Male sex has been identified as a risk factor for higher mortality rates. This difference has primarily been attributed to the complex role of sex hormones. In this prospective cohort study, the authors from the United States measured estradiol, testosterone, P450 aromatase, and transmembrane serine protease 2 (TMPRSS2) levels in patients hospitalized for COVID-19 to determine possible association between their levels and disease severity or mortality.

Two host-cell factors are important for SARS-CoV-2 entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which is bound by S-protein, and TMPRSS2, which cleaves S-protein, leading to membrane fusion of the viral capsid with the eukaryotic membrane. The androgen receptor regulates TMPRSS2. Testosterone activates the androgen receptors, promoting TMPRSS2 transcription and increasing viral entry into cells. Enzyme cytochrome P450 aromatase regulates testosterone concentration converting androgens into estrogens and maintaining the balance of endogenous sex hormones in the body.

Deng Qu and associates confirmed in their study the transcriptional regulation of SARS-CoV-2 receptors ACE2 and TMPRSS2 by androgens in mouse and human cells. TMPRSS2 inhibitor camostat blocked the cleavage of pseudotype SARS-CoV-2 spike protein without disrupting the TMPRSS2-ACE2 interaction, and researchers stated that higher TMPRSS-2 levels increase disease severity in men with COVID-19. As TMPRSS2 inhibitor camostat and anti-androgens reduced SARS-CoV-2 cell entry, the authors proposed that androgen depletion/blockade or TMPRSS2 inhibition may be a therapeutic strategy for COVID-19. (Deng et al., iScience 2021, 24, 102254) https://doi.org/10.1016/j.isci.2021.102254

Contrary to this hypothesis, a study by Dhindsa S. *et al*. found that men with hypogonadism were 2.4 times more likely to be hospitalized for COVID-19 than men with eugonadism. This increased risk was independent of other factors that increase the risk of hospitalization for COVID-19, such as advanced age, comorbid conditions, and immunosuppression. Also, men with hypogonadism receiving testosterone therapy (TTh) were less likely to be hospitalized for COVID-19 than men with hypogonadism who were not receiving Tth. Similarly, men receiving inadequate Tth had a higher risk of hospitalization than those receiving adequate Tth. (Dhindsa S et al. Association of Male Hypogonadism With Risk of Hospitalization for COVID-19. *JAMA* Network Open. 2022;5(9):e2229747)
https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2795874

Other studies have also shown that testosterone concentration is lower in men with a severe course of COVID-19 than in men with a milder course. A measurement of testosterone

concentration in men hospitalized for COVID-19 revealed subnormal testosterone concentrations in 89% of patients at admission. Accordingly, it was hypothesized that testosterone in men might be an independent factor associated with the course of COVID-19.

About the Study and Results

Blood samples were collected from 265 hospitalized COVID-19 patients who met inclusion and exclusion criteria. They were categorized based on WHO COVID-19 severity classification, as either moderate to severe (hospitalized and requiring supplemental oxygen) or critical (in ICU on ventilator/artificial life support). 33% of COVID-19 patients were admitted to the ICU.

41% were women. The mean age of women was 54.3 years (ranging from 19 to 95 years). 59% of women were above 51 years old (average age of menopause). The mean age of men was 52.8 years (ranging from 22 to 101 years), and 56% were above 51 years. In the hospitalized COVID-19 patients, there was no significant difference in the demographics, past medical history, or mortality between genders.

The authors used enzyme-linked immunosorbent assay (ELISA) to measure total testosterone, estradiol, aromatase, and TMPRSS2 levels. CRP values were taken from hospital records at the time of admission.

Results showed that TMPRSS2 and testosterone levels were higher in men than women. Moreover, TMPRSS2 and testosterone were independently associated with gender after adjusting for age in a multivariate model. CRP levels after admission were also higher in men than in women. There was no gender difference in estradiol and P450 aromatase levels in hospitalized COVID-19 patients, which, according to the authors, may be due to the enrollment of more post-menopausal women.

COVID-19 patients were then categorized based on disease severity into either moderate to severe (n = 146) or critical (n = 119) disease. There were no differences in comorbidities, history of hypertension, diabetes, obesity, hyperlipidemia, or smoking between the two groups. Patients with critical disease were older and had higher CRP levels at admission.

Patients with the critical disease had significantly higher concentrations of TMPRSS2, estradiol, and P450 aromatase and lower concentrations of testosterone than those with moderate to severe disease. In addition, testosterone, TMPRSS2, and P450 aromatase were independently associated with COVID-19 severity after adjusting for age, sex, CRP, obesity, hypertension, hyperlipidemia, and diabetes in a multivariate logistic regression model. The authors noted that their results are consistent with other studies that have found lower testosterone concentrations in patients with critical COVID-19 compared to patients with moderate to severe disease, as the abovementioned study by Dhindsa S. *et al*. This supports the theory that in severe inflammation caused by COVID-19 testosterone is actively converted to estradiol by aromatase as a compensatory defense mechanism. Alternatively, the reduction in testosterone levels could represent transient hypogonadism or a stress response, as documented in other severe diseases. It is worth noting here that previous data have shown that persistent hypotestosteronemia in COVID-19 or long COVID syndrome could be of hypothalamic origin due to impaired gonadotropin-releasing hormone (GnRH) function, or hypogonadotropic hypogonadism.
https://discovermednews.com/hypotestosteronemia-men-covid-19-post-covid-hypothalamic-origin/

The group of critical COVID-19 patients was then dichotomized based on mortality into non-survivors ($n = 64$) and survivors ($n = 55$). Non-survivors had significantly higher TMPRSS2, estradiol, and P450 aromatase levels than survivors. In addition, TMPRSS2 and P450 aromatase were independently associated with mortality after adjusting for several risk variables (including age, sex, CRP, hypertension, hyperlipidemia, and diabetes) in a multivariate logistic regression model. CRP levels were also higher in survivors than in non-survivors. Since P450 aromatase levels were higher in COVID-19 non-survivors, and its levels were independently associated with mortality after adjusting for several risk

variables, it indicates that the activity of P450 aromatase increases with disease severity.

Conclusion

In this study, hospitalized patients with COVID-19 were found to have higher TMPRSS-2 and P450 aromatase levels and lower testosterone levels. Testosterone, TMPRSS2, and P450 aromatase were independently associated with COVID-19 severity, and TMPRSS2 and P450 aromatase were independently associated with mortality after adjusting for several risk variables in a multivariate logistic regression model. These results indicate that TMPRSS2, testosterone, and P450 aromatase can be independent markers of poor prognosis or increased COVID-19 severity.

These findings highlight the influence that complex dynamics of sex steroid aromatization and androgen receptor activity have on the TMPRSS2 expression and SARS-CoV-2 cell entry. Longitudinal studies at different time points may help further understand the role of this aromatization pathway.

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