table>

**Table 2. Polymorphism distribution among population.**

<table>
<thead>
<tr>
<th>TYPE OF STUDY POPULATION</th>
<th>TYPE OF POLYMORPHISM</th>
<th>PRIMER 1*</th>
<th>PRIMER 3**</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASE</td>
<td>DD</td>
<td>44% (4/9)</td>
<td>0% (0/9)</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>33% (3/9)</td>
<td>38% (3/9)</td>
</tr>
</tbody>
</table>
The present study was conducted in the Multi-disciplinary research unit (MRU) and Department of Biochemistry Pt. J. N. M. Medical College, Raipur, in collaboration with the Dept of Respiratory Medicine and Dept of Anesthesiology Dr. B. R. A. M. Hospital, Raipur from March 2022 to November 2022. It comprised of 18 Covid positive cases, 9mildly affected patients as(control) and, a

<table>
<thead>
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<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRESENCE OF GENOTYPE DD*</td>
<td>44% (4/9)</td>
<td>14% (1/9)</td>
</tr>
<tr>
<td>ABSENCE OF GENOTYPE DD**</td>
<td>56% (5/9)</td>
<td>86% (8/9)</td>
</tr>
</tbody>
</table>

*Genotype (DD)**Genotype (ID+II)
P=0.0783
Odds ratio=6.4000
The major allele of rs 4646994 which indicates the 490 bp fragment that embraces the 287 bp Alu sequence, known as an insertion (II), The 190 bp fragment represents the minor allele with the deletion (DD) of the Alu sequence. Heterozygosity (ID) specifies the combination of both major and minor fragments, that is, 490/190 bp(Fig.1).

**DISCUSSION**

The present study was done with an aim to assess the role of ACE II receptor gene polymorphism in outcome of Covid 19 in Chhattisgarh population by comparing the clinical outcome with the ACE II genotype between case and control groups. ACE2 has been established as the functional host receptor for SARS-CoV-2,Both ACE andACE2 receptor genes have numerous genetic variations including functional polymorphism of I/D for ACE and rs4646994 and rs2285666 polymorphisms for the ACE2 receptor gene. The D allele shows higher ACE activity and polymorphisms in the ACE2 receptor gene affect circulating ACE2 receptor levels. Considering the role of ACE in COVID-19 pathogenesis and the variation in disease severity, ACE I/D, and ACE2 receptor gene variants have attracted the attention of researchers, as concluded by Suryamohan K, Diwanji D, Stawiski EW, Gupta R, Miersch S, Liu J, et al. The viral spike (S) protein engages the human angiotensin-converting enzyme 2 (ACE2) receptor to invade host cells with ~10–15-fold higher affinity compared to SARS-CoV S-protein, making it highly infectious. Here, we assessed if ACE2 polymorphisms can alter host susceptibility to SARS-CoV-2 by affecting this interaction.

The study was conducted in the Multi-disciplinary research unit (MRU) and Department of Biochemistry Pt. J. N. M. Medical College, Raipur, in collaboration with the Dept of Respiratory Medicine and Dept of Anesthesiology Dr. B. R. A. M. Hospital, Raipur from March 2022 to November 2022. It comprised of 18 Covid positive cases, 9mildly affected patients as(control) and 9severely affected patients (cases). The mean (±SD) age was 37±13 in cases, and 29 ± 14 in control groups, respectively. The middle-aged individuals where infected more as compared to old aged but the severity was more in old age individuals. Also, it was found that gender differences affect COVID-19 severity and men tended to be affected more seriously than women. The percentage of males was 56 % in the severe groups and 44% for females similar to the finding from work done by Pinto BG, Oliveira AE, Singh Y, Jimenez L, Gonçalves AN, Ogava RL, et al which suggest initial findings show that older men are more likely to have severe COVID-19 compared to women, indicate that ACE2 expression in the lung may be sex biased. From Table 1 we can interpret that older males are more prone to be affected severely with COVID 19 infection as (P=0.02) similar to the finding is similar to study done by Koch CM, Prigge AD, Anekalla KR, Shukla A, Do Umehara HC, Setar L, et al. The reason might be less amount of ACE 2 receptors in children than adult which leads to low viral entry via ACE 2 receptors. This invariably resulting in lower viral load in young patients compared to adults. Another factor may be the mucosal immune system in children which is considered to be better than that of adults. Also, children were less exposed socially owing to the Covid lockdown and thus were less exposed to infected individuals, as opposed to adults.

The distribution of each genotype for ACE I/D, ACE 2 rs4646994, and rs2285666 in the COVID-19 patients according to the severity of the disease are shown in Table 2. We had observed increased association of rs 4646994 DD genotype with severe form of COVID 19. It suggests that “DD” genotype has the potential to predict susceptibility for severe form of COVID 19 outcome & this conforms the earlier study in INDIA done on north Indian population by Verma S, Abbas M, Verma S, Khan FH, Raza ST, Siddiqi Z, et al, however our study was started at end stage of pandemic so we were not able to collect enough samples for getting statistically significant result.

**CONCLUSION**

We have observed increased association of rs 4646994 DD genotype with severe form of COVID 19. It suggest “DD” genotype has the potential to predict susceptibility for severe form of COVID 19 outcome, however our study was started at end stage of pandemic so we could not able to collect enough samples for getting statistically significant result was a major drawback of our study. If we find correlation between the polymorphism and outcome, then we can predict the vulnerable population and can limit the spread of infection by providing special care such as prioritizing immunization and for infected patients special wards with...
facilities like 24 hours uninterrupted oxygen supply, better ICU care etc can be arranged so that appropriate treatment could be administered, before the infection becomes fatal.

REFERENCES: