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Estrogen shields women from COVID-19 complications by reducing ER stress

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A B S T R A C T

Estrogen hormone acts as a potential key player in providing immunity against certain viral infection. It is found to be associated in providing immunity against acute lungs inflammation and influenza virus by modulating cytokines storm and mediating adaptive immune alterations respectively. Women are less affected by SARS-CoV-2 infection because of the possible influence of estrogen hormone as compared to men. We hypothesized that SARS-CoV-2 causes stress in endoplasmic reticulum (ER) which in turn aggravates the infection, estrogen hormone might play key role in decreasing ER stress by activating estrogen mediated signaling pathways, results in unfolded protein response (UPR). Estrogen governs degradation of phosphotidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol triphosphate (IP_3) with the help of phospholipase C. IP_3 start in-fluxing Ca^{+2} ions that helps in UPR activation. To support our hypothesis, we analyzed the data of 162,392 COVID-19 patients to determine the relation of this disease with gender. We observed that 26% of women and 74% of men were affected by SARS-CoV-2. It indicated that women are less affected because of the possible influence of estrogen hormone in women.

Introduction

Crown shaped virus has caused deadly destruction in the beginning of 2020. Despite living in the most advanced era of science and technology, nations are struggling to cope with losses which COVID-19 has caused. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the family of coronaviridae, a single stranded RNA virus. Human corona virus (CoV) belongs to second genera of coronaviridae family while first and third genera constitute pigs and bats coronavirus respectively [1]. The outer membrane of CoV is composed of three surface protein. These are S glycoprotein spikes on their envelope which form peplomer on its membrane and gave it appearance of crown. S spike protein allows the viral entry into host cell, while other two proteins are M (membrane protein) and E (small hydrophobic protein) [2]. Before COVID-19 outbreak, it was considered that CoV causes infection via animal to human transmission but deaths at alarming rate by COVID-19 assured its contagious nature and human to human transmission. Carriers of SARS-CoV-2 are real source of COVID-19 outbreak [3].

Coronavirus causes infection by inserting its single stranded RNA genome in host cell and starts its replication [4]. Translation of its genome produces structural and nonstructural proteins and their subsequent foldings occur in host endoplasmic reticulum (ER) [5]. CoV produces ER stress in three ways by; forming double membrane vesical (DMV), glycosylating viral proteins and depleting ER membrane lipids [6]. Replication and translation of CoV result in replication-translation complex (RTC) formation [7,8]. After protein modification in ER, these newly synthesized proteins induce modification in intracellular membrane of ER and form DMV, which halts protein modification function of ER [9]. ER becomes overstressed by frequent viral protein foldings and DMV formation. CoV also overloads ER by glycosylating its structural proteins. CoV spike is composed of S glycoprotein, which exists in trimeric form and is required for viral entry into the host cell [10–12]. Glycosylation of S protein occurs in ER with the help of chaperon "calnexin". Calnexin is involved in folding and maturation of S glycoprotein [13]. Replication of CoV requires large number of proteins, which burden ER. Budding of CoV after multiple replications leads to its release from host cell endoplasmic reticulum–golgi intermediate compartment (ERGIC), which is in continuation with ER [14]. Budding results in depletion of ER membrane lipids, affects ER integrity thus increases ER stress [6].

Estrogen hormone lowers the burden of ER stress by activating unfolded protein response (UPR) [15]. Activation of UPR by estrogen hormone involves multiple signaling pathways. Upon ER stress, estrogen hormone binds to its receptor ERα present in cytoplasm. Estrogen binding causes rapid activation of phospholipase C enzyme that cleaves its substrate PIP_2 (phosphotidylinositol 4,5-bisphosphate) into DAG (diacylglycerol) and IP_3 (inositol triphosphate). Newly synthesized IP_3 binds to its receptor present on ER membrane and starts influx of calcium ions in ER. Ca^{+2} ions activate UPR by modulating its three

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sensors; protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) [16]. Estrogen regulates the expression of X-box binding protein 1 (XBP-1) and 78 kDa glucose-regulated protein (GRP78) [17]. Both genes are involved in UPR activation. *XBP1* regulates the function of other genes i.e., *DNA1, EDEM and PDI*, which are involved in releasing ER stress by activating UPR transducers [18,19]. *XBP-1* performs its function with the help of *SRC-1* (steroid receptor co-activator 1). *SRC-1* itself is regulated by estrogen hormone via *EGFR* (epidermal growth factor receptor) and *TGFα* [20]. GRP78 is known as master regulator of ER stress. It also activates UPR sensors by starting cascade of signaling pathways and results in reducing the ER stress [21]. The current study aimed at the investigation of a possible link that how estrogen protects women from the COVID-19 complications.

Hypothesis

We hypothesized that estrogen might have potential role in decreasing ER stress caused by infection of SARS-CoV-2, consequently women are less affected by SARS-CoV-2 as compared to men. Recently, Vaninov (2020) reported that COVID-19 infection is characterized by cytokine storm which results in adaptive immune response failure after 7–10 days of viral infection onset [22]. Estrogen modulates cytokines storm by suppressing IL-1β and IL-6 production, thus lowers the risk of acute lungs inflammation in women [23]. Estrogen might also play a major role in lowering the exhaustion of T cells caused by cytokines storm [24]. Protective effect of estrogen by reducing ER stress is not reported yet. For this purpose, we proposed a model how estrogen plays an important role in reducing the ER stress, caused by SARS-CoV-2 infection (Fig. 1). Translation of CoV genome starts right after its infection to host cell. Translation results in production of structural and nonstructural proteins of CoV. Both types of proteins cause stress in ER by forming DMV, glycosylation and depleting ER lipids. ER stress leads to activation of estrogen mediated signaling pathways which activates UPR. Estrogen governs degradation of PIP_2 into DAG and IP_3 with the help of phospholipase C. IP_3 starts in-fluxing Ca^{+2} ions that helps in UPR activation. To support our hypothesis, we examined the incidence of COVID-19 in Pakistani population on both genders to determine if women are less affected by SARS-CoV-2 infection.

Evaluation of hypothesis

We used COVID-19 data from official sources of government of Pakistan to update and curate our data set. Government sources include Health Services Academy, Ministry of National Health Services, Regulations and Coordination, Pakistan. We analyzed the data of 162,392 confirmed COVID-19 Pakistani patients collected by June 19, 2020. Patients belonged to both urban and rural areas of Pakistan, we collected data on following basis: (a) Key dates; includes the date of onset of disease, date of admission to hospital, date of confirmation of infection and date of travel. (b) Demographic information about age and gender. (c) Geographic information (Punjab, Sindh, Balochistan, Gilgit Baltistan, Khyber Pakhtunkhwa, Azad Jammu Kashmir and Islamabad). We performed descriptive analysis and reported the results as frequencies. This study was part of the public data collected by government of Pakistan officials and was not considered research to be approved by institutional review board. No written informed consent by participants was required.

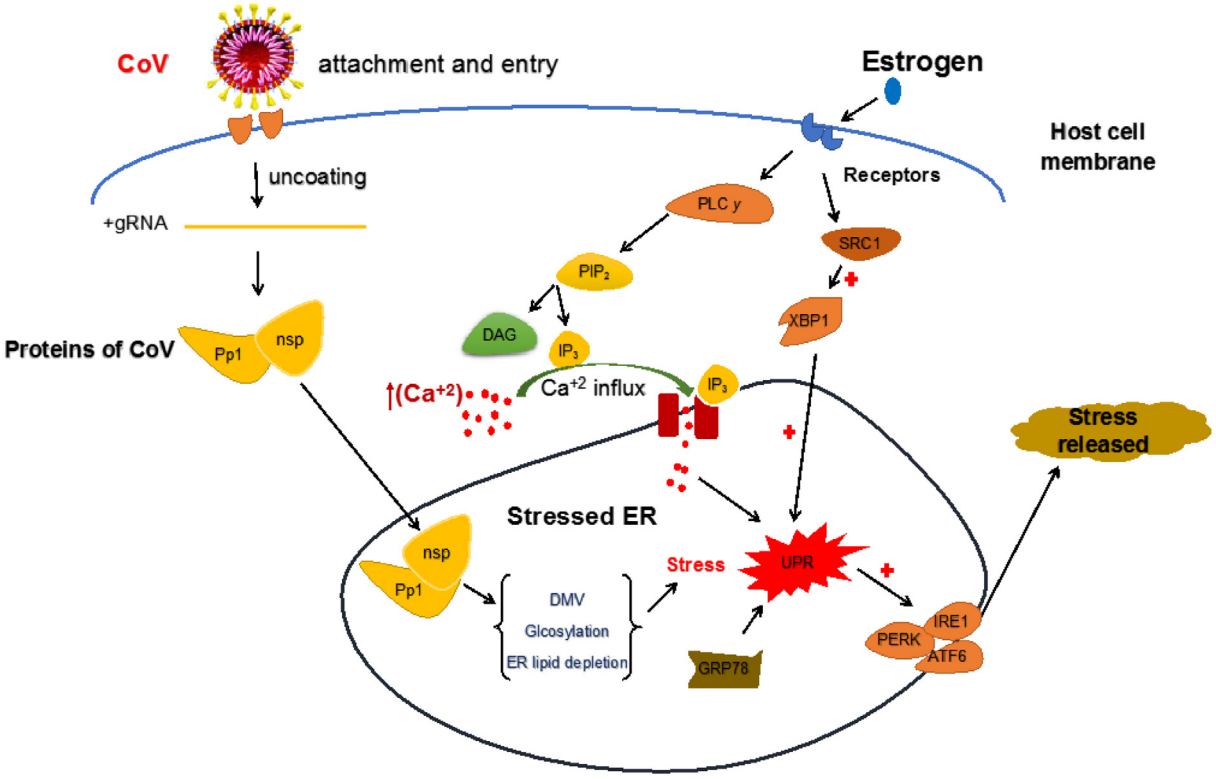

Fig. 1. Proposed mechanism for estrogen-mediated pathway for release of ER stress caused by SARS-CoV-2 infection. Translation of CoV genome starts right after its infection to host cell. Translation results in production of structural and nonstructural proteins of CoV. Both types of proteins cause stress in ER by forming double membrane vesical, glycosylation and depleting ER lipids. ER stress leads to activation of estrogen mediated signaling pathways, which activate UPR. Estrogen governs degradation of PIP_2 into DAG and IP_3 with the help of phospholipase C. IP_3 starts in-fluxing Ca^{+2} ions that helps in UPR activation. *GRP78* and *XBP1* also help in activation of UPR under the influence of estrogen. DAG = diacylglycerol, $PIP_{2=}$ phosphotidylinositol 4,5-bisphosphate, IP3 = inositol triphosphate, XBP1 = X-box binding protein, GRP78 = 78 kDa glucose-regulated protein genes, Pp1 = structural ptotein, nsp = nonstructural proteins of CoV, ATF6 = activating transcription factor 6, PERK = PKR like endoplasmic reticulum kinase and IRE1 = inositol requiring enzyme 1.

Table 1
Clinical Status of COVID-19 cases in Pakistan till June 19, 2020.

Province/ Territory	Lab Status		Hospitalized		Recovered	Deaths
	Tested	Positive	Admitted	Critical		
Azad Jammu Kashmir	73,275	803	469	05	317	17
Balochistan	98,193	9162	5773	07	3289	100
Gilgit Baltistan	15,657	1253	416	10	816	21
Islamabad	18,976	9279	7414	19	2770	95
Khyber Pakhtunkhwa	15,585	18,790	14,378	14	5623	789
Punjab	603,084	62,216	44,905	24	17,964	1347
Sindh	301,458	62,163	31,425	32	32,725	1013
Total	**1,042,787**	**162,392**	**104,780**	**111**	**63,504**	**3382**

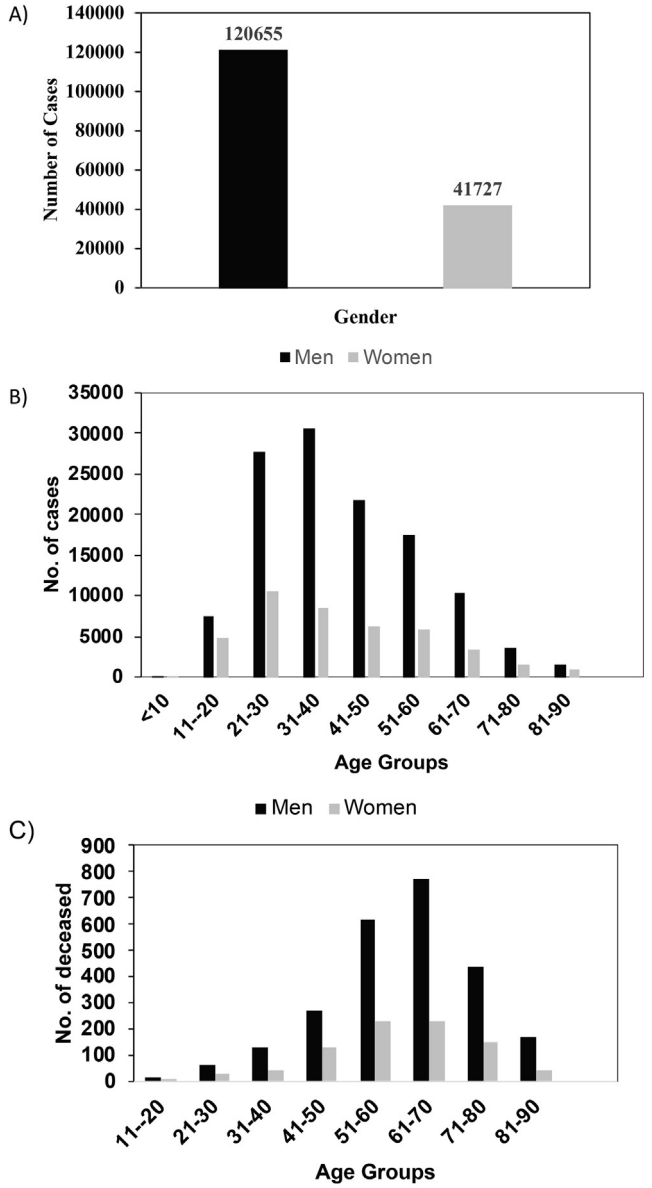

Fig. 2. A) Total number of COVID-19 cases by gender B) Total number of COVID-19 cases in each age group and gender C) Total number of deceased by COVID-19 in each age group and gender.

Results

Our study was based on analysis of 162,392 COVID-19 patients (Table 1). Out of which 120,665 were men, 41,727 were women (Fig. 2A). A total 74% of affected population comprised of men and 26% of women were affected by SARS-CoV-2 infection. Highest cases were reported in age group 31–40 followed by 21–30 (Fig. 2B). Death rate was also high in men as compared to women (Fig. 2C). Death rate was highest in men of age group 60 and above.

Discussion

Coronavirus causes ER stress by forming DMV, glycosylating S protein and depleting ER membrane lipid [25,26]. Studies have reported that estrogen plays important role in reducing ER stress by activating UPR. UPR activation involves PIP_2, PERK and XBP-1/GRP78 pathways [27]. Estrogen has been reported to play important role in suppressing the infection caused by many viruses. Estrogen suppresses hepatitis B virus (HBV) infection by suppressing viral replication [28]. A study conducted on mice showed that estrogen provide immunity against influenza virus by mediating adaptive immune alteration against viral infection [29]. Estrogen also has cardioprotective effect; estrogen-mediated cardioprotective role and ER stress release mechanism share a common signaling pathway as proposed in Fig. 1. GRP30 activation by estrogen lowers the extent of ischemia/reperfusion injury [30]. It also provides cardio-protective effect by reducing LDL oxidation and oxidative stress [31]. Hence, estrogen is the potential key player in shielding women from COVID-19 by reducing ER stress. To support our hypothesis, we conducted this study if Pakistani women are less affected by COVID-19. Nationwide study of hospitalized COVID-19 infected Pakistani cases showed that 26% women were affected as compared to 74% of effected men. Men to women case fatality rate (CFR) ratio was 2.73% vs 1.1% respectively. As mentioned above, estrogen plays important role in releasing ER stress thus provides protective effect, so women are less prone to SARS-CoV-2 infection. Across the globe, SARS-CoV-2 has infected more men as compared to women. In Italy, 80% of their SARS-CoV-2 infected population comprised of men with only 20% of women [32]. China the very first hub of this outbreak shows a similar trend, 60% of infected population were men [33]. This trend was associated with high rate of smoking in men than women, but smoking cannot form the only reason because 1.4% of infected men were smoker in Chines population [34]. According to World Health Organization (WHO), in China men to women CFR ratio was 4.7% vs 2.8% respectively. According to UN women reports, Denmark, Germany, Spain and Republic of Ireland shares the same ratio of deaths. Men to women death ratio in these countries are 64% vs 36% ± 0.5% respectively. In Greece 72% of deceased are men with only 28% women died due to complications of SARS-CoV-2 infection.

Our findings are consistent with our hypothesis in all age groups. With increasing cases reported from different ethnic and genetic

backgrounds worldwide, our hypothesis can be better examined to establish how it contributes to incidence of COVID-19 on the basis of gender.

The comprehensive study of signaling pathways responsible for SARS-CoV-2 infection and its relationship with the estrogen will further support our hypothesis.

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Conflict of interest statement

All authors disclose that there is no conflict of interest.

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