The Resilient Child: Sex Hormones and COVID-19 Incidence in Pediatric Patients

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Abbreviations: Angiotensin-Converting Enzymes, ACE; Acute Respiratory Distress Syndrome, ARDS; Androgen Receptor, AR; Androgen Receptor Elements, AREs; Angiotensin II Type I Receptor, AT_1R; Center for Disease Control, CDC; Corona Viruses CoV; Corona Virus Disease-2019, COVID19; Cytokine Release Syndrome, CRS; Influenza A Virus, IAV; Influenza B Virus, IBV; Intensive Care Unit, ICU; Induced-Pluripotent Stem Cells, iPSC; Length of Stay, LOS; Receptor Mas, MasR; Middle East Respiratory Syndrome, MERS; Receptor Binding Protein, RBP; Renin-Angiotensin System, RAS; Ribonucleic Acid, RNA; Respiratory Syncytial Virus, RSV; Replication-Transcription Complex, RTC; Severe Acute Respiratory Syndrome, SARS; Single Nucleotide Polymorphism, SNP; Type-II Transmembrane Serine Protease-2, TMPRSS2; World Health Organization, WHO

Abstract

COVID-19, a disease caused by the SARS-CoV-2 infection, has become an unprecedented global health emergency, with fatal outcomes among adults of all ages in the United States, and the highest incidence and mortality in adult males. As the pandemic evolves there is limited understanding of a potential association between symptomatic viral infection and age. To date, there is no knowledge of the role children (pre-pubescent, ages 9 to 13) play as "silent" vectors of the virus, with themselves being asymptomatic. Throughout different time frames and geographic locations, the current evidence on COVID-19 suggests that children are getting infected at a significantly lower rate than other age groups - as low as 1%. Androgens upregulate the protease TMPRSS2 (Type-II Transmembrane Serine Protease-2), which facilitates efficient virus-host cell fusion with the epithelium of the lungs, thus increasing susceptibility to SARS-CoV-2 infection and development of severe COVID-19. Due to low levels of steroid hormones, pre-pubertal children may have low expression of TMPRSS2, thereby limiting the viral entry into host cells. As the world anticipates a vaccine against SARS-CoV-2, the role of pre-pubescent children as vectors transmitting the virus must be interrogated to prepare for a potential resurgence of COVID-19. This review discusses the current evidence on the low incidence of COVID-19 in children and the effect of sex steroid hormones on SARS-CoV-2 viral infection and clinical outcomes of pediatric patients. Upon reopening society at large, schools will need to implement heightened health protocols, with the knowledge that children as the "silent" viral transmitters, can significantly impact the adult populations.

Key words: Puberty, Androgens, Estrogens, Immune System, Children

Introduction

In February 2020 the World Health Organization (WHO) formally named the novel coronavirus outbreak triggered by 2019-nCoV as coronavirus disease 2019 (COVID-19). The Internal Committee of Taxonomy of Viruses then named the disease Severe Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), another β-coronavirus cluster related to Severe Acute Respiratory Syndrome (SARS) of 2003 and Middle East Respiratory Syndrome (MERS) of 2012 (1, 2). As of May 5, 2020, the global pandemic had caused over 3.5 million cases and 240,000 deaths, with numbers rising each day (3).

Person-to-person transmission of the virus includes droplet inhalation transmission and contact transmission through oral, nasal, and eye mucous membrane contacts (4). Symptoms of the virus vary depending on the patient but often include fever and dry cough, while others suffer from fatigue, dyspnea, nasal congestion, nausea, or diarrhea (5). The inflammation-driven damaging phase involves viral-induced tissue destruction, compounded by the development of cytokine release syndrome (CRS) causing debilitating effects (6). Cases worsen and lead to Acute Respiratory Distress Syndrome (ARDS) or the development of pneumonia. However, diagnosis is often complicated by a large portion of patients who are asymptomatic (7). Those with pre-existing conditions, such as diabetes, hypertension and pulmonary-, cardiac-, and kidney-disease are considered to be at higher risk of developing a severe form of the disease (8–10). Sex and age have also been important risk factors, as men and older populations are most at risk of contracting the infection and developing a more serious disease (11–14). The potential involvement of certain HLA haplotypes in increasing susceptibility to infection, supports a genetic pre-disposition (6). This review discusses the current evidence on the incidence of COVID-19 in children and the role of sex steroid hormones in the rate of SARS-CoV-2 infection and the clinical outcomes in pediatric patients.

COVID-19 Clinical Presentation in Pediatric Patients

The incidence of COVID-19 in children has been significantly lower compared to adults, with clear underrepresentation when taking into account the age proportions of the overall population. Comprehensive CDC data reported that between February 12th and April 2nd 2020, patients younger than 18 years old accounted for only 1.7% of US cases (11). Among all 2,572 COVID-19 cases in children aged <18 years, the median age was 11 years (range 0–17 years); 59% were ages 10-17, 26% were ages 1-9, and 15% were ages <1 year (11). Early reports from China echoed the recent US findings. A study presenting the 72,314 cases diagnosed by the Chinese Center for Disease Control and Prevention showed that most cases were in the adult age range of 30 to 79 years old (87%), with only 1% aged 10 to 19 years, and 1% aged 9 years or younger. There were also no deaths that occurred in children less than 9 years old (15). The current evidence on COVID-19 indicates that children are getting infected at a significantly lower rate than other age groups – as low as 1% of reported cases in most case studies (11, 16, 17). Similar trends were seen during the SARS epidemic, as children <12 years old had less severe cases of the disease, no deaths reported, and the disease was seen to increase in severity with age (18).

This trend may be due in part to the lack of testing in pediatric patients, as a large number of infected individuals are asymptomatic (5, 11, 16, 19, 20). Children are just as likely to be exposed to and contract SARS-CoV-2 but are less likely to be symptomatic for COVID-19 and thus are less likely to be tested as well (21). As similar rates were detected among the pediatric population during the SARS epidemic, there may also be an underlying molecular mechanism decreasing the susceptibility of children to developing a severe form of COVID-19 disease. Thus, case count is not always an accurate measurement of incidence, as there are asymptomatic children who are impacted by COVID-19 but are not part of the case count due to the lack of testing (19, 20). Among symptomatic children who were tested, the incidence of COVID-19 was still low. In an early report from the Wuhan Children's Hospital, 1,391 children who had upper respiratory symptoms resembling those of SARS-CoV-2 infections were

assessed and only 171 (12.3%) had COVID-19, with the median age of COVID-19-positive patients being 6.7 years, a younger median age than the CDC report from the United States due to the younger population studied (17). Death count, which is another more standardized measurement that has been used to control for testing capabilities, has also been low in the pediatric population, with some case reports reporting 0-0.2% deaths in patients under 19 years old (11, 22). Furthermore, 80% of the reported severe cases in children by the CDC were children that had an underlying condition, with the most common being chronic respiratory problems like asthma (11). The incidence, case count, severity of symptoms, and death rate, are all lower in pediatric patients compared to adult populations (Table 1) (22–28).

The pediatric experience at Mount Sinai supports the concept that children are less severely affected by COVID-19 than adults. Between March 1st through April 24th – representing the height of the pandemic in New York City – 29 children were admitted to the Kravis Children's Hospital at Mount Sinai with COVID-19. Ages ranged from 4 weeks to 21 years of age. There were 16 male and 13 female patients. The average length of stay (LOS) for this group was 4.5 days. Five of the 29 patients received ICU-level care. None required intubation and there were no deaths in the cohort. Two of the patients were babies in the NICU, where the average LOS was 2 days. At the end of April 2020, past the initial adult peak in New York City, reports began emerging of children with symptoms unlike the respiratory symptoms of cough and shortness seen in adults. Children have presented with skin rashes, conjunctivitis and an inflammatory vascular phenomenon that involves the heart. Initially high fever, tachycardia and hypotension were noted, in some cases precipitous decompensation requiring ICU admission occurred. As of this report, seven patients with this syndrome have required ICU care, including one that required ECMO; no fatalities have been seen. Resemblance to the pediatric illness Kawasaki's disease has been noted. Interestingly, one of these patients tested negatively on three occasions for COVID-19, only to have a later bronchial lavage sample be positive. On May 3rd, 2020, an

international conference confirmed the pathophysiology in a similar cohort of pediatric patients in Europe.

Typical symptoms in children include fever, cough, and shortness of breath (Table 1) (11, 22, 23, 29). Symptoms among pediatric patients tend to compare in nature to those in adults, though the overall severity of the symptoms and frequency of manifestation is lower (22). However, the sex discrepancy in the incidence of COVID-19 amongst adults is not reflected in the pediatric population. Adult males have significantly higher risk of ICU admission and death than adult females diagnosed with COVID-19 (12). In the pediatric population, there have been no significant differences in the number or severity of COVID-19 cases across sex (Table 1) (16). Thus, the different incidence rates between sexes only appears with adulthood, suggesting hormonal changes and sexual maturity may affect viral transmission and clinical symptom manifestations.

The low case number for pediatric patients being treated for COVID-19, presents a limitation in determining transmission patterns among this young population. In a case report tracing the transmission cluster of one pediatric case in France, a child who tested positive for COVID-19 visited three schools, and 169 individuals had been in contact with the student. Of these contacts, 70 individuals presented with respiratory symptoms. After testing 73 of these contacts, only 13 tested positive for COVID-19 (30). The low incidence amongst the contacts of confirmed positive children raises the possibility that SARS-CoV-2 follows a different transmission process in children than in adults, impacting the rate of viral infection and clinical manifestation of COVID-19 (22, 30). Moreover it was reported that the virus in pediatric patients is present in higher levels in stool samples than in the respiratory epithelium, raising the possibility that the virus may be transmitted through fomites from contaminated feces instead of the suspected transmission through respiratory droplets in this population (31).

In efforts to define the transmission dynamic among pediatric patients, one must also consider the potential spread through expecting mothers. A recent systematic review of cases in pregnant women recommended that pregnant women be treated as a high priority patient due to their immunosuppression (32, 33). Others have reported no severe or lethal cases in pregnancy, with some even suggesting a protective effect by pregnancy (34–37). In one report, it was suggested that there was no vertical transmission of the viral from mother to fetus, due to the absence of the virus in the amniotic fluid, cord blood samples, and breast milk, as well as swabs from the newborns (37). Of the reported cases of vertical transmission, the positively testing neonates recovered safely (22, 38, 39). Similar patterns were also shown in SARS, with no evidence of vertical transmission from infected mothers to their children (18).

The question thus arises as to a cut-off age for children for SARS-CoV2 infections and viral propagation. In addition to the lower case counts among pediatric patients, there is particularly lower incidence among younger children. In a large CDC cohort, 25% of pediatric patients were 1-10 years old, whereas nearly 60% of pediatric patients were ages 10-19 years old (11). This could be caused by the immunological changes and increasing hormone levels associated with puberty, but it is also important to understand the effects of adrenarche and the production of androgens in this process. Adrenarche is an early stage in sexual maturation in both sexes, in which the adrenal glands secrete increased levels of weak adrenal androgens including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S) and androstenedione (A4) without increased cortisol levels (40). It is a process related to puberty but distinct from hypothalamic-pituitary-gonadal (HPG axis) maturation and function (41). Adrenarche occurs on average at age 6 to 8 years and precedes puberty by about 2 years (40). Puberty in girls usually lasts from around 8-14 years of age, and for boys, it lasts from 10-16 years of age on average. The similar sex compositions across ages among those COVID-19 pediatric patients suggest effects from adrenarche rather than puberty, as the synchronous hormonal changes increase

susceptibility across both sexes until adulthood when men are at increased risk due to higher androgen levels (42, 43).

Molecular Mechanisms of SARS-CoV-2 Infection

Structural Spike (S) proteins drive entry of coronaviruses SARS-CoV and SARS-CoV-2 into target host cells by engaging the cellular receptor, Angiotensin-converting enzyme 2 (ACE2), and facilitating the viral attachment to target cells (44). This step is also functionally associated with activation of cellular TMPRSS2, a type-II transmembrane serine protease, that drives the entry of virus into the target cell and is regulated by Androgen Receptor (AR) signaling. Successful SARS-CoV infection is dependent on the proteolytic activity of TMPRSS2, which results in cleavage of SARS S protein at multiple sites (45). Proteolytic cleavage of SARS S protein by TMPRSS2, known as S priming, mediates efficient virus-host cell fusion and decreases virus sensitivity to neutralizing antibodies (46). ACE2 depletes Angiotensin I and II l (Ang I and II) evels by directly catalyzing the compounds and converting Ang I to Angiotensin 1-9 and Ang II to Angiotensin 1-7, known vasodilators acting through MasR with anti-fibrotic, anti-proliferative, and anti-inflammatory effects (8, 47–49). ACE2 expression is elevated in patients with cardiovascular conditions, diabetes, and hypertension, comorbidities that confer higher risk of mortality due to COVID-19 (50–52). Thus, inhibition of the Renin-Angiotensin System (RAS) may affect COVID-19 outcomes by decreasing proinflammatory activity of Ang II or increasing virulence in the heart and lungs due to the increased ACE2 expression (10).

Both ACE2 and TMPRSS2 are co-expressed on ciliated bronchial epithelial cells and type II pneumocytes, the epithelium of small intestine, and podocytes and the brush border of proximal tubule cells of the kidney, facilitating routes for SARS-CoV-2 infection (53). Compared to women, males have higher ACE2 expression and activity in the kidneys and higher expression of TMPRSS2 in the lungs (54, 55). There is also the potential for sex hormones to affect levels of activity of these receptors (56). Not

144 only might this impact the gender disparities in disease incidence, it could also have implications for the
145 infection rate children who physiologically have low levels sex hormones (57). It is critical to understand
146 the underlying mechanisms of the low incidence of COVID-19 in pediatric patients and their capacity to
147 be silent vectors while being asymptomatic (11, 16).
148
149 **Mechanistic Considerations in Diagnosis in Children**

150 ACE2 has been a common target of potential therapies against COVID-19; however, it is
151 unknown whether this important viral receptor contributes to the lower incidence of COVID-19 in
152 pediatric patients (58, 59). Several studies have reported no significant differences in lung ACE2
153 expression levels across sexes, implying that sex-related differences in ACE2 expression are not driving
154 the sex-related difference in disease severity (55, 56). It is interesting to note that of the 3,984 exomes
155 for ACE2 obtained from a large Italian cohort, there were no significant differences in the burden of rare
156 deleterious variants as compared to European and East Asian cohorts (55). The most common single
157 nucleotide polymorphism (SNP) difference in variants between the European populations and East Asian
158 populations was rs2285666 (also called G8790A) (55). This variant has been studied as a potential risk
159 factor for hypertension, type 2 diabetes, and coronary artery disease – comorbidities that increase
160 susceptibility to SARS-CoV-2 infection and severe virulence (60, 61). Therefore, ACE2 and genetic
161 variations of its target genes are important to the predisposition of certain populations to higher SAS-
162 CoV-2 infection, although independent of sex steroid hormones.

163 TMPRSS2 is essential for entry of a variety of viruses, such as SARS-Cov2, MERS-Cov, HCov-229E,
164 influenza A virus (IAV), and influenza b virus (IBV), in the primary human type II pneumocytes and
165 enhances the virulent effects on the host (46, 62, 63). Subsequently, there is emerging therapeutic
166 potential of TMPRSS2 inhibitors in the treatment of COVID-19 (46, 64). The TMPRSS2 gene, located on
167 human chromosome 21, has several androgen receptor elements (AREs) and TMPRSS2 protein is

expressed in an androgen-dependent manner in both prostate and lung cancer cells (65, 66). Androgen-regulated TMPRSS2 in prostate cells has been demonstrated to play a role in both normal male reproduction and in progression and metastasis of prostate cancer (67). Its expression in the lung and sputum has been found to be higher in men than in women (62, 68). This is consistent with increased susceptibility of men to COVID-19, as the increased TMPRSS2 allows for increased viral entry (64). TMPRSS2 also has an estrogen-responsive promoter, a mechanistic step that may be of significance in the hormonal control in pediatric patients as compared to adults (69). As pre-pubertal children have lower levels of sex steroid hormones, they will then have lower TMPRSS2, resulting in a lower disease incidence and severity in this age population.

While there is evidence that estrogens may influence TMPRSS2 activity, the main driver of expression of TMPRSS2 appears to be androgens (62). Adrenarche, as mentioned previously, is then an important time marker for increasing susceptibility in pediatric patients. Data supports that children ages 10 years and older of both sexes have higher incidence of COVID-19, suggesting the entrance into adrenarche and production of androgen may increase susceptibility to the virus (11). The presence of androgens may be even more important than pre-existing conditions, such as asthma, in the pediatric population. The TMRPSS2 expression in sputum was found to be no different in asthma patients as compared to healthy patients, yet was still increased in adult men as compared to adult women (68). Because of these androgenic effects, there is potential to decrease viral entry of host cells and prevent severe disease in high risk individuals through the use of androgen synthesis inhibitors, used in treatment of advanced prostate cancer (70). The evidence so far supports that with lower levels of androgens and estrogens, pediatric patients have low expression of TMPRSS2, limiting the extent of viral entry into host cells due to decreased protease activity; the lower viral load would lead to less severe symptoms among children (71).

SARS-CoV-2 Infection in Children: Environmental and Hormonal Reasoning

In addition to the potential molecular mechanisms contributing to the decreased incidence and severity of COVID-19 in pediatric patients, other physiological and environmental factors may be at play. In general, the pediatric population is exposed to fewer harmful environmental factors, such as smoke and air pollution, which allows them to have healthier respiratory tracts (72). However, prior studies in low income communities have shown differential susceptibility to some infectious diseases in children based on existing environmental, economic and psychological factors (73, 74). Psychological stress is higher among low-income families and their children, resulting in impaired immune function and hence higher susceptibility to epidemic diseases (75, 76). Malnutrition in children is also a driving mechanism for acute respiratory infection and related mortality (77–79). Disparities in vaccine uptake rates could cause differential susceptibility once exposed to the virus. Vaccine uptake rates are likely to differ by socioeconomic status, including access to health insurance (73, 74).

The child population is constantly exposed to a variety of coronaviruses that make up colds and flus that circulate endemically. These prior exposures could increase the resilience of children's immune systems (18). A study in France tracking infections spread among schools and family clusters found greater dissemination of picornaviruses and influenza amongst the pediatric population of both groups. The results suggest these infections are more easily transmitted and potentially more infectious than COVID-19 in children (30). These results were confirmed in a hospital study of pediatric patients in a hospital in Wuhan China. Of 366 patients with respiratory infections, 6.3% were detected to have influenza A, 5.5% had influenza B, and only 1.6% tested positive for COVID-19 (80). Inversely, exposure to other viruses and viral interference could potentially make these patients *more* susceptible to contracting the novel coronavirus, a trend seen by the interaction between H1N1 influenza virus and RSV (81).

COVID-19 and Educational and Psychosocial Health Challenges in Pediatric Patients

Despite lower incidence in the pediatric population, children have suffered from the disease in other indirect ways. Global preventive measures of COVID-19 through social-distancing have prompted the closure of schools (82). Though reducing COVID-19 incidence and mortality rates, the impact of school closures can have significant health, economic, and societal consequences for children (85, 86). These effects are likely to be more prominent in disadvantaged families and their children and may result in mental health challenges, more compromised nutrition, and economic costs to families who have suffered wage loss because of COVID-19 (84, 87). For many children, the COVID-19 crisis will mean falling academically further behind their peers, further increasing existing gaps in educational inequalities due to the lack of or limitation in internet access and availability of learning materials. In spite of the reduced COVID-19 risk in children, reopening schools may increase risk of this disease in teachers and ultimately increasing the risk for the larger community (86).

According to the Human Rights Watch, even though children are less likely to experience severe symptoms of or die with COVID-19 compared to different age groups, job and income loss and economic insecurity among families are likely to increase rates of child labor, sexual and physical exploitation (87). The high rates of COVID-19 mortality are also likely to result in large numbers of children losing significant family members, thereby increasing their psychological and physical vulnerability. For children living in institutional environments such as refugee camps, justice systems, immigration detention centers, or orphanages, the risk of COVID-19 and its physical and psychological consequences are higher due to the close proximity to other infected individuals, limited access to water and sanitation, and other environmental and health conditions that contribute to the lack of proper health care (87).

Future Directions

The concept that androgens correlate directly with TMPRSS2 activity and vulnerability to severe SARS-CoV-2 infection requires evaluation at the cellular and epidemiological level. It will be significant to determine if children with higher androgen levels – whether endogenous, as in intersex/DSD conditions (congenital adrenal hyperplasia) or exogenous, as in transgender adolescents who undergo cross-sex induction of puberty – demonstrate increased susceptibility to COVID-19 compared to their age- and chromosomal-sex-matched peers. Conversely, children with hypogonadism, whether natural or induced by puberty blockade, may be relatively protected from infection. If patients with hyperandrogenism are determined to be more vulnerable to infection, they and their families would be empowered to assume extra precautions.

When proposing the study of sex-gender minority populations, especially children who cannot provide their own informed consent to research, it is vital to consider not only the scientific merit of the project, but also its moral ramifications. For patients with intersex/DSD and transgender conditions, COVID-19 reinforces the social isolation and alienation from a culture that conflates sex with gender and perceives both as a male/female binary. Historically, many of these patients experienced their involvement in research and clinical care as stigmatizing and traumatic, issues that are further challenged by the isolating measures of the ongoing pandemic (88).

As the COVID-19 pandemic evolves implodes globally, the current knowledge at the molecular level implicates that this disease is biologically under sex steroid control, allowing investigators to better understand why most children are clinically "silent" carriers of the virus. In addition to TMPRSS2 activity, driving increased susceptibility to and cell internalization of SARS-CoV-2, further mechanistic insights into the hormonal regulation of immunological responses also invite an exploration of sex- and age-related differences. The direct action of androgens (driven by local precursor synthesis and metabolic alterations) in human lungs must be investigated. The strategy to address these issues will involve the

potential use of induced-pluripotent stem cells (iPSC) technology as a means to develop individual-specific lung epithelial cell lines and organoids that could be used to exploit the SARS-CoV-2 entry into human cells directly. Infected patients' specific derived alveolar cells dully differentiated under "reprogramming factors" could potentially populate tissue engineered lungs, provide a cell model for functional interrogation of lungs from COVID19-patients and drug testing against the disease (89). Induced to pluripotency stem cells can be differentiated to alveolar epithelium through exposure to a variety of different culture conditions and growth media. The ultimate success of differentiated cells for translational medicine applications will depend on further advances in the understanding of the effect of steroid hormones and SARS-CoV2 infection in human lungs, using *in vitro* cultures and organoids, from different iPSC to cells resembling respiratory epithelium *in vitro*.

At the time of submission of this review (early May 2020), there were emerging reports on a pediatric multisystem post-infectious inflammatory syndrome (myocarditis, high fever, and hypotension) resembling the pediatric Kawasaki's disease in SARS-CoV-2 infected children; this clearly merits further investigation immediately. Global initiatives and policies are needed to understand the COVID-19 pandemic to strengthen the health protection and social wellness for the most innocent and yet apparently most resilient members of our population, the children.

References

1. Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence. *J Med Virol*. Published online 2020:10-13. doi:10.1002/jmv.25722

2. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol*. 2020;92(4):418-423. doi:10.1002/jmv.25681

3. World Health Organization. Coronavirus disease (COVID-19): Situation Report – 106 Data. *World Heal Organ*. Published online 2020. doi:10.1001/jama.2020.2633

4. Perico L, Benigni A, Remuzzi G. Should COVID-19 Concern Nephrologists? Why and to What Extent? The Emerging Impasse of Angiotensin Blockade. *Nephron*. 2020;24126:1-9. doi:10.1159/000507305

5. Guan W, Ni Z, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. Published online 2020:1-13. doi:10.1056/nejmoa2002032

6. Shi Y, Wang Y, Shao C, et al. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ*. Published online 2020. doi:10.1038/s41418-020-0530-3

7. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507-513. doi:10.1016/S0140-6736(20)30211-7

8. Patel AB, Verma A. COVID-19 and Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers. *Jama*. 2020;2019. doi:10.1001/jama.2020.4812

9. Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int*. Published online 2020. doi:10.1016/j.kint.2020.03.005

10. Hanff TC, Harhay MO, Brown TS, Cohen JB, Mohareb AM. Is There an Association Between COVID-19 Mortality and the Renin-Angiotensin System—a Call for Epidemiologic Investigations. *Clin Infect Dis ciaa329,*. 2020;ciaa329. https://doi-org.eresources.mssm.edu/10.1093/cid/ciaa329

11. CDC COVID-19 Response Team. Coronavirus Disease 2019 in Children–United States, Februar 12–April 2, 2020. *Morb Mortal Wkly Rep*. 2020;69(56):1-8. doi:10.15585/mmwr.mm6914e4external icon.

12. Webb K, Peckham H, de Gruijtter N, et al. Sex-bias in COVID-19 : a meta-analysis and review of sex differences in disease and immunit. *Res Sq*. Published online 2020. doi:10.21203/rs.3.rs-23651/v1

13. Jin J-M, Bai P, He W, et al. Gender differences in patients with COVID-19: Focus on severity and mortality. *medRxiv*. Published online 2020. doi:10.1101/2020.02.23.20026864

14. Conti P, Younes A. Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. *J Biol Regul Homeost Agents*. 2020;34(2). doi:0.23812/Editorial-Conti-3

15. Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. *JAMA - J Am Med Assoc*. 2020;323(13):13-16. doi:10.1001/jama.2020.2648

16. Dong Y, Mo X, Hu Y, et al. Epidemiological Characteristics of 2143 Pediatric Patients With 2019 Coronavirus Disease in China. *Pediatrics*. Published online 2020:e20200702. doi:10.1542/peds.2020-0702

17. Lu X, Zhang L, Du H, et al. SARS-Cov-2 Infection in Children. *J Infect Dis*. Published online 2020. doi:10.1056/NEJMc2005073

18. Li AM, Ng PC. Severe acute respiratory syndrome (SARS) in neonates and children. *Arch Dis Child Fetal Neonatal Ed*. 2005;90(6):461-465. doi:10.1136/adc.2005.075309

19. Kam K, Yung CF, Cui L, et al. A Well Infant With Coronavirus Disease 2019 With High Viral Load. *Clin Infect Dis*. Published online 2020:1-3. doi:10.1093/cid/ciaa201

20. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3):1-8. doi:10.2807/1560-7917.ES.2020.25.3.2000045

21. Bi Q, Wu Y, Mei S, et al. Epidemiology and Transmission of COVID-19 in Shenzhen China: Analysis of 391 cases and 1,286 of their close contacts. *medRxiv*. Published online 2020. doi:10.1101/2020.03.03.20028423 .

22. Morand A, Fabre A, Minodier P, et al. COVID-19 virus and children: What do we know? *Arch Pédiatrie*. 2020;27(3):117-118. doi:10.1016/j.arcped.2020.03.001

23. Wang D, Ju X, Xie F, et al. [Clinical analysis of 31 cases of 2019 novel coronavirus infection in children from six provinces (autonomous region) of northern China]. *Chinese J Pediatr*. 2020;58(4):E011. doi:10.3760/cma.j.cn112140-20200225-00138

24. Liu Z, Bing X, Zhi X, et al. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2020;41:145-151.

25. National Institute of Infectious Diseases. *Field Briefing: Diamond Princess COVID-19 Cases, 20 Feb Update*.

26. Tian S, Hu N, Lou J, et al. Characteristics of COVID-19 infection in Beijing. *J Infect*. Published online 2020. doi:10.1016/j.jinf.2020.02.018 [pii: S0163- 4453(20)30101-8]

27. Tang A, Xu W, Shen M, et al. A retrospective study of the clinical characteristics of COVID-19

infection in 26 children. *medRxiv*. Published online 2020. doi:10.1101/2020.03.08.20029710 .

28. Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. *Lancet Infect Dis*. Published online 2020. doi:10.1016/s1473-3099(20)30198-5

29. Cai J, Xu J, Daojiong L, et al. A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features. *Clin Infect Dis*. 2020;ciaa198. doi:0.1093/cid/ciaa198

30. Danis K, Epaulard O, Benet T, et al. Cluster of coronavirus disease 2019 (Covid-19) in the French Alps, 2020. *Clin Infect Dis*. 2020;ciaa424. doi:10.1093/cid/ciaa424

31. Xing Y-H, Ni W, Wu Q, et al. Prolonged Viral Shedding in Feces of Pediatric Patients with Coronavirus Disease 2019. *J Microbiol Immunol Infect*. Published online 2020. doi:10.1016/j.jmii.2020.03.021

32. Liu H, Wang LL, Zhao SJ, Kwak-Kim J, Mor G, Liao AH. Why are pregnant women susceptible to COVID-19? An immunological viewpoint. *J Reprod Immunol*. 2020;139(103122). doi:10.1016/j.jri.2020.103122

33. Hasan MZ, Al Kibria GM, Alam T. Pregnancy during the evolving pandemic Coronavirus Disease 2019 (COVID-19): A rapid scoping review of evidence in the published literature. *Res Sq*. 2019;2019:1-34. doi:10.21203/rs.3.rs-23407/v1

34. Chen H, Guo J, Wang C, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet*. 2020;395(10226):809-815. doi:10.1016/S0140-6736(20)30360-3

35. Wang X, Zhou Z, Zhang J, Zhu F, Tang Y, Shen X. A case of 2019 Novel Coronavirus in a pregnant woman with preterm delivery. *Clin Infect Dis*. Published online 2020. doi:10.1093/cid/ciaa200

36. Liu W, Wang Q, Zhang Q, et al. Coronavirus disease 2019 (COVID-19) during pregnancy: a case series. Published online 2020. doi:10.20944/preprints202004.0004.v1

37. Vasylyeva O. Pregnancy and COVID-19, a brief review. *Int J Integr Pediatr Environ Med*. 2020;5:8-13. doi:10.36013/ijipem.v5i1.71

38. Lu Q, Shi Y. Coronavirus disease (COVID-19) and neonate: What neonatologist need to know. *J Med Virol*. Published online 2020:564-567. doi:10.1002/jmv.25740

39. Zeng L, Tao X, Yuan W, et al. [First case of neonate infected with novelcoronavirus pneumonia in China]. *Zhonghua Er Ke Za Zhi*. 2020;58:E009. doi:10.3760/cma.j.issn.0578-1310.2020.0009

40. Parker LN. Adrenarche. *Endocrinologist*. Published online 1993. doi:10.1097/00019616-199311000-00005

41. Palmert MR, Hayden DL, Mansfield MJ, et al. The longitudinal study of adrenal maturation during

gonadal suppression: Evidence that adrenarche is a gradual process. *J Clin Endocrinol Metab*. Published online 2001. doi:10.1210/jcem.86.9.7863

42. Warne GL, Carter JN, Faiman C, Reyes FI, Winter JSD. Hormonal changes in girls with precocious adrenarche: A possible role for estradiol or prolactin. *J Pediatr*. 1978;92(5):743-747. doi:10.1016/S0022-3476(78)80141-3

43. Longcope C, Kato T, Horton R. Conversion of blood androgens to estrogens in normal adult men and women. *J Clin Invest*. 1969;48(12):2191-2201. doi:10.1172/JCI106185

44. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science (80)*. 2020;367(6483):1260-1263. doi:10.1126/science.aax0902

45. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. *bioRxiv*. Published online 2020. doi:10.1101/2020.01.31.929042

46. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020;181:1-10. doi:10.1016/j.cell.2020.02.052

47. Patel VB, Zhong J-C, Grant MB, Oudit GY. Role of the ACE2/Angiotensin 1–7 axis of the Renin-Angiotensin System in Heart Failure. *Circ Res*. 2016;118(8):1313-1326. doi:10.1161/CIRCRESAHA.116.307708

48. de Farias Lelis D, de Freitas DF, Machado AS, Crespo TS, Santos SHS. Angiotensin-(1-7), Adipokines and Inflammation. *Metabolism*. 2019;95:36-45. doi:10.1016/j.metabol.2019.03.006

49. Gonzalez L, Novoa U, Moya J, et al. Angiotensin-(1-9) reduces cardiovascular and renal inflammation in experimental renin-independent hypertension. *Biochem Pharmacol*. 2018;156:357-370. doi:10.1016/j.bcp.2018.08.045

50. Anguiano L, Riera M, Pascual J, Soler MJ. Circulating ACE2 in Cardiovascular and Kidney Diseases. *Curr Med Chem*. 2017;24(30):3231-3241. doi:10.2174/0929867324666170414162841

51. Qiao W, Wang C, Chen B, et al. Ibuprofen attenuates cardiac fibrosis in streptozotocin-induced diabetic rats. *Cardiol*. 2015;131(2):97-106. doi:10.1159/000375362

52. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol*. Published online 2020. doi:10.1038/s41569-020-0360-5

53. Xu D, Zhang H, Zhou W, Wang LH, Cui XG. Identification of a potential mechanism of acute kidney injury during the COVID - 19 outbreak : a study based on single-cell transcriptome analysis. *Intensive Care Med*. Published online 2020:2-4. doi:10.1007/s00134-020-06026-1

54. Liu J, Ji H, Zheng W, et al. Sex differences in renal angiotensin converting enzyme 2 (ACE2) activity

412 are 17β-oestradiol-dependent and sex chromosome-independent. *Biol Sex Differ*. 2010;1(1):1-11.
413 doi:10.1186/2042-6410-1-6

414 55. Asselta R, Paraboschi EM, Mantovani A, Duga S. ACE2 and TMPRSS2 variants and expression as
415 candidates to sex and country differences in COVID-19 severity in Italy. *medRxiv*. Published online
416 2020.

417 56. Brosnihan KB, Hodgin JB, Smithies O, Maeda N, Gallagher P. Tissue-specific regulation of
418 ACE/ACE2 and AT1/AT2 receptor gene expression by oestrogen in apolipoprotein E/oestrogen
419 receptor-α knock-out mice. *Exp Physiol*. 2008;93(5):658-664.
420 doi:10.1113/expphysiol.2007.041806

421 57. Maruyama Y, Aoki N, Suzuki Y, Ohno Y, Imamura M, Saika T. Sex-steroid-binding plasma protein
422 (SBP), testosterone, oestradiol and dehydroepiandrosterone (DHEA) in prepuberty and puberty.
423 *Eur J Endocrinol*. 2020;114(1):60-67. doi:10.1530/acta.0.1140060

424 58. Sampson AK, Moritz KM, Denton KM. Postnatal ontogeny of angiotensin receptors and ACE2 in
425 male and female rats. *Gend Med*. 2012;9(1):21-32. doi:10.1016/j.genm.2011.12.003

426 59. Kruse RL. Therapeutic strategies in an outbreak scenario to treat the novel coronavirus
427 originating in Wuhan, China. *F1000Research*. 2020;9:72. doi:10.12688/f1000research.22211.2

428 60. Yang M, Zhao J, Xing L, Shi L. The association between angiotensinconverting enzyme 2
429 polymorphisms and essential hypertension risk: A metaanalysis involving 14,122 patients. *JRAAS -*
430 *J Renin-Angiotensin-Aldosterone Syst*. 2015;16(4):1240-1244. doi:10.1177/1470320314549221

431 61. Chaoxin J, Daili S, Yanxin H, Ruwei G, Chenlong W, Yaobin T. The influence of ACE2 gene
432 polymorphisms on type 2 diabetes mellitus and coronary heart disease. Published online
433 2013:2654-2659.

434 62. Bertram S, Dijkman R, Habjan M, et al. TMPRSS2 Activates the Human Coronavirus 229E for
435 Cathepsin-Independent Host Cell Entry and Is Expressed in Viral Target Cells in the Respiratory
436 Epithelium. *J Virol*. 2013;87(11):6150-6160. doi:10.1128/jvi.03372-12

437 63. Limburg H, Harbig A, Bestle D, et al. TMPRSS2 Is the Major Activating Protease of Influenza A
438 Virus in Primary Human Airway Cells and Influenza B Virus in Human Type II Pneumocytes. *J Virol*.
439 2019;93(21):1-22. doi:10.1128/jvi.00649-19

440 64. Brenner SR. Covid-19, TMPRSS2, and Whether Android Regulation Affects Pandemic Virus Gender
441 Incidence and Age Distribution of Disease. *Med Hypotheses*. 2020;140:109773.
442 doi:https://doi.org/10.1016/j.mehy.2020.109773

443 65. Lin B, Ferguson C, White JT, et al. Prostate-localized and Androgen-regulated Expression of the
444 Membrane-bound Serine Protease TMPRSS2. *Cancer Res*. 1999;59:4180-4184.

445 66. Mikkonen L, Pihlajamaa P, Sahu B, Zhang FP, Jänne OA. Androgen receptor and androgen-

dependent gene expression in lung. *Mol Cell Endocrinol*. 2010;317(1-2):14-24. doi:10.1016/j.mce.2009.12.022

67. Lucas JM, Heinlein C, Kim T, et al. The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate cancer metastasis. *Cancer Discov*. 2014;4(11):1310-1325. doi:10.1158/2159-8290.CD-13-1010

68. Peters MC, Sajuthi S, Dedord P, et al. COVID-19 Related Genes in Sputum Cells in Asthma: Relationship to Demographic Features and Corticosteroids Michael. *Am J Respir Crit Care Med*. Published online 2020. doi:10.1164/rccm.202003-0821OC

69. Baena E, Shao Z, Linn DE, et al. ETV1 directs androgen metabolism and confers aggressive prostate cancer in targeted mice and patients. *Genes Dev*. 2013;27(6):683-698. doi:10.1101/gad.211011.112

70. Sharifi N, Ryan CJ. Androgen hazards with COVID-19. *Endocr Relat Cancer*. Published online 2020. doi:10.1530/erc-20-0133

71. Liu Y, Yan LM, Wan L, et al. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis*. Published online 2020. doi:10.1016/S1473-3099(20)30232-2

72. Lee P-I, Hu Y-L, Chen P-Y, Huang Y-C, Hsueh P-R. Are children less susceptible to COVID-19? *J Microbiol Immunol Infect*. Published online 2020. https://doi.org/10.1016/j.jmii.2020.02.011

73. Quinn SC, Kumar S, Freimuth VS, Musa D, Casteneda-Angarita N, Kidwell K. Racial disparities in exposure, susceptibility, and access to health care in the US H1N1 influenza pandemic. *Am J Public Health*. 2011;101(2):285-293. doi:10.2105/AJPH.2009.188029

74. Kumar S, Crouse Quinn S, Kim KH, Musa D, Hilyard KM, Freimuth VS. The Social Ecological Model as a Framework for the Determinants of 2009 H1N1 Vaccine Uptake in the US. *Health Educ Behav*. 2012;39(2):229-243. doi:10.1177/1090198111415105.The

75. Cohen S, Janicki-Deverts D. Who's Stressed? Distributions of Psychological Stress in the United States in Probability Samples from 1983, 2006, and 2009. *J Appl Soc Psychol*. 2012;42(6):1320-1334. doi:10.1111/j.1559-1816.2012.00900.x

76. Cohen S, Doyle WJ, Skoner DP. Psychological stress, cytokine production, and severity of upper respiratory illness. *Psychosom Med*. Published online 1999. doi:10.1097/00006842-199903000-00009

77. Monto AS, Ullman BM. Acute Respiratory Illness in an American Community. *JAMA*. 1974;227(2):164-169.

78. Rajatonirina S, Razanajatovo NH, Ratsima EH, et al. Outcome Risk Factors during Respiratory Infections in a Paediatric Ward in Antananarivo, Madagascar 2010-2012. *PLoS One*. 2013;8(9):1-9.

doi:10.1371/journal.pone.0072839

79. Blumenshine P, Reingold A, Egerter S, Mockenhaupt R, Braveman P, Marks J. Pandemic influenza planning in the United States from a health disparities perspective. *Emerg Infect Dis*. 2008;14(5):709-715. doi:10.3201/eid1405.071301

80. Liu W, Zhang Q, Chen J, et al. Detection of Covid-19 in Children in Early January 2020 in Wuhan , China. *N Engl J Med*. 2020;328(14).

81. Casalegno JS, Ottmann M, Bouscambert-Duchamp M, Valette M, Morfin F, Lina B. Impact of the 2009 influenza a(H1N1) pandemic wave on the pattern of hibernal respiratory virus epidemics, France, 2009. *Euro Surveill*. 2010;15(6):2. doi:10.2807/ese.15.06.19485-en

82. UNESCO. COVID-19 Educational Disruption and Response. Published 2020. Accessed March 17, 2020. https://en.unesco.org/covid19/educationresponse

83. UNESCO. Adverse consequences of school closures. Published 2020. Accessed March 15, 2020. https://en.unesco.org/covid19/educationresponse/consequences

84. Lindzon J. School closures are starting, and they'll have far-reaching economic impacts. Fast Company. Published 2020. Accessed March 22, 2020. https://www.fastcompany.com/90476445/school-closures-are-starting-and-theyll-have-far-reaching-economic-impacts

85. Lough R. Coronavirus deprives nearly 300 million students of their schooling: UNESCO. The Telegram.

86. Bryant J, Dorn E, Hall S, Panier F. Safely back to school after coronavirus closures. McKinsey & Company. Published 2020. https://www.mckinsey.com/industries/social-sector/our-insights/safely-back-to-school-after-coronavirus-closures

87. Human Rights Watch. COVID-19 and Children's Rights.

88. Karkazis K. *Fixing Sex: Intersex, Medical Authority, and Lived Experience*. Duke University Press; 2008.

89. Ghaedi M, Niklason LE, Williams JC. Development of Lung Epithelium from Induced Pluripotent Stem Cells. Published online 2015:81-89. doi:10.1007/s40472-014-0039-0

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516 **Table 1: Summary of Current Literature on COVID-19 Incidence in Pediatric Patients**

Study	Location	Time Frame in 2020	Pediatric Count	Ages (Range)	Sex	Symptoms	Days to Symptom Onset (Range)	Deaths	Comorbidities
Cai et al. 2020	Shanghai, China	January 19, - February 3,	10	Mean 6.1 yo (3 mo - 11 yo)	1:1.5	- Fever 8 (80%) - Cough 6 (60%) - Sore Throat 4 (40%) - Nasal Congestion 3 (30%) - Sneezing and Rhinorrhea 2 (20%)	Mean 6.5 days (2-10 days)	0	
CDC 2020	United States	February 12-April 2	2,572/ 149,802 (1.7%)	Median 11 yo (0-17 yo)	57% male, 53% female	- 73% fever, cough, or shortness of breath		3	Of 345 cases with information: - 80 (23%) at least one - 40 chronic lung disease (including asthma) - 25 cardiovascular disease - 10 immunosuppression
Dong et al.	Mainland	January 16 - February	2,143	Median 7	57% males,	- Asymptomatic 94 (4.4%) - Mild 1091 (50.9%) - Moderate 831 (38.8%)	Median 2 days (0-42	1	

2020	China	8		yo	43% female		days)	
Liu et al. 2020	Wuhan, China	January 7 - January 15	6	Median 3 yo (1 - 7 yo)	33% male, 67% female	- High Fever 6 (100%) - Cough 6 (100%) - Vomiting 4 (66.7%)		0	
Lu et al. 2020	Wuhan, China	January 28 - February 26	171	Median 6.7 yo (1 day - 15 yo)	61% male, 39% female	- Asymptomatic 27 (15.8%) - Cough 83 (48.5%) - Pharyngeal Erythema 79 (46.3%) - Fever 71 (41.5) - Diarrhea15 (8.8%) - Fatigue 13 (7.6%) - Rhinorrhea 13 (7.6%) - Vomiting 11 (6.4%) - Nasal Congestion 9 (5.3%)		1	
Qiu et al. 2020	Zhejiang, China	January 17 - March 1	36	Mean 8.3 yo (1-16)	64% male, 36% female	- None 10 (28%) - Dry cough 7 (19%) - Dyspnoea or tachypnoea 1 (3%) - Pharyngeal congestion 1 (3%) - Sore throat 2 (6%) - Vomiting or diarrhea 2 (6%) - Fever 13 (36%) - Headache 3 (8%)		0	None
Tang et al. 2020	Shenzhen, China	January 16 - February 8	26	Mean 6.9 yo (0-13yo)	35% male, 65% female	- None 9 (35%) - Fever 11 (42%) - Cough 12 (46%) - Rhinorrhea 2 (8%) - Vomiting 2 (8%) - Diarrhea 10 (38%)		0	None had underlying conditions
Wang et al. 2020	6 provinces in mainland	January 25 - February	31	Mean 7.1 yo (6 mo -	48% male, 52%	- Fever 20 (65%) - Cough 14 (45%) - Fatigue 3 (10%) - Diarrhea 3 (9%)	Mean 5 days	0	

	China	21		17 yo)	female			
Xia et al. 2020	Wuhan, China	January 23 - February 8	20	Median 2.13 yo (1 day - 14.6 yo)	65% male, 35% female	- Fever 12 (60%) - Cough 13 (65%) - Diarrhea 3 (15%) - Nasal Discharge 3 (15%) - Sore Throat 1 (5%) - Fatigue 1 (5%) - Vomiting 2 (10%) - Tachypnea 2 (10%)	0	7 (35%) had history of congenital or acquired disease
Xu et al. 2020	Guangzhou, China	- February 20	10	(2 mo - 15 yo)	60% male, 40% female	- Asymptomatic 1 (10%) - Coughing 5 (50%) - Sore Throat 4 (40%) - Nasal Congestion/Rhinorrhea 2 (20%) - Diarrhea 3 (3%)	0	

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