

Premenopausal and postmenopausal women during the COVID-19 pandemic

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Abstract

The current global COVID-19 mortality rate is estimated to be around 3.4%; however, it is dependent on age, sex, and comorbidities.

Epidemiological evidence shows gender disparities in COVID-19 severity and fatality, with non-menopausal females having milder severity and better outcomes than age-matched males. However, the difference vanishes when comparing postmenopausal women with age-matched men.

It has been suggested that, to some extent, this is due to the protective role of female hormones, such as anti-Müllerian hormone and oestradiol (E2), in non-menopausal women.

Oestrogens have been hypothesized to be crucial in modulating viral infection and the progression of the disease via an action on immune/inflammatory responses and angiotensin-converting enzyme type 2 expression. Hence, the most likely explanation is that, because the levels of oestrogen in females after menopause decrease, oestrogen no longer offers a beneficial effect as seen in younger females.

The COVID-19 pandemic has highlighted the serious negative effects arising from the state of E2 deficiency. Therefore, hormone replacement therapy gains further support as the damaging effect of the decline in ovarian function affects many biological systems, and recently with the COVID-19 pandemic, oestrogen's vital role within the immune system has become quite clear.

However, additional clinical investigations regarding hormone replacement therapy are urgently needed to further verify the protective and therapeutic effects of E2 on menopausal women with COVID-19.

Key words: postmenopause, oestrogen, COVID-19, premenopausal women.

Introduction

On 31 December 2019, the WHO was informed of cases of pneumonia of unknown cause in Wuhan City, China. A novel coronavirus was identified as the cause by Chinese authorities on 7 January 2020 and was temporarily named "2019-nCoV" [1]. By mid-March 2020, the WHO European Region had become the epicentre of the epidemic, reporting over 40% of globally confirmed cases. As of 28 April 2020, 63% of global mortality from the virus was from the region. According to the WHO, by the end of 2020, nearly 100 million patients worldwide had been diagnosed with COVID-19, with more than 2 million deaths. By September 2021, almost 2 years after COVID-19 was first identified, there had been more than 200 million confirmed cases and over 4.6 million lives lost to the disease [1].

At the time of writing (February 2022), the number of new COVID-19 cases remains similar to that reported in the last week of January 2022, while the number of new deaths increased by 9% [2]. Across the 6 WHO regions, over 22 million new cases and over 59,000

new deaths were reported. As of 30 January 2022, over 370 million confirmed cases and over 5.6 million deaths have been reported globally. At the regional level, increases in the number of new cases were reported by the Western Pacific (37%) the Eastern Mediterranean (24%) and the European (7%) regions, while a decrease was reported by the Region of the Americas (20%) and the South-East Asia Region (8%) [2].

COVID-19 epidemiological data

The current global mortality rate is estimated to be around 3.4%; however, it is dependent on age, sex, and comorbidities [3].

Interestingly, a noticeable difference has been observed in various epidemiological studies when cases were analysed by gender, with women showing significant protection against severe disease presentations and related outcomes in response to the COVID-19 infection [4–7]. Early epidemiological observations indicated that severe acute respiratory syndrome coronavirus

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(SARS-CoV-2) infects all age groups, but with a higher rate among men (58.1%) than women (41.9%) [8].

This observation was confirmed by several national organizations of disease control and prevention (China – 4.7%: 2.8%, Italy – 10.4%: 6.2%, and Korea – 2.99%: 1.91% in male vs. female, respectively) [9–11], with similar trends in Iran, Germany, France, the U.S., and the U.K. [12, 13].

In 34 out of the 35 countries that provided sex disaggregated data, the male/female ratio is always above 1.1 (Pakistan is the exception, with a ratio of 0.9), independently of age [14, 15].

Men account for over 50% of total deaths, and almost twice as many men with COVID-19 suffer severe symptoms or death in comparison to women [12, 16]. This was confirmed in a meta-analysis conducted in 2020, which showed, that male sex was associated with the development of severe disease as measured by ICU admission (OR=2.84; 95% CI: 2.06–3.92; $p=1.86 \times 10^{-10}$) and death (OR=1.39; 95% CI: 1.31–1.47; $p=5.00 \times 10^{-30}$) [17].

All these reports suggest that men are more adversely affected and have worse clinical outcomes compared to women, with higher morbidity and mortality [13].

COVID-19 transmission, infection, replication, and clinical effect

The enveloped virus contains a positive-sense, single-stranded RNA genome and a nucleocapsid of helical symmetry of ~120 nm [18]. There are several plausible pathways for viruses to be transmitted from person to person, including virus transmission via direct (deposited on persons) or indirect (deposited on objects) contact and airborne (droplets and aerosols) routes [19–21].

It is now established that the airborne transmission of COVID-19/SARS-CoV-2 is highly virulent and represents the dominant route to spread the disease [18]. This finding was obtained based on the analysis of the trend and mitigation measures in 3 different cities considered epicentres of COVID-19: Wuhan, China, Italy, and New York City, in the period from 23 January to 9 May 2020 [22]. While transmission via direct or indirect contact occurs in a short range, airborne transmission via aerosols can occur over an extended distance and time. Inhaled virus-bearing aerosols deposit directly along the human respiratory tract [18].

In short, SARS-CoV-2 enters the cell via the angiotensin-converting enzyme type 2 (ACE-2) receptor, which is expressed by pneumocytes and leads to the down-regulation of ACE-2 levels. Angiotensin-converting enzyme type 2 is normally responsible for converting angiotensin II (Ang II) into vasodilatory and less immune augmenting variants of angiotensin [23].

Angiotensin II binds type 1 angiotensin receptors (AT1R) in the lung to induce vasoconstriction and inflam-

mation via activation of the nuclear factor κ B (NF- κ B) pathway, which increases cytokine synthesis [24]. Low levels of ACE-2 and high levels of Ang II lead to increased pulmonary vessel permeability, which results in inflammatory damage to the lung tissue [25].

This enveloped positive-sense, single-stranded RNA virus is capable of infecting multiple organ systems in its host, and the density of ACE-2 receptors in each tissue correlates with the severity of organ-specific pathology [26].

The proposed primary reason for severe COVID-19 is the cytokine storm (excessive production of proinflammatory cytokines) [27]. In an attempt to protect the body from SARS-CoV-2, immune cells infiltrate the lungs, causing hyperactivation of monocytes and macrophages, and elevated production of proinflammatory cytokines (e.g. interleukin-6 (IL-6), interleukin-1 β (IL-1 β), tumour necrosis factor α (TNF- α)), and chemokines (e.g. monocyte chemoattractant protein-1 (MCP-1/CCL2)) [27]. These patients rapidly develop respiratory distress syndrome, and lung oedema and failure (often associated with hepatic, myocardial, and renal injury and haemostasis alteration) [28]. Interestingly, when compared with non-intensive care patients, intensive care patients have higher levels of IL-2, IL-7, and TNF [28]. Many cytokines detected in these patients belong to the Th17 type response. The consequent IL-17-related pathway promotes broad pro-inflammatory effects by induction of specific cytokines, such as IL-1 β , IL-6, TNF (responsible for systemic inflammatory symptoms), chemokines, and matrix metalloproteinases (responsible for tissue damage and remodelling) [29]. Moreover, pro-inflammatory cytokines, including IL-1 β and IL-6, are directly induced by SARS-CoV-2 by interaction between viral components (probably nucleocapsid proteins) and toll like receptors of the host cells [30]. Increased production and elevated local and systemic IL-6 is hypothesized to be central to the development of the cytokine storm [31, 32], resulting from an unchecked inflammatory response that damages the lung tissue. This could be worsening some patients' condition severely enough to require assisted ventilation and eventually causing death in a substantial percentage of cases [33].

Pathophysiological context and the role of oestrogens

Sex-specific infection and mortality rates have been documented in humans [23]. As mentioned earlier, the number of deaths due to COVID-19 infection is lower in women than in men [12, 16, 34, 35]. When considering men and women of all ages, women seem to be infected at similar rates to men, but the infection is less lethal to women [36]. Additionally, in the cohort of their study, Liu *et al.* found that female patients had lower

disease severity and mortality than male patients, especially aged ≤ 55 years old. The authors believe that the influence of oestrogen, especially E2, on the regulation of inflammatory response and immune cell function may be one of the protective factors [37]. Moreover, Liu *et al.* reported that among patients in their cohort aged ≤ 55 years, females had a much lower incidence of developing complications than male patients, especially in terms of lung injury (such as dyspnoea or ARDS). However, this difference in incidence between males and females vanished among the patients aged more than 55 years [37]. Again, the authors believe that the most likely explanation is that because the levels of oestrogen in females after menopause decrease, oestrogen no longer offers a beneficial effect as seen in females younger than 55 years old [37].

In the last 2 years, several papers have been published trying to offer an explanation as to why the outcome of human coronavirus infections is strongly sex-dependent [38–40]. Once more, oestrogens have been hypothesized as crucial in modulating viral infection and the progression of the disease via an action on immune/inflammatory responses and ACE-2 expression [35].

Although most of the immune regulatory genes are encoded by X chromosomes, resulting in a generally stronger immune response in women, this sex difference in inflammatory response is postulated to be largely driven by sex hormones [41]. Although oestrogen plays a complex role in modulating the immune system, generally in a dose-dependent manner, it is reported to have an anti-inflammatory effect at normal physiological levels in premenopausal women [42, 43]. Most cytokines, namely, IL-6, IL-8, and TNF- α , are inhibited by periovulatory dosages of oestrogen, while low levels of oestradiol (E2) can augment inflammatory mediators, which could explain the proinflammatory states that most postmenopausal women suffer from (e.g. atherosclerosis) [42].

As mentioned above, SARS-CoV-2 virions use ACE-2 as a host-cell receptor for viral uptake [44].

Entrance is facilitated by a host type 2 transmembrane serine protease, TMPRSS2, that is responsible for priming the viral S glycoprotein. Increased tissue (co-) expression of ACE-2 and TMPRSS2 at the virus entry sites may enhance infection, while downregulation may prevent SARS-CoV-2 binding to target cells [45]. Human ACE-2 is an essential part of the renin-angiotensin system and is encoded on the X chromosome [46]. Angiotensin-converting enzyme type 2 is widely distributed in tissues, including lung alveolar (type II) epithelial cells, the vascular endothelium, heart, kidney, and testis [47]. It has extensive vascular and organ-protective functions mediated via angiotensin (Ang 1–7), by the Ang II receptor type 2, and the Mas receptor (MasR) [48].

As mentioned earlier, expression of ACE-2 is downregulated by E2 [35, 49]. Oestradiol is also able to in-

hibit the production of the TMPRSS2 protein, which is necessary for trimming and activating the SARS-CoV-2 spike protein to bind ACE-2 [50] and to increase the expression of A disintegrin and metalloproteinase, mainly ADAM-17 [14], which is able to cleave the ACE-2 ectodomain with release of highly soluble circulating and SARS-CoV-2-neutralizing ACE-2 [51].

Additionally, oestrogen modulates the cytokine storm by suppressing IL-1 β and IL-6 production, and hence lowers the risk of acute lung inflammation in women [52]. Oestrogen might also play a major role in lowering the exhaustion of T cells caused by the cytokine storm [53].

Indirect evidence of the protective effect of oestrogen has been confirmed by Channappanavar *et al.* in a mouse model, demonstrating that female mice administered with oestrogen receptor antagonist have a higher mortality rate due to SARS-Cov2 when compared with control female mice, while this effect was not demonstrated in male mice. They also showed poor prognosis and extensive lung involvement with pro-inflammatory cytokines/chemokines in ovariectomized/gonadectomized female mice [54].

Oestrogens downregulate the AT1R signalling pathway and inhibit ACE activity [55, 56]. This classical ACE/AngII/AT1R regulatory axis counter-regulates (upregulates) the ACE-2/Ang 1–7/MasR axis, whereas the oestrogen levels are high [57]. 17 β -oestradiol also increases ACE-2 activity in the adipose tissue, kidneys, and myocardium [58, 59].

Summarizing, for most infectious diseases, women have been consistently observed to mount a stronger immune response when compared to men [60]. In general, the female immune system responds more efficiently to pathogens, producing higher amounts of interferons and antibodies; however, this protective effect, mediated primarily by oestrogen, is attenuated in postmenopausal women [42].

COVID-19 infection, menopause, and hormonal therapy

Immunosenescence contributes to a decreased capacity of the immune system to respond effectively to infections or vaccines in the elderly [61, 62], and it is characterized by the inability to mount effective (protective) humoral and cellular immune responses against a pathogen, as well as a systemic low-grade inflammatory state, which contributes to the dysregulation of several components of the innate and adaptive immune systems [63–66].

The aging process affects sexual dimorphism regarding immunocompetence and disease susceptibility [41, 67, 68]. Notably, however, immune-pathological effects may also decrease after menopause; for example, in severe forms of dengue and influenza [69, 70].

The menopause has a distinct impact on the female immune system [41]. Postmenopausal women exhibit a reduced number of total lymphocytes, mainly B and CD4+T lymphocytes [71].

Pronounced endocrine changes alter the expression of inflammatory mediators, thereby elevating plasma IL-1 β , IL-6, IL-10, and TNF- α with menopause [72–74]; however, these levels are reduced with the use of hormone therapy, especially oestrogen-containing types, to premenopausal levels [41].

Additionally, the activated oestrogen receptor, specifically oestrogen receptor- α , has been found to inhibit NF- κ B-mediated inflammation response and cytokine production via immune cells, lymphocytes, macrophages, and neutrophils [75]. The finding that Ang II activates the NF- κ B pathway to increase cytokine synthesis after SARS infection while oestrogen can shut down the NF- κ B pathway holds possible relevance for COVID-19 treatment strategies in female patients [23].

In the last 2 years the relationship between menopausal status and COVID-19 outcomes has become of interest. The first study comparing COVID-19 outcomes of premenopausal and postmenopausal women with men for hospitalized patients based on a well-conducted propensity score matching analysis (retrospectively) was published by Wang *et al.* [76]. In this study, the authors observed that men were significantly more likely to experience severe disease compared to premenopausal women; however, the mortality rates were not significantly different between the 2 groups. Additionally, the odds of experiencing severe disease and mortality were not significantly different between men and postmenopausal women. This data suggests that a menopausal status bias exists in patients with COVID-19 [76].

A similar result was also noted in an Italian study, in which the authors suggested that by acting on the immune system, oestrogens may reduce disease progression and favour virus clearance [38, 40, 77], making COVID-19 infection less lethal in women of reproductive age, whereas the opposite may occur in postmenopausal women due to decreased oestrogen levels [35].

Loss of ovarian function at menopause and the resulting change in the concentration of sex hormones may contribute to the increased risk of COVID-19 [75]. Given that oestrogen plays a crucial role in protecting female mice from SARS-CoV infection and that ovariectomy or oestrogen receptor blockage increases the susceptibility to infection and mortality [54], the results may be explained in part by the protective effect of oestrogen against COVID-19 in premenopausal women [76].

In addition to its immunomodulatory effects, oestrogen modulates the expression of Th1 and Th2 cytokines, deactivates excessive inflammatory processes, and restores homeostatic conditions, thus potentially inhibiting cytokine storm syndrome (a proposed prima-

ry reason for the morbidity and mortality in COVID-19) in women [78–80]. Additionally, *in vitro* data suggest that oestrogen might exert a direct antiviral effect on SARS-CoV-2 by downregulating the expression of ACE-2 mRNA in bronchial epithelial cells, which has been proven to be the major receptor responsible for mediating virus entry into cells [76, 81].

In support of the above, the data from SARS-CoV-2 indicate that the use of oestrogen therapy could be effective in the fight against COVID-19 [82], also emphasizing the necessity of further research in patients treated with these agents. Additionally, Chanana *et al.* suggested that the reversible effects of the hormones allow short-term hormone therapy treatment in COVID-19 patients, hence avoiding any long-term side effects [83].

Ding *et al.* suggested that menopause is an independent risk factor for female COVID-19 patients [84]. In their paper, the logistic regression analyses showed that the levels of E2 and anti-Müllerian hormone in the non-severe group were higher than those in the severe group, potentially playing vital roles in the progression of COVID-19. Additionally, E2 levels were negatively correlated with IL-2R, IL-6, IL-8, and TNF- α in the luteal phase ($p = 0.033$, $p = 0.048$, $p = 0.054$, and $p = 0.023$) and C3 in the follicular phase ($p = 0.030$), and E2 is attributed to its regulation of cytokines related to immunity and inflammation [84]. The data indicated that non-menopausal females presented milder disease severity and better outcomes than age-matched males, whereas the differences disappeared between menopausal women and age-matched men, indicating that female hormones of premenopausal females may provide protection [84].

Seeland *et al.* in their study focused on the incidence and outcome of COVID-19 infections by considering an age- and sex-disaggregated data analysis [48]. The authors identified a sex-specific distribution of COVID-19 incidence rates, with the highest frequencies being among premenopausal women in the 20–55-year age range. They also found a higher fatality rate of men compared to age-matched women, beginning at 50 years of age, and that E2 hormone use reduced fatality rates for women in this 50+ age range [48].

The data in the Seeland *et al.* study indicate that pre-menopausal women are disproportionately more infected with coronavirus than men in the same age range, but they do not become as seriously ill, as shown by lower fatality rates [48].

Interestingly, among post-menopausal women, Seeland *et al.* observed a significant difference in the rates of death between women with regular E2 use (user group) and those without E2 sex hormone intake (non-user group). This important finding – that the fatality risk for women > 50 years receiving E2 therapy (user group) is reduced by more than 50% (OR 0.33, 95% CI:

0.18–0.62 and hazard ratio 0.29, 95% CI: 0.11–0.76) compared to the non-users group – was described for the first time [48]. Hence, the authors concluded that the chief finding of their study is the strong positive effect of regular E2 hormone therapy on the survival rates of post-menopausal women.

Moreover, based on the main finding of their study, Seeland *et al.* believe there are no concerns regarding continuation of the use of sex hormones that contain E2 prior to SARS-CoV-2 infection [48]. Even though the data indicate that the risk of infection is higher in pre-menopausal women with higher endogenous E2 levels, compared to either men of the same age strata or to post-menopausal women, it should be noted that the clinical course of COVID-19 disease, and the ultimate mortality rate, is lower in women with higher E2 levels [48]. Additionally, higher survival probabilities are particularly evident in post-menopausal women who are infected with SARS-CoV-2 and who regularly use exogenous E2 (e.g. for postmenopausal complaints) [48].

Also, data from the Youn *et al.* study support the hypothesis that oestrogen may be used to alleviate viral infection and cytokine storm-induced endothelial dysfunction, resulting in therapeutic effects to attenuate disease progression, severity, and mortality [85]. They demonstrated that oestrogen-mediated attenuation of NADPH oxidases NOX2 activation, reactive oxygen species production, and monocyte chemoattractant protein-1 MCP-1 upregulation in response to S protein/IL-6 exposure of endothelial cells underlie protection against COVID-19 in females. Hence, these data indicate that oestrogen administration can be used as a robust treatment option for COVID-19 to effectively reduce disease severity and improve survival [85]; however, the pro-coagulant effect cannot be ignored [86].

As mentioned earlier, E2 has receptors on all innate and adaptive immune cells and is a key player in the immune response, which includes both pro-inflammatory and anti-inflammatory functions [87]. Oestradiol is a modulator of the renin-angiotensin-aldosterone system, a major force in the instigation of the inflammatory response and in the resolution of inflammation [88]. Oestradiol plays a major role in regulating lipid mediators and peptides involved in the processes needed for an optimal immune response, improving the likelihood of a successful outcome in the fight against an infectious agent such as SARS CoV-2 [89, 90].

Additionally, in the letter to the Editor of Clinical Infectious Diseases regarding a paper published by Ding *et al.* [84], Gersh *et al.* [90] advocate the use of physiologically dosed human-identical transdermal E2 as a hormone replacement, combined with human-identical cyclic progesterone, in recently menopausal women without contraindications in connection of E2 levels and menopausal status with outcomes from infections with SARS-CoV-2 in women [91].

Their recommendations are based on a significant body of preclinical and clinical data [92], confirming that the findings of a distinctly protective effect of E2 in women with functioning ovaries in the study by Ding *et al.* is in complete alignment with the position of Gersh *et al.* and with scientific reports [91].

Conclusions

The COVID-19 pandemic has highlighted the serious negative effects arising from the state of E2 deficiency. Therefore, the use of hormone replacement therapy has gained further support because the damaging effect of a decline in ovarian function affects many biological systems. Accordingly, the signs and symptoms of menopause include central nervous system-related disorders; metabolic, weight, cardiovascular and musculoskeletal changes; urogenital and skin atrophy; and sexual dysfunction [93], and recently with the COVID-19 pandemic, oestrogen's vital role within the immune system became quite clear. Therefore, in view of the above, it should be emphasized again that appropriate post-menopausal women should be considered for hormone replacement therapy.

However, additional clinical investigations regarding hormone replacement therapy are urgently needed to further verify the protective and therapeutic effects of E2 on menopausal women with COVID-19 [84].

Disclosure

The authors report no conflict of interest.

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