Coronavirus Pandemic

SARS-CoV-2 viral shedding and susceptibility: perspectives on gender and asymptomatic patients

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Abstract
Despite efforts to contain and manage the SARS-CoV-2 outbreak which was declared a public health emergency of international concern in January 2020 by the World Health Organization (WHO), the COVID-19 pandemic still remains a major global challenge. Patients who display the classical symptoms of the infection are easily identified, tested, isolated and monitored. However, many cases of infected asymptomatic patients have been documented. These patients are not easily identified even though many evidences suggest that they can spread the virus to others. How and why these COVID-19 asymptomatic presentations occur remain unclear. The many theories and views are conjectural, and supporting evidences are still needed. In this review, we described the trend in SARS-CoV-2 viral shedding and susceptibility, providing perspectives on gender differences and asymptomatic patients. We further discussed how genetics, gender, viral inoculum, and pre-existing immunity may influence asymptomatic presentations in COVID-19 infections. We hope that this article improves our understanding of asymptomatic SAR-CoV-2 infection and it sheds light on some salient areas that should be considered as the search for a potent vaccine continues.

Key words: COVID-19; SARS-CoV-2; symptomatic; asymptomatic; viral shedding.


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Introduction
In late December 2019, a cluster of patients with pneumonia of an unknown cause was reported by the local health authorities in Wuhan, Hubei Province, China [1]. Subsequently, the identified pathogen, a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for causing coronavirus disease 2019 (COVID-19), was declared a “public health emergency of international concern” by the World Health Organization (WHO) on 30th January 2020 [2]. According to the WHO situation report, a total of 79.2 million confirmed cases and 1.7 million deaths were reported worldwide as at 27th December 2020 [3]. Although the WHO’s media briefing of 13th July 2020 revealed that COVID-19 was worsening as many countries neglected the basic principles of public health [4], the asymptomatic COVID-19 population remained a major challenge. Despite the promise of intensive contact tracing in restricting the spread of the virus, these ‘physically healthy’ but asymptomatic individuals are often neglected. Priority is placed on symptomatic patients, and perhaps from the economic perspective, not every citizen can be tested at all times to ascertain their infection status. However, this could amount to a serious public health consequence.

Most COVID-19 positive patients have reportedly had mild to severe respiratory disease that could occur within 2–14 days of exposure [5]. Many patients, however, are diagnosed with a positive molecular test but are either asymptomatic or minimally symptomatic [6]. Increasing evidence has shown that asymptomatic individuals can spread the virus, and these silent SARS-
CoV-2 spreaders have created difficulties in the management of the pandemic [6,7]. Most of the SARS-CoV-2 studies focused predominantly on symptomatic cases, therefore, data on asymptomatic cases are sparse. During the pre-symptomatic and asymptomatic phases, silent disease transmissions are responsible for more than 50% of the overall spread in COVID-19 outbreaks [8]. These silent transmissions can continue to cause outbreaks, even if all symptomatic cases are immediately isolated [8].

In terms of gender vulnerability to COVID-19, men, regardless of age, are more at risk of worse outcomes and death compared to females [9]. Previous clinical studies have also shown that females are less vulnerable to contracting viral infections, and have decreased production of cytokines [10]. Furthermore, in vivo studies in mice reveal higher expression of angiotensin-converting enzyme 2 (ACE2) in the kidneys of males compared to females [10,11]. This could account for the gender differences in vulnerability and advancement of COVID-19 since ACE2 is an important receptor for the virus. These results may indicate that men are more likely to get infected and die from COVID-19. Our understandings of the COVID-19 clinical features, gender susceptibility, and immune responses of asymptomatic people infected with SARS-CoV-2 are, however, limited.

In this narrative review, we provide a succinct account of SARS-CoV-2 viral shedding and susceptibility as they relate to gender and the asymptomatic population. We presented some salient factors that will not only improve our understanding of the gender and asymptomatic realm of SARS-CoV-2 but will also help shape our perspective and approach to tackling the pandemic. We feel that these elements are critical for a better understanding of the pathophysiology of COVID-19, and should be carefully considered by clinicians and scientists in the field.

Table 1. Duration of SARS-CoV-2 viral shedding in asymptomatic and symptomatic patients. Different sample types showed distinct duration of viral shedding in both symptomatic and asymptomatic patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of sample</th>
<th>Type of patients</th>
<th>Duration of viral shedding (days)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee et al. 2020 [74]</td>
<td>Nasopharyngeal and oropharyngeal swab</td>
<td>Symptomatic (including pre-symptomatic)</td>
<td>19.5 (SE = 0.63)</td>
<td>Viral loads in asymptomatic patients from diagnosis to discharge tend to decrease more slowly in the time interaction trend than those in symptomatic (including pre-symptomatic) patients</td>
</tr>
<tr>
<td>Li et al. 2020 [75]</td>
<td>Nasopharyngeal, oropharyngeal, sputum and anal swab</td>
<td>Pre-symptomatic</td>
<td>11.5 (IQR: 10–14)</td>
<td>Seven patients (38.9%) continued to shed virus after hospital discharge and during recovering phase. Detectable antibodies of SARS-CoV-2 and RNA were simultaneously observed in five patients (27.8%)</td>
</tr>
<tr>
<td>Zheng et al. 2020 [76]</td>
<td>Respiratory (sputum and saliva)</td>
<td>Symptomatic</td>
<td>22 (IQR: 17–31)</td>
<td>The median duration of virus in respiratory samples of patients with severe disease (21 days, IQR: 14–30) was significantly longer than patients with mild disease (14 days, IQR: 10–21; p = 0.04)</td>
</tr>
<tr>
<td>Lu et al. 2020 [77]</td>
<td>Nasopharyngeal swab</td>
<td>Symptomatic</td>
<td>17 (IQR: 12–23)</td>
<td>Prolonged duration of viral shedding in children with COVID-19 was associated with symptomatic infection (particularly fever, pneumonia, and lymphocyte count less than 2.0 x 10⁹/L). Symptom assessment could be a cue to ascertaining viral shedding status in children with COVID-19</td>
</tr>
<tr>
<td>Qi et al. 2020 [78]</td>
<td>Nasopharyngeal swab</td>
<td>Symptomatic</td>
<td>17 (IQR: 12–21)</td>
<td>-</td>
</tr>
<tr>
<td>Ling et al. 2020 [79]</td>
<td>Oropharyngeal swab</td>
<td>Symptomatic</td>
<td>9.5 (IQR: 6–11)</td>
<td>As the clearance of viral RNA in patient’s stools was delayed compared to that in oropharyngeal swabs, it may be pertinent to identify viral RNA in feces during convalescence</td>
</tr>
<tr>
<td>Lo et al. 2020 [80]</td>
<td>Nasopharyngeal swab</td>
<td>Symptomatic</td>
<td>18.2 (SD: 4.6)</td>
<td>-</td>
</tr>
<tr>
<td>Zhang et al. 2020 [81]</td>
<td>Nasopharyngeal and/or oropharyngeal swab</td>
<td>Symptomatic</td>
<td>10 (IQR: 8–17)</td>
<td>-</td>
</tr>
<tr>
<td>Long et al. 2020 [5]</td>
<td>Nasopharyngeal swab</td>
<td>Asymptomatic Mild symptomatic</td>
<td>19 (IQR: 15–26)</td>
<td>The asymptomatic group had a significantly longer duration of viral shedding than the symptomatic group</td>
</tr>
<tr>
<td>Cevik et al. 2020 [82]</td>
<td>Upper respiratory tract (URT)</td>
<td>Symptomatic</td>
<td>17 (95% CI: 15.5–18.6)</td>
<td>Maximum duration of SARS-CoV-2 viral RNA shedding reported in URT, LRT, stool and serum were 83, 59, 35 and 60 days, respectively</td>
</tr>
<tr>
<td></td>
<td>Lower respiratory tract (LRT)</td>
<td>Symptomatic</td>
<td>14.6 (95% CI: 9.3–20.0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Stool</td>
<td>Symptomatic</td>
<td>17.2 (95% CI: 14.4–20.1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>Symptomatic</td>
<td>16.6 (95% CI: 3.6–29.7)</td>
<td>-</td>
</tr>
</tbody>
</table>
SARS-CoV-2 viral shedding

Absolute reliance on viral shedding as a marker of infectivity in COVID-19 infection may be misleading as variations abound in the duration of SARS-CoV-2 viral shedding (Table 1). Similarly, the determination of infectivity based on positive quantitative reverse transcription polymerase chain reaction (RT-qPCR) can be misleading because the test detects both viable and non-viable SARS-CoV-2 [12]. Further studies are required to investigate whether or not SARS-CoV-2 patients with consistent positive RT-qPCR results remain infectious [12]. Depending on factors such as host immune system, amount of viral inoculum, etc., viral shedding and infectivity can differ between asymptomatic and symptomatic patients.

Current evidence indicates that SARS-CoV-2 viral loads in the respiratory tract can peak earlier compared to SARS-CoV [13,22]. In addition, studies show that nose swabs contained higher viral loads compared to swabs taken from the throat [13–15]. Based on symptom, it was found that viral load in an asymptomatic patient was similar to that in symptomatic patients, suggesting the possibility of COVID-19 transmission by asymptomatic or minimally symptomatic patients [13,16]. He et al. reported that patients with COVID-19 could be infectious as early as 2 days prior to the onset of symptoms [17]. Cheng et al. found that the transmissibility of SARS-CoV-2 was higher in contacts whose exposure to the index case began within 5 days of the onset of symptoms compared to those who got exposed at a later time [18]. These data suggest that containment method (i.e., isolation of newly diagnosed cases) alone is not sufficient to halt the spread of the virus. A more generalized measure, such as social distancing, is invaluable in minimizing the spread. In addition, many patients with mild illness are not able to isolate in the hospital and other isolation facilities.

The assessment of respiratory tract samples revealed that SARS-CoV-2 viral shedding persists for up to 63 days after symptom onset, and the median period of viral shedding is placed at 12–20 days [14,19,20]. However, the contagiousness may decrease drastically from the second week after the onset of symptoms, as it becomes impossible to isolate live SARS-CoV-2 [14,16]. Moreover, a study showed that SARS-CoV-2 could not be isolated from samples with less than 106 copies/mL [16]. Severe COVID-19 cases also showed prolonged viral shedding and higher viral loads [16,20]. Patients with coronary heart disease, decreased albumin levels, concomitant hypertension, severe illness at admission, delay in antiviral therapy, invasive mechanical ventilation, old age, and compromised immune system were reported to experience delays in SARS-CoV-2 RNA clearance [12].

Regardless of sample types, the duration of SARS-CoV-2 viral RNA shedding in both asymptomatic and symptomatic populations is comparable (Table 1). Among symptomatic patients, those with severe symptoms and comorbidities had a longer duration of viral shedding compared to pre-symptomatic or mild symptomatic patients [12,20,21]. Among sample types, prolonged detection of SARS-CoV-2 viral RNA was found in stool samples compared to other samples (Table 1), indicating the possibility of SARS-CoV-2 transmission during the convalescence phase. Since RT-qPCR cannot distinguish the viability of SARS-CoV-2, the detection of viral RNA in stools may not necessarily suggest the possibility for transmission or infectivity [22]. However, Wang et al. successfully grew four SARS-CoV-2 with high copy numbers from positive fecal samples and identified viable virus particles from two samples using electron microscopy [15]. These findings need to be further investigated in larger cohorts of patients in order to demonstrate the transmission and infectivity of SARS-CoV-2 from feces.

From a gender perspective, few studies reported males to have prolonged SARS-CoV-2 viral RNA shedding, indicating slower recovery than females [20,23,24]. In addition, Ortolan et al. reported that males are marginally more vulnerable to SARS-CoV-2 infection with a higher risk of presenting with a more serious disease and mortality than females [24]. However, another study involving severe COVID-19 patients did not identify a significant difference in the duration of viral RNA shedding between males and females [25].

The concern of seroconversion is another factor in understanding the infectivity of SARS-CoV-2. The relationship between seroconversion and contagiousness of COVID-19 patients is yet to be elucidated. Studies have shown that, despite seroconversion, viral shedding still occurs [14,26]. For example, Wölfel et al. recorded seroconversion in 50% of patients after a week, and seroconversion achieved in all patients on day 14, but viral loads did not rapidly decline [14].

SARS-CoV-2 susceptibility in asymptomatic population

Asymptomatic patients with COVID-19 often include individuals with abnormal chest computed
tomography (CT) images and positive RT-qPCR results but experience no clinical symptoms [5]. Several studies reported that 40–45% of those infected with SARS-CoV-2 would remain asymptomatic, potentially playing a role in spreading the virus and serving as a likely source of infection within the population [27–29]. Nonetheless, the prevalence of human-to-human transmission of SARS-CoV-2 remains uncertain [29].

Asymptomatic COVID-19 patients are reported to have an immune system that responds differently to SARS-CoV-2 infection. Although with abnormal radiological findings in one or both lungs, asymptomatic patients showed lower inflammatory response characterized by reduced cytokines and chemokines concentrations compared to symptomatic patients [5]. Garcia et al. mentioned that asymptomatic patients could have low SARS-CoV-2 viral load and an excellent immune response status, leading to rapid viral clearance [30]. It remains to be fully understood how and why some infected COVID-19 patients develop asymptomatic conditions. However, many factors, including environmental and host factors, appear to play a role in the establishment of asymptomatic COVID-19 infection (Figure 1).

**Impaired Type I Interferon (IFN) function and production**

In a viral infection, the innate immune system involves in early defense by releasing pro-inflammatory cytokines. Type-1 interferons (IFN-α and IFN-β), considered one of the essential classes of cytokines, inhibit viral replication, and induce extensive IFN-stimulated gene (ISG) expression that exert antiviral functions [31]. IFN-I development is rapidly triggered in the host by the identification of pathogen-associated molecular patterns (PAMPs) such as viral nucleic acids [32]. IFN-I mediated signaling, which involves interaction with transcription factors (e.g., TYK2, JAK1, STAT1, STAT2, and IRF9), rapidly stimulates the expression of hundreds of ISGs along with other IFN-I-controlled downstream molecules such as pro-inflammatory cytokines [33]. According to previous SARS-CoV studies, there are different SARS-CoV strategies that can inhibit IFN-I production and suppress its response to viral infection [34]. Similar to SARS-CoV, SARS-CoV-2 is thought to induce IFN-I immune response suppression and interrupt host innate immunity, resulting in failure to control viral infection at the early stage of infection [35].

Lokugamage et al. showed that SARS-CoV and SARS-CoV-2 exhibited marked differences in their ability to antagonize the IFN-I response. SARS-CoV-2 was more sensitive to IFN-I as activation of IFN-I pathways led to considerable impairment of viral replication [36]. Similarly, another study suggests that the use of IFN-α can reduce SARS-CoV-2 infection rate by inhibiting virus infection and replication [37]. In addition, Blanco-Melo et al. reported that IFN-I could efficiently restrict SARS-CoV-2 replication in vitro [38]. An investigation of the expression of ISG showed that there is significant elevation in the expression of ISGs with robust IFN response in SARS-CoV-2 infection [39]. In general, SARS-CoV encrypts at least 10 proteins which enable the virus to either evade or prevent the activation and antiviral action of IFN [33]. In SARS-CoV-2, suppression of IFN-I signaling is facilitated by ORF3a, Nsp1, and ORF6 proteins. The ORF3a protein promotes interferon alpha and beta receptor (IFNAR I) degradation, Nsp1 reduces STAT1 phosphorylation, and ORF6 sequesters karyopherin alpha 2 and beta 1, which are associated with STAT1 phosphorylation [40–42].

Although impaired type I IFN is understood to impact viral clearance and disease severity in COVID-19 infection, data to substantiate its role in the different presentations of symptomatic and asymptomatic cases is sparse. Recent evidence, however, points at mutation in IFN-associated genes. In the study, the genome of 659 hospitalized patients with life-threatening COVID-
19 pneumonia and 534 subjects with benign or asymptomatic infection was analyzed [43]. Among the COVID-19 patients, rare loss-of-function gene variants were found in 13 gene loci known to direct Toll-like receptor 3 (TLR3)- and interferon regulatory factor 7 (IRF7)-dependent type 1 IFN immunity to severe influenza virus infection. The absence of this mutation in the asymptomatic patients further strengthens the speculation that asymptomatic COVID-19 presentation is dependent on an intact IFN function.

Presence of autoantibodies could be another underlying factor. Autoantibodies are known for their ability to aberrantly identify, bind to, and neutralize self-proteins. In a recent study published in October 2020, the authors found immunoglobulin G (IgG) autoantibodies against IFN-α and/or IFN-ω in 101 patients out of the 987 with severe cases of COVID-19 [44]. These autoantibodies were absent in all the 663 patients with mild or asymptomatic cases of COVID-19 and present in only 4 of 1227 healthy subjects sampled prior to the pandemic. The presence of highly active autoantibodies may substantially deplete the needed IFN, leading to disastrous disease outcome. While this may in part explain the observed asymptomatic presentations in some of the COVID-19 patients, more studies are required to support the claim.

**HLA variability**

In certain populations, genetic differences play a role in the establishment and progression of some diseases. For example, variation in human leukocyte antigen (HLA) has been associated with the SARS-CoV-2 infection. HLA plays a crucial role in adaptive immunity and is a classical molecule that presents small pathogen-originated peptides on the surface of infected cells [45]. Generally, all Class I and Class II HLA proteins are translated from multiple genes, giving rise to several thousand genetically diverse alleles [46]. This accounts for different degrees of affinity to various pathogenic peptides and the competence of the HLAs to present them. Thus, an individual’s HLA genetic profile can influence the intensity of immune response to an intrusive pathogen, as the encoded HLA molecules may display different peptide-binding properties [46]. Barquera et al. reported that HLA-A*02:02, HLA-B*15.03, and HLA-DRB1*01:02 are the strongest HLA binders that bind more than 1% of peptides derived from all pandemic viruses, including SARS-CoV-2 [46]. This result indicates that individuals with these HLA genetic profiles have the ability to present highly conserved SARS-CoV-2 peptides, thereby facilitating adaptive T-cell cross-protective immunity in the course of the infection. Thus, a reduction in viral load is expected in such patients.

Up to now, there is no published evidence comparing genomic HLA profiles of asymptomatic and symptomatic COVID-19 patients. While such a study is still anticipated, it can be assumed that asymptomatic patients exhibit HLA alleles with strong binding ability. In contrast, individuals with HLA-B*46.01 have the weakest binding capacity to peptides of SARS-CoV-2 [47], suggesting a higher likelihood of disease progression and symptom development. Furthermore, although in SARS infection, a previous study ascribed infection severity to increase in HLA-B*46.01 allele [48]. Studies to improve our understanding of the role of HLA as it relates to asymptomatic and symptomatic COVID-19 presentations is pertinent, especially as the race for a potent vaccine continues.

**Pre-existing immunity (T cell memory)**

One concept proposes that some people have partial immunity to the SARS-CoV-2 due to the existence of memory T cells. Several studies have reported that certain people have pre-existing reactivity to SARS-CoV-2 though they do not have previous exposure to SARS-CoV-2 [49,50]. Although the immune response mechanisms underlying this concept are uncertain, prior exposure to circulating common cold coronaviruses might be involved. Mateus et al. reported that the pre-existing reactivity against SARS-CoV-2 comes from memory T cells and that cross-reactive T cells can specifically detect a SARS-CoV-2 epitope together with the homologue epitope from a common cold coronavirus such as (HCoV)-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1 [50]. Grifoni et al. also found SARS-CoV-2-reactive CD4+ T cells in approximately 40%–60% of healthy donors (recruited between 2015 and 2018) prior to the SARS-CoV-2 outbreak [49]. However, Le Bert et al. reported the existence of SARS-CoV-2-specific memory T cells in individuals with no history of SARS-CoV and COVID-19 and have not been in contact with people who had suffered SARS-CoV or COVID-19 infection in the past [51].

Sekine et al. reported robust T cell immunity in convalescent people with asymptomatic or mild COVID-19. In their study, a robust specific memory T cells population against SARS-CoV-2 was detected in seronegative exposed family members and convalescent people with asymptomatic and mild COVID-19, though several months after infection [52]. Memory T cell responses against SARS-CoV-2 were also found in healthy blood donors who donated blood
before (2019) or during the pandemic (2020) [52]. In line with their observations, none of the convalescent people in the study, including those with previous mild disease, experienced further recurrent episodes of COVID-19 [52]. The immune system of individuals with this pre-existing immunity would apparently ward off or reduce SARS-CoV-2 infection and/or severity, hence their asymptomatic presentations. However, more studies are needed to support this concept.

**SARS-CoV-2 viral inoculum**

The quantity of viral inoculum and the condition of the immune system during the incubation period (presymptomatic), real asymptomatic, and pseudo-asymptomatic periods can influence the susceptibility to and severity of COVID-19 infection. Given that disease severity was associated with infectious dose of influenza A virus in animal model, Paulo et al. used a simulation model to demonstrate the association of infectious dose and the rate of case fatality [53]. They found that the fewer the number of infectious individuals in the population, the lower the number of severe infections and case fatality rate. This observation was in part attributed to limited simultaneous contacts between susceptible persons and infectious ones. Thus, one possible theory to explain asymptomatic presentation in COVID-19 is the relationship between viral dose inoculation and the use of facemasks. The use of facemasks can considerably reduce exposure to the SARS-CoV-2 [54], which could result in mild infection due to limited dose of the viral particles.

Following the first reported case of fever in a cruise ship SARS-CoV-2 outbreak, all the passengers and crew utilized surgical or N95 masks in addition to isolation protocols. Although 128 of the 217 passengers and staff were ultimately positive with SARS-CoV-2, 81% of the infected people were asymptomatic [55]. On the contrary, on the Diamond Princess cruise ship where masks were not used, asymptomatic infections were observed in 47% of those infected by the virus [56].

An in vivo study by Chan et al. demonstrated non-contact transmission in 66.7% of exposed naïve hamsters following exposure to SARS-CoV-2 infected hamsters [57]. Interestingly, the use of surgical mask partition to contain the exhaled respiratory droplets from infected hamsters significantly decreased the transmission rate of SARS-CoV-2 to 16.7% ($p = 0.019$) [57]. In addition, unlike serious clinical manifestations observed in the challenged index hamsters, infected naïve hamsters had lower clinical scores, milder histopathological changes, and lower viral nucleocapsid antigen expression in respiratory tissues. The aforementioned findings support the assertion that mask-wearing reduces the transmission of SARS-CoV-2.

Although it is plausible that reduction in the viral inoculum is insufficient to prevent infection, masks can lower the required infectious dose. Lower SARS-CoV-2 viral inoculum suggests that infection takes time to build up while providing the immune system sufficient time to respond [58]. In contrast, higher SARS-CoV-2 viral inoculum implies higher viral load which could easily overwhelm the immune system, thus, facilitating disease severity. According to Virlogeux et al., a shorter incubation period could be indicative of a higher infective dose, contributing to faster or higher viral replication, an out-running adaptive immune response, or a more aggressive and damaging inflammatory responses, ultimately leading to more serious diseases [59].

**SARS-CoV-2 and gender susceptibility**

The rate of SARS-CoV-2 infection among males and females appears to vary. In a meta-analysis based on 43 studies published between 24th January 2020 and 28th February 2020, the percentage of male patients ranged from 29.0-77.0% (median 56.5 %) [60]. Based on epidemiological data across 38 countries, the case fatality rate among males is 1.7 times higher than females, with an increased risk of death for males above 30 years old [61].

Several studies suggested that the biological differences between males and females, such as sex chromosomes, sex hormones, genome, and epigenetics may affect SARS-CoV-2 infection [61]. The difference in the copy number of X-linked genes (TLR-7, TLR8, CD40L, FOXP3, and CXCR3) may account for any other possible sex advantage for disease susceptibility. These X-linked immunoregulatory genes modulate the innate and adaptive immune response to virus infection and influence the immune response [62]. It was suggested that women would have over-expression of these X-linked immunoregulatory genes because of the extra X chromosome. Therefore, women may have higher levels of antibodies and CD4+ T cells and lower viral load levels than men [63]. In this context, the role played by the Toll-like receptor 7 (TLR7) is very important [64]. TLR7 recognizes viral single-stranded RNA and is therefore likely to be implicated in the clearance of SARS-CoV-2. The expression level of TLR7 in innate immune cells is greater in females than in males [62], which leads to higher immune response and viral infection resistance. In COVID-19, the
occurrence of high levels of IL-6 and other inflammatory cytokines (the cytokine storm) is linked to a worse prognosis [65]. Interestingly, in females, the production of inflammatory IL-6 after a viral infection is lower than in males and is often associated with an improved life expectancy [66].

Sex hormones can be considered important determinants of SARS-CoV-2 susceptibility. Estrogen is a positive stimulator of the immune response, particularly with increasing the activity and proliferation of T-cells [67]. Estrogen increases humoral responses from B lymphocytes, producing more antibodies in females than males through enhancing IgG and IgM antibodies [68]. Also, estrogen increases neutrophil activation in non-infectious states while reducing the expression of TLR4 [68], whereas testosterone suppresses innate immune responses by reducing cytokine production and proliferation of lymphocytes [68]. Men with higher testosterone levels exhibited lower titers of antibodies after vaccination compared to women who have lower testosterone levels [68]. Therefore, the levels of testosterone and estrogen in men and women could predispose individuals to different levels of severity in COVID-19 symptoms [10].

SARS-CoV-2 susceptibility has also been ascribed to the association between SARS-CoV-2 and ACE2, the target receptor expressed in the virally exposed epitheliums [69]. This receptor is primarily involved in the first step of viral entry [70]. As ACE2 receptors are a significant part of the COVID-19 pathogenesis, genetic disparities can affect their expressions, and ultimately alter the normal interaction between SARS-CoV-2 and the host. The gene that codes for the ACE2 receptor is located on the X chromosome [71]. Increased estrogen levels will down-regulate the expression of ACE2 receptor [61]. Nevertheless, in female mice, it was found that the expression of ACE2 seems to increase with menstrual cycle and age. It was then hypothesized that the elderly and pregnant patients may increase their risk for COVID-19 because of the increased expression of ACE2 expression [10]. Generally, the increased estrogen in females reduces their chance of viral infections from increased macrophage/neutrophil/dendritic cell activity, humoral response, and T-cell function compared to males. It is believed that the increased immune response in females reduces their susceptibility to COVID-19. The biological mechanism behind these differences also needs further elucidation.

Qian et al. reported that there is heterogeneity in ACE2 expression in human alveolar type II cells from different individuals, suggesting variation in susceptibility to SARS-CoV infection [72]. Ortiz-Fernández and Sawalha reported a substantial heterogeneity in the genetic determinants of ACE2 and transmembrane protease serine 2 (TMPRSS2) expressions in different sex groups in the South Asian and East Asian populations [73]. In addition, a genetic predisposition for lower expression levels of ACE2 and TMPRSS2 genes are seen in African populations [73]. These data indicate that host genetic profiles influence inter-individual variability in vulnerability and severity of COVID-19.

Conclusions
Globally, several new cases of COVID-19 infection are recorded daily. This pandemic has triggered severe public health issues, and healthcare systems around the world, for the most part, have been overwhelmed. Management and control have been largely through isolation and contact tracing – an effort that seemed to have been sabotaged by asymptomatic COVID-19 patients, as they continue to silently spread the virus. In this review, SARS-CoV-2 viral shedding and susceptibility as it relates to asymptomatic COVID-19 patients were discussed. More investigations on asymptomatic populations are needed to broaden our understanding of the pathogenesis of the virus.

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