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IMPACT OF 17β-estradiol (E2), IN INCREASING IMMUNITY AGAINST COVID-19 AND MITIGATING SEVERE COVID-19 OUTCOMES

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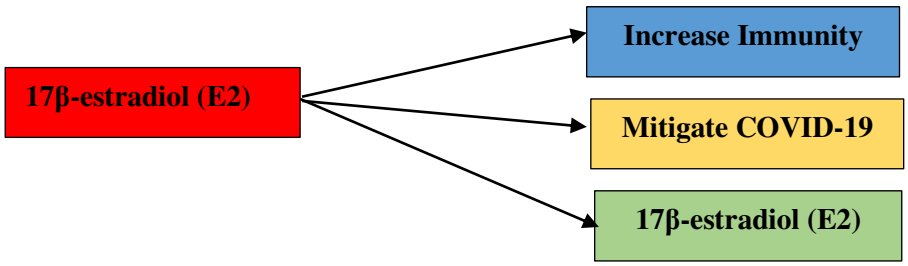
Graphical Abstract.



Highlights.

- COVID-19 attacks more males as compared to females.
- The concentration of 17β-estradiol (E2) is high in women than men.
- Estrogen increased the immune system.
- 17β-estradiol (E2) helps in increasing immunity against COVID-19 and mitigating severe COVID-19 outcomes.

Abstract. COVID-19 attacks more males as compared to females. There is a need to search out an element that gives immunity to women. Substantial amounts of estrogens (17B-ESTRADIOL) are found in men and women. To see the current situation of the COVID-19 pandemic the study aims to find the impact of 17β-estradiol (E2), in increasing immunity against COVID-19 and mitigating severe COVID-19 outcomes. A total of 113 pharmacists and doctors from different hospitals, in different provinces of Pakistan, respond to questionnaires. 73.5% of doctors and pharmacist's response positively to using 17β-estradiol (E2) as an element of increasing immunity against COVID-19 while 8.8 percent disagreed on that point and 17.7% of those answered go with the possibility. We got a significant result from our study. After the analysis of the whole research, we can conclude that Yes! 17β-estradiol (E2) aids in raising tolerance against the COVID-19 attack and minimizing extreme COVID-19 outcomes since estrogen works directly on the respiratory immune system through increasing phagocyte production. These cells will kill the COVID-19 if it is attacked, stopping it from spreading into the lower respiratory region.

Keywords. Estrogen, COVID-19, Vaccine, Coronavirus, Progesterone, Immune Function.

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1. Introduction

The COVID-19 pandemic in the city of Wuhan, China, was confirmed to be a case of unexplained pneumonia, measured at global elevated levels at the end of December 2019 [1]. Coronavirus is named due to its pneumonic symptoms which are analyzed by the Center for Disease Control (CDC). Hoehl, 2020 [2] stated that one of the most common viruses nowadays in human respiratory systems is coronavirus. Zhu, 2019 [3] says that Chinese researchers called the virus 2019-COVID. The new coronavirus has been subsequently called the Special Committee for Virus Taxonomy (SARS-CoV-2) as Serious Acute Respiratory Syndrome Coronavirus-2. [4]

On 30 January 2020, COVID 19 was declared as the 6th (WHO disaster services public safety outbreak. [6][5] COVID-19 is not the first case of the corona-virus but Past infections also had an epidemic of SARS-COV and the Middle East Respiratory Syndrome Coronavirus (MERS-COV) outbreak, respectively. [7] Overall, 15,012,731 COVID-19 verified cases, from which 619,150 deaths, have been registered by WHO on 23 July 2020. COVID-19 is the spread of a total of 213 nations. The maximum confirmed cases for Pakistan are 260906. [8] The first COVID-19 epidemic impacted border countries, like China to Pakistan. With COVID-19 in the North, Italy is the worst in the West, while Iran is the lowest after Italy. [9]

Estrogens are essential hormones, especially in women, for sexual and reproductive growth. These are also classified as sex hormones for women. Estrogens are primarily developed in the ovaries in women. Ovaries are uterine grapes that are components of the endocrine system. The hormone estrogens activate the reaction of the nasal mucosa by evaluating hypertrophy and decreases the nasal mucosal content of mucins. These substances have anti-viral and antibacterial actions which are fundamental in contrasting with upper airway infections [10]

In the prevention or diagnosis of COVID-19 WHO may not allow self-medication, including antibiotics. However, numerous research trials of both conventional and Western therapies are also in development. When the study findings fall into the country, WHO must direct initiatives to produce vaccinations and medications for the prevention and care of COVID-19. [11] Men are likely to die from COVID-19 more often than women, so researchers thinking to treat them with female hormones. Substantial amounts of estrogens are found in men and women but, these exist in considerably higher proportions in women after sexual maturity (starting from childhood cycles) until menopause (stop menstrual phases at a reproductive age). [12]

From the start of the 21st century, there is an attack of 2 deadly coronaviruses the SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV). The attack of these viruses was more in men as compared to women. As per the researcher [13] due to SARS-CoV the cases of the death rate of women were 13% and men were 22% in Hong Kong. Also, as per the researcher [14], 38% of women cases are reported in Saudi Arabia as compared to 52% of men in MERS-CoV outreach. As per a new study [15] COVID-19 attack on China, Europe, and the United States, explore more cases of the man attacked by COVID-19 in comparison to women. 42% of women cases are reported in China as compared to 58% of men cases. As per [16] researcher in the Italy area of Lombardy presents only 18% of women cases. In New York as per researcher [17], 39% of death of women is recorded.

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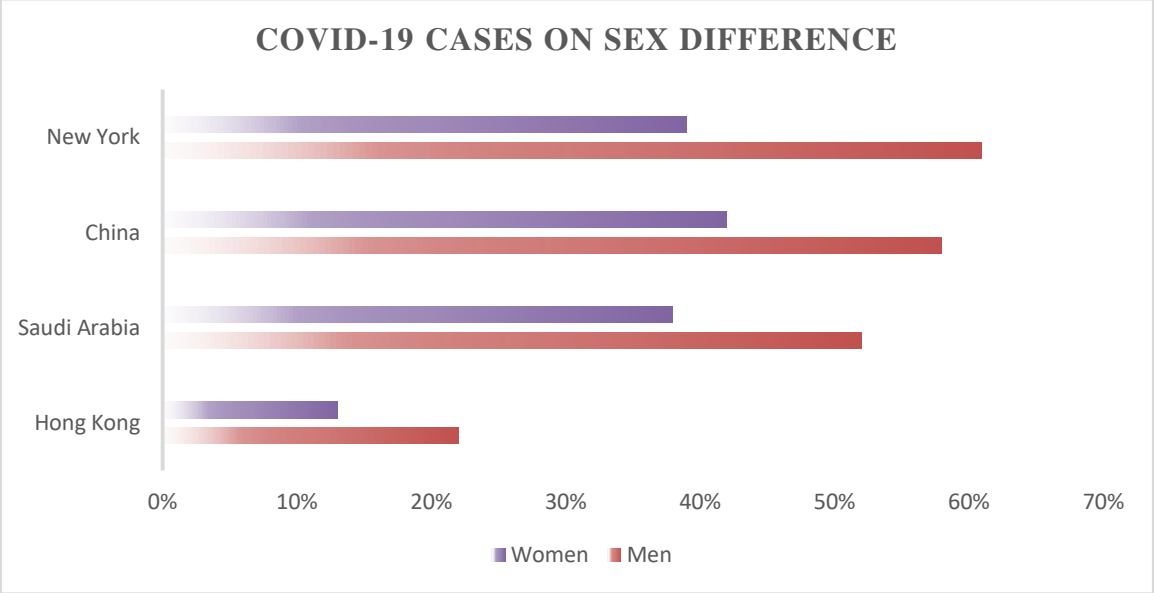

Figure 1: COVID-19 cases on Sex Difference

We interpret these data present in figure 1 that COVID-19 has attacked men more than women. Here from the interpretation, a question is arising states that what are the factors that act as protective measures to mitigate the effect of COVID-19. In comparison to men, women's bodies elevated more immune responses. Researchers have already provided some factors that cause more immunity in women than a man like a researcher [18] explain that IgG and IgM concentration is more in women than man, as per researcher [19], The response of protective antibody in women is twice as strong as in men. Women are even more common in CD4 + T than men [20].

An additional explanation as mentioned by the researcher [21] is XX chromosome in women. Sexual steroid hormones such as estrogen levels, P4, and androgens between women and men likely influence the immune response and inflammatory effects of COVID-19. There is clear evidence from previous studies that estrogen induces immune functions. As per [22] study, it was stated that estrogen impedes the pathogenesis of disease by blocking the development of IL-6. In a study of the researcher [23], the male rat is induced with estrogen which developed more IL-6 from liver Kupffer cells which in return provides more resistance to HCC than women. In this study the estrogen act as a shield in protecting male rat.

High levels of E2 may increase the synthesis of proinflammatory cytokines in innate immune cells and promote anti-inflammatory responses. Acute E2 therapy can blunt endogenous infections and facilitating B-cell responses and immune complexes without any major side effects [24, 25]. Below figure 2 shows how 17β-estradiol mitigates COVID-19 impact.

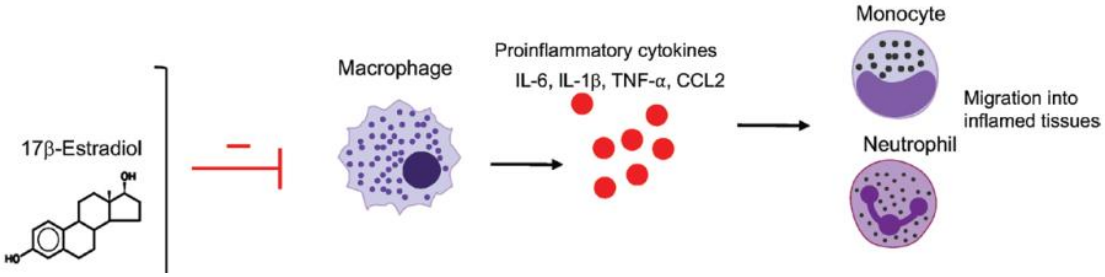

Figure 2: 17β-estradiol impact on mitigating COVID-19

The depth of awareness about their therapeutic effectiveness and potency that has accrued from decades of therapeutic and fundamental trials is a vital benefit of estrogen. Millions of women use estrogen therapy for fertility and the treatment of menopausal symptoms. It is easily accessible, affordable, manufactured to size, and can be instantly used in hospitals. 2 clinical trials are independently testing E2 (ClinicalTrials.gov code NCT04359329) in COVID-19 patients as this analysis is being written. Figure 3 shows that how 17β-estradiol helps in the production of antibodies.

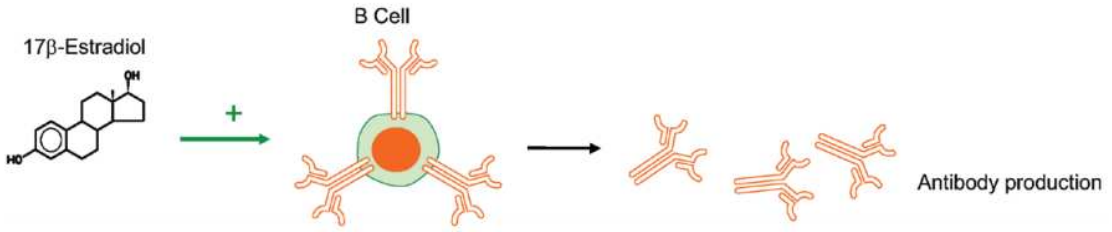

Figure 3: 17β-estradiol helps in the production of antibodies

The researchers are trying to mitigate the impact of COVID-19 in different ways. We also suggest an element that can be used to mitigate the impact of COVID-19. So, our study aims to focus on the 17β-estradiol (E2), which is a steroid hormone and has a high power of providing immunity. This paper discusses the E2 as a reliable therapeutic option to fight against COVID-19. We study the impact of 17β-estradiol (E2), in increasing immunity against COVID-19 and mitigating severe COVID-19 outcomes.

2. METHODS AND MATERIALS

2.1. Study population:

To test the immunity raising agent 17β-estradiol (E2), against COVID-19 an exploratory analysis using a cross-sectional online survey was performed. The research period was between 20 April and 24 May 2020. The samples from both doctors (n=53) and pharmacists (n=60) of every country (Baluchistan, Khyber Pakhtunkhwa, Punjab, and Sindh) in Pakistan were conducted to ensure a well-spread pool of interviewees. Students got daily reminders of a survey's invitation via Google Forms via WhatsApp messages. Confidentiality of their replies was guaranteed to the participants. This research included specific demographics: gender, age, designation, a form of work, and ethnicity.

2.2. Study Instrument:

As there was a lockdown so we selected the interview method for our study. WhatsApp was the research tool that is used in this study. We have used this analysis tool to study, as our research is online survey research, it was simply because of its all-around access.

2.3. Methodology:

We firstly made a questionary with all relevant questions regarding the evidence related to 17β-estradiol (E2), which is a powerful agent in immunity against COVID-19. We discuss this question with the respondent that E2 acts as a therapeutic agent and can be a viable option to mitigate severe COVID-19 outcomes. The questionary was divided into three sections, in the first section, we provide an overview of the case (COVID-19 penetration, Estrogen role (17β-estradiol (E2), Impact of sex on lungs, Gender variation) so that all the doctors and pharmacists will know what are they answering and what are the study all about.

In the second section, we have a demographic study section, from where we selected the doctor's data and pharmacists and excluded the response from nurses, the common public, and other health workers. In the third section, questions are asked regarding the study. Interview sections were conducted from December 2019 to February 2020). Each participant was interviewed for at least 20 minutes. A hard copy of their responses is sent to them to check the reliability of the responses. Their response or outcomes are automatically recorded on the internet. The summary of the methodology is mentioned below (Figure 4):

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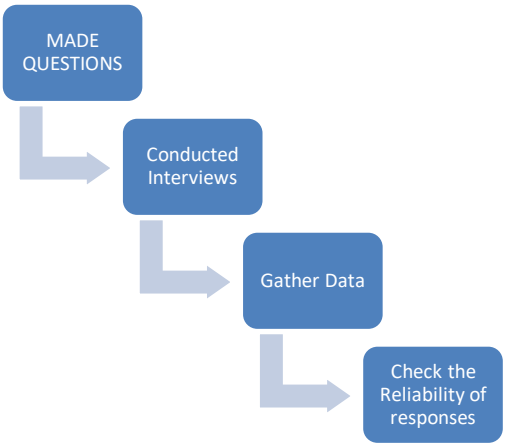

Figure 4: Summary of methodology

2.4. **Statistical Analysis:**

We apply descriptive statistics, chi-square test, Pi Square test, frequency distribution, and
In this data set firstly, we apply descriptive statistics and then we apply the test of association or chi-square test to evaluate our results.

3. Results:

3.1. Demographic Analysis

In this study, a total of 113 responses were received. The demographic characteristics of the respondents are shown in Table 1. Among the 113 respondents, 18.5% were females, the majority (81.5%) were male. The majority of the respondents are from above 25 years age (44.2%), 37.2% in the age group of 19–25 years, and less were in 17-18 years (18.6%). The proportion of respondents from Punjab is greater than others (53.0%) in Punjab as compared to Sindh (35.5%), Baluchistan (9%), and Khyber-Pakhtunkhwa (2.63%). In terms of the field of Designation, about 47% were Doctors while 60% are a pharmacist. 64% of the respondents are from Government jobs while 36% respondents from private jobs.

Table 1: Demographic analysis of responses

Variable	Frequency	Percentage
Gender		
Female	21	18.5
Male	92	81.5
Age		
17–18 years	21	18.6
19–25 years	42	37.2
Above 25 years	50	44.2
Ethnicity		
Punjab	60	53.0
Sindh	40	35.5
Baluchistan	10	9
Khyber-Pakhtunkhwa	3	2.63
Designation		
Doctors	53	47
Pharmacist	60	53.0
Job Type		
Government	73	64
Private	43	36

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3.2. Responses on 17β-estradiol (E2) as a powerful agent in immunity against COVID-19

The frequency distribution of responses was evaluated to analyze 17β-estradiol (E2), as a powerful agent in immunity against COVID-19. 73.5 percent of respondents agreed with the argument and 8.8 percent of respondents disagreed with the argument while 17.7% of those answered go with the possibility. The mean and standard deviation of 1.4425 and 0.77848.

Table 2: Descriptive measures on responses

Responses	Frequency	Percent	Mean	Standard deviation
Yes	83	73.5	1.4425	.77848
No	10	8.8		
Maybe	20	17.7		
Total	**113**	**100.0**		

3.3. Pi Square test on Responses:

Figure 5 shows the response of doctors and pharmacists on steroid hormones 17β-estradiol (E2), which can be a reliable and safe agent in fighting against the COVID-19.

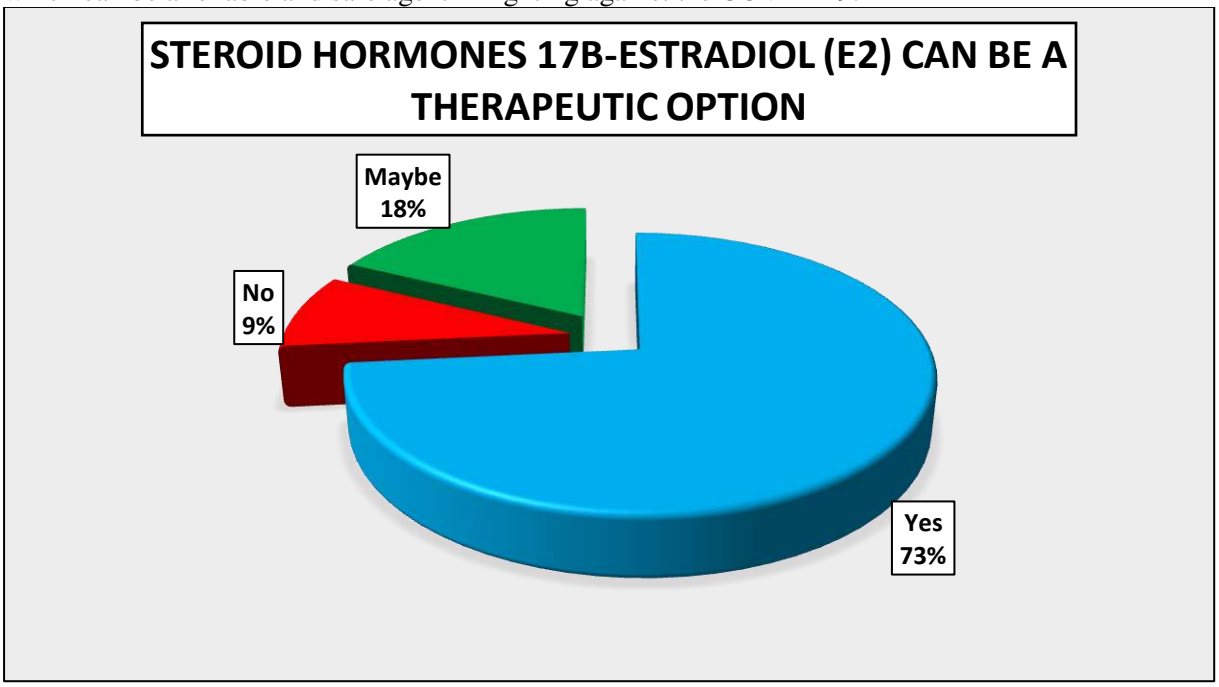

Figure 5: Pi Square test on Responses

3.4. Chi-square test on Responses

In table 2, the frequency distribution of the estrogen can be an element of the vaccine for COVID-19 is checked. Now We compare our result with the p-value and apply the chi-square test to our data. If the p-value is less than 0.05 then our result is significant otherwise non-significant. from the below table, we can see that there is a significant result because the p-value is less than 0.05.

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Table 3: Chi-square on data of 17β-estradiol (E2) as a powerful agent in immunity against COVID-19

Gender	Steroid hormones 17β-estradiol (E2) can be a therapeutic option			Total
	Yes	No	Maybe	
Male	66	8	18	92
Female	17	2	2	21
Total	83	10	20	113

Chi-square value = 10. 3 d.f = 4 P-value = 0.034

3.5. Frequency Distribution test on responses:

Figure 6. demonstrate frequency on data of 17β-estradiol (E2) as a powerful agent in immunity against COVID-19.

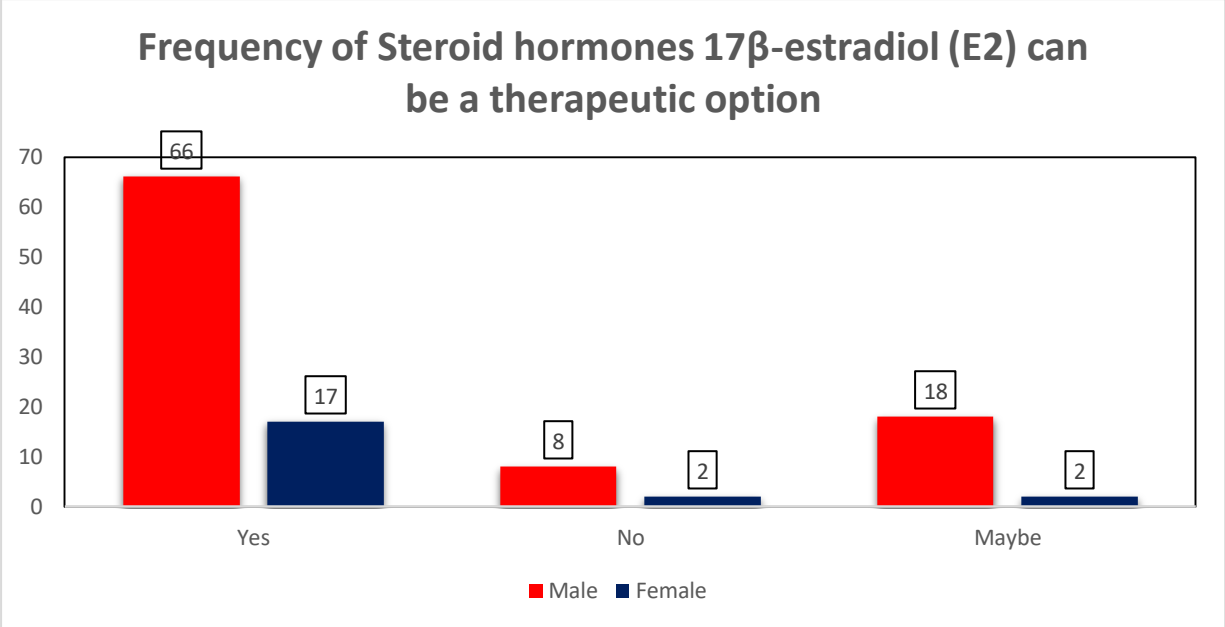

Figure 6: Frequency on data of 17β-estradiol (E2) as a powerful agent in immunity against COVID-19

4. Discussion:

Studies show that the extent of infection with coronavirus could be slightly more severe in men than in women. [27] Studies have shown that estrogen inhibits both the replication of the influenza virus in human female nasal epithelial cells and enhances the cytokine storm in murine models of this infection, a life-saving effect removed by oophorectomy and restored when estrogen is given to female gonadectomy mice. [27] Estrogen receptor signal controls immune response to respiratory viral infection in both male and female respiratory viral infection respondents Ligand enabled ERs are hubs of the mammalian genomics network that control both somatic and reproductive cellular functions. [28] Changes in ER activation or ER-regulated transcription processes may cause powerful compensatory acts, whereas uncompensated deregulation of ER signaling leads to severe chronic diseases like cancer. [29]

As transcription factors, hormone-activated hormone receptors (ERs) control the development of immune cells and the pathways of the innate and adaptive immune system. [30] In contrast, changes in androgen receptor (AR) signaling do not have significant influences on inflammatory and repairing immune reactions [31]. Consequently, the greater ER signaling

upregulation in both male and female patients, the more successful the response to respiratory viruses is. Greater immune responses in women are due to their greater oestrogen-ER-aromatase-estrogen regulatory chain, which reflects their tremendous reproductive functions. [32] During women's reproductive life, estrogen rates are higher, and ER signals are far greater than men matched during the age, thus immune response against respiratory viruses reveals noticeable gender differences. [33] Estrogen levels and ER signals are also crucial in adult men for the maintenance of reproductive capacity and somatic health, whereas estrogen deficiency is severe. [33]

ERs play a key role in preserving the homeostasis of glucose; however, there are few known human cases of highly deficient male ER signals. [34] The case of a male patient with genetic mutations in both ERα alleles was documented. This patient displayed ER resistance to glucose intolerance causing estrogen, hyperinsulinemia, extreme osteoporosis, and premature serious cardiovascular disease providing proof of the broad role of ER signaling in male physiology for the genome. Both male and female ERα knockout mice exhibit insulin resistance, compromised glucose tolerance, and obesity suggesting the role of ER signaling in both sexes in sustaining glucose homeostasis. [35]

Autoimmune nephritis, myeloid leukemia, and Sjögren syndrome have been identified in ERα knockout mice showing that ERs play key functions in immune system regulation. [36] These findings support that the progression of autoimmune disorders can correlate with an ER signaling deficiency, rather than with its extreme upregulation. The androgen excess and the predominant androgen receptor signal mistakenly suggest that, instead of estrogens, androgens can play a crucial role in both innate and adaptive immune responses in men. Nevertheless, gonadectomy or anti-androgen treatment could not affect either morbidity or mortality in male mice infected with a lethal dose of SARS-CoV, indicating that a lack of androgens did not affect immune responses. At the same time, infection with sublethal SARS-CoV dramatically decreased serum testosterone levels in male mice. [37] Rapid conversion of androgens to estrogens through increased expression of aromatase can be an effort to silence the cytokine storm of virus-infected lungs in male mice which leads to depletion of testosterone.

Treatment with estrogen prevents osteoporosis by inhibiting the cytokine pathway needed to differentiate and activate the osteoclasts. Estrogens modulate the receptor response and the production of inflammatory cytokines which can be very harmful when released in excess and can lead to death. [38] The immune system can, therefore, be modulated by estrogens which bind alpha or beta receptors to the ER (estrogen receptor). ER-alpha is expressed among all immune cells and is involved in their regulation and maturation, as well as being immune protective as it is liable for IFN type I production and NK cell activation. ER-beta has an opposite reaction to ER alpha and participates in inflammatory phenomena. [39]

Peripheral estrogen-treated mononuclear blood cells (PBMCs) respond better to the antigen and express Toll-like 7 (TLR7) with higher efficiency. CoV-19 binds macrophages, dendritic cells, and mast cells to TLRs (the key molecules in triggering innate immunity), triggering an inflammatory cytokine storm with respiratory infections. CoV-19 activation of TLR in the lung can promote vascularization and hyperemia by generating inflammatory cytokines and chemokines that aggravate the patient's condition with COVID-19. [40]

Roni. 2020 [41] by noting in his study that estrogens will affect an angiotensin-converting enzyme 2 (ACE2) protein, which does support our results. Coronavirus uses cell surfaces with ACE2 receptors as the pathway and ACE2 is considered in men and women differently. In experiments with rats, Dr. Sandberg and her collaborators have also shown that estrogen can decrease ACE2 protein expression in their kidneys, as well as the hormone, which can significantly decrease ACE2 expression for males.

Conclusion:

One of the most common viruses nowadays in human respiratory systems is coronavirus. Which is nowadays known as COVID-19. Since estrogen treatment in animal studies silences the inflammatory reactions and lowers the virus titers resulting in an increased survival rate, it seems to be a perfect

immunity increasing technique against COVID-19. In our study, 73.5% of doctors and pharmacists response positively to using 17β-estradiol (E2) as an element of vaccine for COVID-19 while 8.8 percent of doctors disagreed on that point and 17.7% of those answered go with the possibility. Overall, the mean and standard deviation are (1.4425) and (.77848). We may conclude that Indeed, after reviewing the entire analysis! This 17β estradiol (E2) helps to improve the immunity from the COVID-19 attack. These phagocytotic cells will destroy the virus if it is activated, stopping it from spreading into the lower respiratory region. For this study, clinical trials are recommended.

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Conflict of interest statements:
Author Saba Malik (Corresponding author) declares that he has no conflict of interest. Anwaar UL Huda, Aqsa Munir, Rabia Shakeel, Touqeer Hussain, and Saddam Hussain declare that they have no conflict of interest.

CRediT author statement:
Saba Malik: Conceptualization, Writing- Original draft preparation, Supervision. Aqsa Munir: Methodology. Rabia Shakeel: Data collection, Results. Touqeer Hussain: Data collection, Results, Discussion. Anwaar UL Huda: Visualization, Investigation. Saddam Hussain: Reviewing and Editing.

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